STUDIES ON THE ACCUMULATION OF TRACE METALS IN TROPICAL ANGUILLID EELS FROM VIETNAM

(ベトナムにおける熱帯ウナギの微量金属蓄積に関する研究)

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Abbreviation

Cd:	Cadmium
Co:	Cobalt
Cr:	Chromium
Cu:	Copper
Hg:	Mercury
Mn:	Manganese
Pb:	Lead
Sr:	Stromium
Zn:	Zinc
V:	Vanadium
MeHg:	Methylmercury
QT:	Quang Tri
QN:	Quang Ngai
BD:	Binh Dinh
PY:	Phu Yen
PEC:	Probable effect concentration
E.P.A	Environmental Protection Agency
TL:	Total length
GW:	Gonad weight
LW:	Liver weight
GSI:	Gonad-somatic index
CS:	Sea eel
CE:	Estuarine eel
CR:	River eel

Summary

Vietnam is an agricultural based country, which has a very strategic position at the center of the Southeast Asian region. The usage of chemicals for agriculture was extensive in the past and has remained until very recently. In the last decade, furthermore, the rapid domestic growth and industrialization since the introduction of the renovation policy in 1986 have caused serious environmental pollution as the results of increase in industrial and municipal waste. The discharge of wastewater from human activities inputted to rivers and coastal areas have severely impacted on aquatic systems in many places for recent years. Among pollutants, trace metals are great concern because of their toxicity, persistence and prevalence. Although environmental monitoring systems to assess the water system quality in Vietnam have been done since 1990s, the systems are still under development and merely monitor the water systems along the coastal areas. A lack of advanced studies examines the effects of contamination by toxic chemicals to aquatic organisms, especially fish species. As a consequence, pollutants discharged in the aquatic environment are likely to accumulate in fish and represent a potential risk not only to the fish, but also to other fish consumers, particularly humans.

Catadromous eels, widely distributes throughout the world, are one of the top predator in freshwater ecosystems and they are abundant in Vietnam. The anguillid eels are also commercial fishes for both local consumption and international trade in Vietnam. Due to the long-life cycle and the specific biological and ecological features of anguillid eels, they are vulnerable to adverse impacts from nature and human activities. Therefore, the eel populations have declined dramatically in recent years and the causes are attributed to over-fishing, construction, climate change, other environmental factors, especially environmental pollution. Otherwise, a little information available for using tropical eels as bio-indicator in Asian countries including Vietnam though anguillid eels are abundant in aquatic system of the countries.

Therefore, in the present study, trace metal accumulation in tropical anguillid eels are examined,

which is important first step to understand the mechanism of trace metal accumulations in the tropical anguillid eels and to determine whether anguillid eels can be used as bioindicator or biomonitor in aquatic system in Vietnam as well.

Firstly, the study investigated the accumulation of trace metals in sediment of the rivers from central part of Vietnam in order to assess environmental quality. The concentrations of 10 trace metals in surface sediments of the Ba River and Thach Han River were examined. The Mn levels were the most abundant metal in the sediment, followed by Zn, V, Cr, Sr, Pb, Cu and Co. The lowest levels were found to be Cd and Hg. While the concentrations of trace metals in the sediment did not vary among sites in the Ba River, excepted for Sr, the metal levels in the sediment, however, differed among sites in Thach Han River. The high levels of V, Cr, Cu, Cd Pb and Co observed in upper part of the Thach Han River might result from anthropogenic sources. In contrast, trace metal levels in the Ba River might originate from the natural environment. Although the mean levels of Cd, Cu, Zn and Pb in Thach Han River were higher than those in Ba River, the level of V, Cr, Mn, Co and Sr in the Thach Han River were slightly lower than those in the Ba River. The metal concentrations in sediment from both rivers were comparable with the Environmental Protection Agency (EPA) criteria and background concentration, except for Mn, Zn and Pb in sediment, these trace metal levels, however, do not show the probable effect levels to aquatic environment.

Secondly, the study investigated the distribution of trace metal accumulation in various organs of the maturing eel *Anguilla marmorata* from the Ba River to understand the target organs for metal accumulation and metabolism. The results indicated that the liver and kidney were dominant organs for almost all trace metals, whereas muscle tended to highly accumulate Hg and approximately 87.4-100% of Hg was methylmercury. Interestingly, a strict link of metal accumulations between liver and gonad related to the Zn levels in these organs and the elevated Cd burden in gonad suggest that hepatic trace metals, both essential and nonessential, can transfer to gonad during gonadal maturation. Though almost none of the metal concentrations in the muscle exceeded the reference doses (RfDo) of the U.S.EPA (2008), approximately 80% of the eels from the river

contained mercury exceeding the recommended levels (0.30 μg/g) of the U.S.EPA, and might present a risk for human consumption.

Thirdly, in order to understand the present status of trace metal contamination and the risk associated with human consumption, commercial freshwater eels in Vietnam were examined. The concentration of ten elements (V, Cr, Mn, Co, Cu, Zn, Sr, Cd, Pb, and Hg) was determined in muscle and liver tissues of tropical eel *Anguilla marmorata* collected from four provinces, Quang Tri (QT), Quang Ngai (QN), Binh Dinh (BD), and Phu Yen (PY), in the central part of Vietnam. The results indicated that both muscle and liver tissues reflected the higher potential metal pollution in QT when compared to other sites. Hg levels in muscle significantly correlated to body size. None or negative relationships between other trace metal levels in muscle and body sizes existed, which likely related to somatic growth dilution. Additionally, the maximum metal levels in the muscle of the yellow eel were found to be far below RfDo guideline values of U.S.EPA for human consumption. Thus, the muscle tissue of yellow eel from the central part of Vietnam may not currently cause any serious health risk for human consumption.

Fourthly, whether metal accumulations relates to the maturity stages of tropical anguillid eels, *Anguilla marmorata* and *A. bicolor pacifica*, was examined. The level of nine trace metals in liver and muscle were determined in both yellow and silver stages of the eels. The results indicated that the elevated levels of essential metal of Zn in tissues of both species related to the maturation of anguillid eels. The levels of other essential metals such as Cr, Co, Mn and Cu accumulated in silver eels were higher than those in yellow eels but the differences were depended on the organ tissues and the species. Nonessential metals such as Cd and Pb in tissues exposed no significant difference between maturity stages of eels. Although two species resided in the same river, the Hg levels in *A. marmorata* found to be higher than that in *A. bicolor*. The difference seems to be caused by the difference in the food items between the two species rather than ambient environment.

Finally, in order to understand whether the metal concentrations in eels are related to migratory types using Japanese eel *Anguilla japonica* as a substitute for tropical eel. Nine elements were

analyzed in the livers of three migratory types of the eels collected from Tokushima Prefecture (south Japan). Japanese eels were collected from two sites of the Katsuura River; the upper reach and the estuary 1-2 km from the river mouth. The eels (silver stage) were also collected from Kii Channel. Three migratory types of silver eels were classified by examining the Sr:Ca ratio in otoliths. All types were found 'sea eels', 'estuarine eels', and 'river eels' from the channel, whereas only 'river eels' in the two sites of the river. The results showed that there were significant differences in V, Cr, Cd, and Pb concentrations among the migratory types. Maturing silver sea-eels show a higher risk of metal accumulation than other types of eels, and the concentrations of Mn, Cu, and Zn in maturing eels were significantly higher than those in immature eels. Furthermore, finding of correlations between metal accumulations and otolith Sr:Ca ratios suggested that the sea eels seem to be higher potential risk of metal pollution than other migratory types of eels. Therefore, the study suggests that migratory types of anguillid eels can be considered as a useful tool to aid the interpretation of metal pollution in fresh, brackish and coastal waters.

Hence, the findings in this study provide the useful information on trace metal accumulations in anguillid eels, which are considered to be good indicators to assess water system quality in Vietnam and other tropical countries where the eel distributes as well.

Chapter 1

General Introduction

1.1. Environmental pollution status in Vietnam

Vietnam has more than 3000 km coastal lines, hundreds of rivers draining to the sea along the coast with diverse biological and ecological properties. The country is famous with the two large river deltas: the Red River in the north and the Mekong River delta in the south. These are among the largest plains in the East Asian region and also among the most populous regions in the world (Figure 1.1). Between the two large river deltas of the coastal line, the central part of Vietnam is the narrowest part of the country covering a mixed landscape of mountains, hills, and rivers with a strong seaward slope and coastal plains. Although there has no advantages of geography like the north and south, the region has played important roles in domestic rice production and the economic development of the central coastal provinces for the last decades. The usage of chemicals for agriculture was extensive in the past and has remained until very recently in Vietnam. In the last decade, furthermore, the rapid domestic growth and industrialization since the introduction of the renovation policy in 1986 have caused serious environmental pollution as the results of increase in industrial and municipal waste. The discharge of wastewater from human activities inputted to rivers and coastal areas have severely impacted to aquatic systems in many places for recent years (Ho and Egashira 2000; Hoang et al., 2006; Nguyen et al., 2007; Phuong et al., 2010). Among pollutants, trace metals are great concern because of their toxicity, persistence and prevalence. The subsequent problems have received considerable scientific attention for many years. Various abnormalities have been observed in wildlife (Weber et al., 1993; Mela et al. 2007) and, even in humans (Murata and Nakagawa, 1958; Tsuchiya et al., 1969; Inaba et al. 2005), have been proved to be linked with the high degree of exposure to toxic metal contaminants. Therefore, environmental monitoring activities play an important role in assessing and predicting the impact

of contaminants to environment. Although environmental monitoring systems to assess the water system quality in Vietnam have been done since 1990s, the systems are still under development and merely monitor the water systems along the coastal areas.

Recent studies demonstrated relatively high magnitude of contamination of trace metals such as As, Cu, Zn, Ni, Cd, Pb in aquatic systems of the rivers in the north and south areas (Nhan et al., 1999; Ho et al., 2000; Nguyen et al. 2007; Minh et al., 2007). Due to the industrial and municipal waste-water is directly discharged to rivers, consequently, the rivers have been heavily polluted by trace metals (Ho et al., 2000; Hoang et al., 2006). Ho et al. (2000) examined sediment in three rivers in Hanoi area indicated that concentration of the total trace metals ranged from the background levels to over the maximum permissible levels to crop growth. Nguyen et al. (2007) also found that To Lich and Kim Nguu Rivers in the North Vietnam were heavily polluted with trace metals, and the metal concentrations all exceed the Vietnamese surface water standard. They also suggested that the metal concentrations in environment closely related to the type of manufacturing plants located along the rivers. While Hoang et al. (2006) reported environmental pollution status in South Vietnam indicated that the rivers and canals received untreated wastewater from domestic and industrial sources. They demonstrated that the chemical composition of these aquatic sediments contains very high concentrations of several "urban" metals such as Cd, Cr, Cu and Zn. Most of the samples have exceeded the U.S. EPA's toxicity reference values for Cu, Zn and Cr.

Unlike the north and south region, the central region was underdeveloped in the past. However, recently the governmental and local authorities have encouraged the economic development projects in the region. Furthermore, the rapid economic growth of urban areas (Da Nang or Nha Trang) as economic spill-over influence to other adjacent areas. Urban and industrial zones have been constructed in many cities in the central region such as Dung Quat (Quang Ngai province), Hoa Hiep (Phu Yen province), Quan Ngang (Quang Tri province) and so on. While manager capacity and ability of the authorities do not meet the economic development in the region in particularly and Vietnam in generally,

therefore the environment in the region has been great concern in recent. However, there is a little information available of metal pollution in the central part of Vietnam. Alternatively, the studies have been simply investigated the status of the pollutants in various environmental media (Thuy et al., 2000; Hoang et al., 2006; Nguyen et al., 2007). Almost all of them lacked advanced studies to examine the effects of contamination by these toxic chemicals to aquatic organisms, especially fish species that are considered top consumers in aquatic ecosystems. As a consequence, pollutants discharged in the aquatic environment are likely to accumulate in fish and represent a potential risk not only to the fish, but also to other fish consumers, particularly humans. Thus, many studies have been done with fish as bioindicator to assess the health of aquatic system and fish food quality in recent (Agusa et al., 2005; 2007; Has-Schon et al., 2008).

1.2. Why are tropical anguillid eels chosen for this study?

The catadromous anguillid eels are widely distributed throughout the world and are one of the top predators in freshwater ecosystems. In Vietnam, tropical anguillid eels were found from Nghe An to Binh Thuan, however they are abundant in the central region from Quang Tri to Phu Yen (Figure 1.1). The eels are commercial fishes for both local consumption and international trade in Vietnam (Hoang 2006). Anguillid eels are also well-known for their mysterious life-cycle. They spend their lives in freshwater and return to the ocean to spawn. After hatching in ocean, the larvae, leptocephali, are drifted by the ocean currents to continents where they grow to glass eel. When glass eels enter estuaries they become pigmented and are known as elvers. Elvers travel upstream in freshwater rivers where they grow to adulthood for several years to a few decades depended on species and environmental condition (Tesch, 2003). Due to the long-life cycle and the specific biological and ecological features of anguillid eels, they are vulnerable to adverse impacts from nature and human activities (Robinet and Feunteun 2002; Pierron et al. 2007; 2008). Therefore, the eel populations have declined dramatically in recent years and the causes are attributed to

over-fishing, construction, climate change, other environmental factors, especially environmental pollution (Brusle 1990; Castonguay et al. 1994; Robinet and Feunteun 2002; Wirth and Bernatchez 2003; Pierron et al. 2007; 2008; Bonhommeau et al. 2008). Hence, anguillid eel has been widely used as bioindicator for environmental monitoring to assess the aquatic system quality in many countries (Edward et al. 1999; Langston et al. 2002; Usero et al. 2003; Maes et al., 2007).

Since the 1990s, many countries in Europe have used eels in monitoring the metal contaminant loads in the environment (Brusle 1990; Barak and Mason 1990; Batty et al. 1996; Linde et al. 1996). Brusle (1990) published a review on trace metals in different eel species focused on two main toxicological topics: observation on contaminant levels in wild eels from sampling sites, mainly from contaminated water, and experimental studies under laboratory conditions. Batty et al. (1996) used the eel as sentinel species not only to determine the source of metal pollution, but also to evaluate the ecological risk for fish consumers from Camargue region of France. Up to recent years, many studies focused on the insight of the contaminant on physiological or behavioral responses of the fish and the studies demonstrated the sensitive responses of fishes to anthropogenic pollution in molecular levels such as proteins, enzymes, genetics (Langston et al., 2002; Teles et al. 2004; Bird et al., 2008; Oliveira et al. 2008). However, these studies were limited in laboratory work and it is difficult to apply in fieldwork because in wild, a lot of pollution sources can affect the molecular responses of fish (Ron van der Oost et al., 2003; Van den Broek et al. 2010). In addition, when in laboratory experiments using wild eels, it sometimes happens that the contaminant levels of the control eels are higher than that in the experimental groups because some fish contain the contaminants derived from their original habitats. The results of monitoring work in field by using eels as bioindicator are, therefore, rather useful to assess the health of aquatic system, fish consumers and risk for human consumption (Batty et al., 1995; Belpaire et al. 2007, 2008; Maes et al., 2008). However, the use of European eel *Anguilla anguilla* as bio-indicator or bio-monitor has mostly been done in EU countries for decades (Edward et al. 1999; Langston et al. 2002; Usero et al. 2003; Maes et al., 2007) and few studies on *A. japonica* (Yang and Chen, 1996;

Ohji et al., 2006; Le et al., 2010). Moreover, less information is available for the use of tropical eels as bio-indicator though tropical eels are abundant in aquatic system of Asian countries including Vietnam.

1.3. Research Objectives

In the present study, I characterized the patterns of trace metal accumulation in various tissue organs and also its correlation with the spatial distribution, migratory types and maturity stage of anguillid eel are examined to understand the mechanism of trace metal accumulations in the tropical anguillid eels. This is the important first step to prove the usefulness of anguillid eels as models for biomonitoring of aquatic (eco)system in Vietnam. In addition, the study also assesses the ecological risk for eel population and safety for human consumption.

Firstly, the study examined the environmental status in the central part of Vietnam through evaluating the trace metal levels accumulated in sediments along two rivers, Ba River and Thach Han River, where eels are abundant (Chapter 2). Secondly, the study investigated the distribution of trace metals in various organs of the eel *Anguilla marmorata*. It shows the overview of the metal distribution in soft tissue organs of eels and indicates that which organ is suitable for the purpose of environmental monitoring (Chapter 3). The chapter also presents the levels of methylmercury (MeHg) in liver and muscle of eels. Thirdly, the metal levels were determined in muscle and liver of the tropical eel *Anguilla marmorata* from different sampling sites in center part of Vietnam (Chapter 4). It also investigated the correlation between metal levels in muscle and body size classes of eel to evaluate the potential risk for eel population and human consumption. Fourthly, whether the amounts of accumulated trace metals were related to growth stages (the yellow and silver stages) in two anguillid eel species to re-evaluate the results of the monitoring works using eels (Chapter 5). Finally, whether the differences of metal accumulation levels in eels related to migratory types were examined (Chapter 6). Recent studies based on Sr:Ca profile in otolith

indicate that Japanese eels do not only live in freshwater after recruiting in continental but also reside in brackish or sea water for their whole life (Tsukamato et al., 1998; Tzeng et al., 2000; Arai et al.2004, 2006; Shiao et al., 2003, Kotake et al., 2003, 2005). Therefore, comparison of the contaminant levels in the eels collected at different sites without considering the migratory patterns might not reflect the actual contaminant status, because eels collected in a brackish water area might be visitors from seawater or freshwater areas. Thus, this query will be elucidated in chapter 6. Finally, General Discussion is to discuss overview of the study and to discuss the advantage and disadvantage in use of the anguillid eels as bioindicator or biomonitor. The chapter is also devoted to discussion on the risk assessment of contamination of anguillid eels and human consumption effect.

Figure 1.1. The map of anguillid eels' distribution (red circle) in Vietnam

Chapter 2

Trace metals in surface sediment of the two rivers from the central part of Vietnam

2.1. Introduction

As described the environmental status in Vietnam in Chapter 1, this chapter is focused on examining the trace metal distribution in the rivers from central region of Vietnam, especially the Ba River and Thach Han River. The basins of two rivers exhibit typical geology of the region. These rivers also play important roles to maintain the stock for aquaculture and wild fish resources because of the abundance of commercial fish distribution such as freshwater anguillid eels or giant goby (*Oxyeleotris marmorata*). However, the increase in urban populations and civic centers along the rivers has resulted in an increase in industrial and municipal waste in recent years. The increase in population has also necessitated an increase in agricultural food production, for local consumption and export of high value product. This has resulted in an increase in agricultural waste such as pesticides and fertilizers, which contain high levels of certain metals such as Cu, Cd, As, Hg, etc. These may be potential sources of pollutants input to rivers though run off from human activities by heavy rains. Metal pollutions are attracting attention of scientists and regulatory authorities in Vietnam (Ho et al., 2000; Nguyen et al., 2007; Nguyen and Ohtsubo, 2007), because trace metals are toxic, persistent and /or bioaccumulative (Shriadah, 1999; Tam and Wong, 2000). In river water body, sediments form an important repository of trace metals, due to adsorption, hydrolysis and co-precipitation; only a small portion of free metal ions stay dissolved in water and a large quantity of them get deposited in the surface sediment (Ranjbar, 1998; Kamau, 2001). Therefore, the metal pollution of aquatic system is often most obviously manifested in higher metal levels in sediment than those in water (Jain et al., 2005). Hence, river sediment monitoring has been widely used to assess environmental quality (Shriadah, 1999; Singh et al., 2002; Gaur et al., 2005). However, trace metals are not monitored in most the local monitoring programmes in

Vietnam, particularly in the central region (Brown, 2009), and limited data is available for trace metals (Ho and Egashira, 2000; Kikuchi et al., 2009), although the health of rivers have been of great concern in Vietnam in recent.

Thus, the aim of study is to investigate the distribution of metal loads in sediment and to assess the environmental quality from the two rivers.

2.2. Materials and Methods

2.2.1. Site description

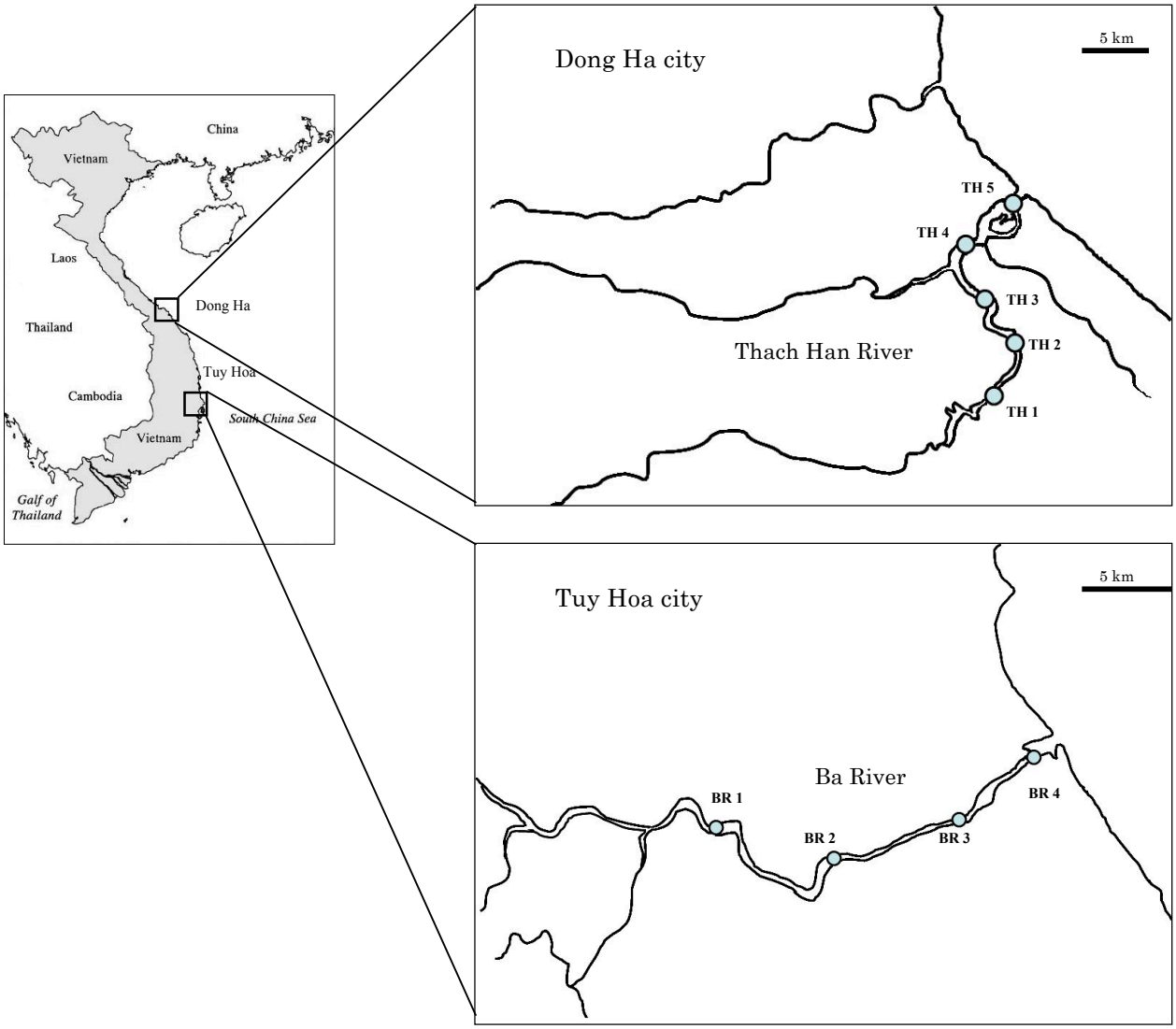
The Ba River (Quang Tri) and Thach Han River (Phu Yen) are the two of the largest rivers in the central part of Vietnam. Although the Ba River is located in the north central part and Thach Han River is in the south central part, the hydrodynamic characteristics of the rivers are under similar the local climate and natural conditions. The central part of Vietnam is covered by a mixed landscape of mountains, hills, and rivers. The rivers are generally short and strong seaward slope and originate from Truong Son mountains near the border of Laos and Cambodia. Although the mountains and highlands here are composed of granulites and igneous rocks (Tran et al. 2001; Nguyen and Egashira, 2008) and extend quite close to the coast, the region also has the small scales of delta fluvial plain along the rivers. There are annually two main seasons in the region, dry and rainy season. In the rainy season, heavy rains often occur in a large scale and lasts for a few weeks to months, which cause with flooding, usually from September to December. During this season, the rivers receive an overflow of expanses of freshwater that submerge surrounding areas. In dry season, in contrast, the rivers generally lack freshwater supply from highland and the seawater invades further in upper fluvial areas. Although the rivers are rather similar, the rivers have different topographic characteristics. The Ba River has strong seaward slope and high fluvial flow velocity because the Ba River basin lies on the lowland area being surrounded by many mountains such as Mt. Kon Tum, Mt. Binh Dinh and Cho Ho Mu. Thus, the Ba River estuary is less affected

by tidal movement in the downstream area and the freshwater dominates in the river. The mixed area of freshwater and seawater is 4-6 km from river mouth, where the variation of salinity is quite high and falls in the range of 0 - 8 ppt in dry season, especially the maximum of salinity can reach 18 ppt some days in drought period (Nguyen, 2008). In contrast, the lowland of Thach Han River is located in flat area, the fluvial flow velocity of this river is weaker than that in the Ba River. Thus, the seawater routinely invades about 15 – 20 km in upper part of the river from river mouth in dry season. The salinity of water increases from 0 ppt in the site 1 to 22 ppt in the site 5 of Thach Han River and the freshwater just predominates in main rainy season that occurs from September to November (Fig. 2.1).

2.2.2. Sampling

Sediment samples were collected at four and five sites from river mouth to upper area of the Ba River (Phu Yen) and Thach Han River (Quang Tri) in June 2009, respectively (Fig. 2.1). In both rivers, the sampling site was started collecting at 1 km from river mouth and other sites were continued towards to the upper reach of the rivers. The distance between the sites was about 5 - 6 km, thus, the last sampling site is about 25 – 30 km from river mouth. The surface sediment samples (1 cm depth) were taken by hand to avoid contamination at a 30-50 cm depth of the river. The wet sediment was homogenously mixed and then sieved through 63 μm plastic mesh sieve for trace metal analysis. Size fraction <63 μm is the most commonly recommended size because trace metals are detected mainly in clay/silt particles in most cases. After air drying at room temperature overnight, the sieved samples were stored in polyethylene z-clock bags at -20^{0}C until analysis.

Figure 2.1. Sampling sites of environmental and eel samples from the Ba River, Vietnam



2.2.3. Chemical analysis

The sediment samples were digested by using aqua regia as described by Loring and Rantala (1992) with modification. In brief, the sediment samples were dried in an oven at 60^0C until constant weight and then ground with an almina motor. Approximately 100 mg of dried sediment samples were microwave-digested in Teflon bombs using 3 ml of aqua regia acid (HNO_3: HCl; ratio 1:3). After heating by microwave oven, the bombs were cooled to room temperature in an ice bath. The bombs were carefully opened, HNO_3 40% was added in the supernatant, and the mixed solution was then heated on a hot plate at 90^0C to dry up, the dried residues were dissolved in 100 ml of 1% HNO_3 into polyethylene tubes. The solutions were stored at 4^0C until metal analysis.

Before measuring the nine trace metals (V, Cr, Mn, Co, Cu, Zn, Sr, Cd, and Pb) in sediment samples by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent-7500cs, Agilent Technologies, USA), internal standard solution including Scandium (Sc), Indium (In), and Bismuth (Bi) was added to each sample to correct the matrix effects and instrumental drift in ICP-MS during measurement. Hg was determined with a cold vapor atomic absorption spectrometer (Model MA-2000, Nippon Instruments Corporation, Japan).

Detection limits (in sediment and eel samples) of V, Cr, Mn, Co, Cu, Zn, Sr, Cd, Pb and Hg were estimated to be 0.0001, 0.001, 0.001, 0.0001, 0.001, 0.01, 0.002, 0.0001, 0.0001 and 0.001 μg/g, respectively.

Reference material SRM 1646a (estuarine sediment, National Institute of Standards and Technology, USA), was prepared in the same way. Recoveries of all trace metals in sediment samples ranged from 89 – 105 %.

2.2.4. Sediment quality assessment

Several sediment quality guidelines were recently developed to provide interpretative tools for assessing pollutants such as U.S. EPA (2009) or McDonald (2000). However, Geoaccumulation index (I_{geo}) was even calculated for some toxic metals in sediment introduced by Muller (1981) and

the equation is showed below:

$$I_{geo} = Log_2(C_n \div 1.5 \times B_n) \quad (1)$$

Where C_n is concentration of trace metals n in sediment, B_n is geochemical background value in average shale of element n (Turekian et al., 1961), 1.5 is the background matrix correction in factor due to lithogenic effects. The studied metals in sediment are evaluated and compared to Geo-accumulation index that consists of seven grades ranging from "unpolluted" to "very strongly polluted" (Table 2.1).

2.2.5. Statistical analysis

The results are expressed as mean ± SD. ANOVA and Tukey tests were performed to reveal any significant differences in metal concentrations between the rivers. The statistical analyses were performed using STATISTICA 5.5 for Windows (Statsoft, Inc., USA).

2.3. Results

The metal levels in sediment from the rivers were showed in Table 2.2. The metal levels indicated the same trend in the both rivers. The concentrations of Mn were the most abundant metal in the rivers, followed by Zn, V, Cr, Sr, Pb, Cu and Co. The levels of Cd and Hg were found to be the lowest. In the Ba River, the concentrations of metals in the sediment did not vary among sites, except for Sr. Sr levels were slightly increased from the site BR 1 to the site BR 4 in the Ba River. In contrast, the metal levels in the sediment varied among sites in Thach Han River. The highest levels of V, Cr, Cu, Cd Pb and Co were observed in upper part of the river (site TH 1). While most metal levels were found to be lowest in the sites 2 and 3. Unlike the Ba River, Sr levels showed clear increasing trend from upper to lower part in the Thach Han River and the highest level of Sr were observed in the river mouth site (TH5).

Comparison the metal mean levels between the rivers indicated that only Cd level in Thach Han

River was significantly higher than that in Ba River ($p<0.05$). Although average levels of Cu, Zn and Pb in Thach Han River were slightly higher than those in the Ba River and those of V, Cr, Mn, Co and Sr in the Thach Han River were slightly lower than those in the Ba River, these differences were not significant (Figure 2.2).

Table 2.1. Geoaccumulation index (Muller, 1981) of metal concentrations in sediment

I_{geo}	Pollution Intensity
<0	Background concentration
0 ~ 1	Unpolluted to moderately polluted
1 ~ 2	Moderately unpolluted
2 ~ 3	Moderated to strongly polluted
3 ~ 4	Strongly polluted
4 ~ 5	Strongly to very strongly polluted
> 5	Very strongly polluted

Table 2.2. Trace metal concentrations (μg/g dry wt.) in sediment collected in the Thach Han and Ba Rivers

Sites	V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg
TH1	90.5	56.4	1038.1	16.2	27.2	148.6	25.7	0.46	36.9	0.041
TH2	71.3	43.9	919.6	10.9	18.7	94.8	34.4	0.27	20.2	0.047
TH3	73.2	45.3	1119.1	11.7	19.3	118.5	37.9	0.22	23.9	0.054
TH4	81.7	50.2	901.6	12.9	24.2	107.0	46.2	0.30	22.5	0.049
TH5	88.7	54.1	1012.9	12.2	23.2	155.5	50.1	0.19	27.8	0.056
Mean	**81.1**	**50.0**	**998.3**	**12.8**	**22.5**	**122.9**	**38.8**	**0.29**	**26.2**	**0.050**
BR1	81.7	50.1	1151.3	16.5	20.4	102.8	53.7	0.11	24.5	0.039
BR2	87.5	47.9	1212.7	16.6	21.5	115.7	57.3	0.18	22.6	0.048
BR3	90.0	57.0	1180.5	18.6	23.7	123.3	57.1	0.15	25.6	0.050
BR4	84.1	55.6	1161.9	16.8	18.9	120.6	59.5	0.12	26.4	0.046
Mean	**82.1**	**52.6**	**1176.6**	**17.1**	**21.1**	**115.6**	**56.9**	**0.1**	**24.8**	**0.046**
Mean crust[a]	100*	10-90*	950	18	50	75	Ng	0.3	14	0.002-0.015*
Back.[b]	50	7-13	400	10	10-25	7-38	49	0.1-0.3	4-17	0.004-0.051
PEC[c]	ng	111	ng	ng	149	459	ng	4.98	128	1.06

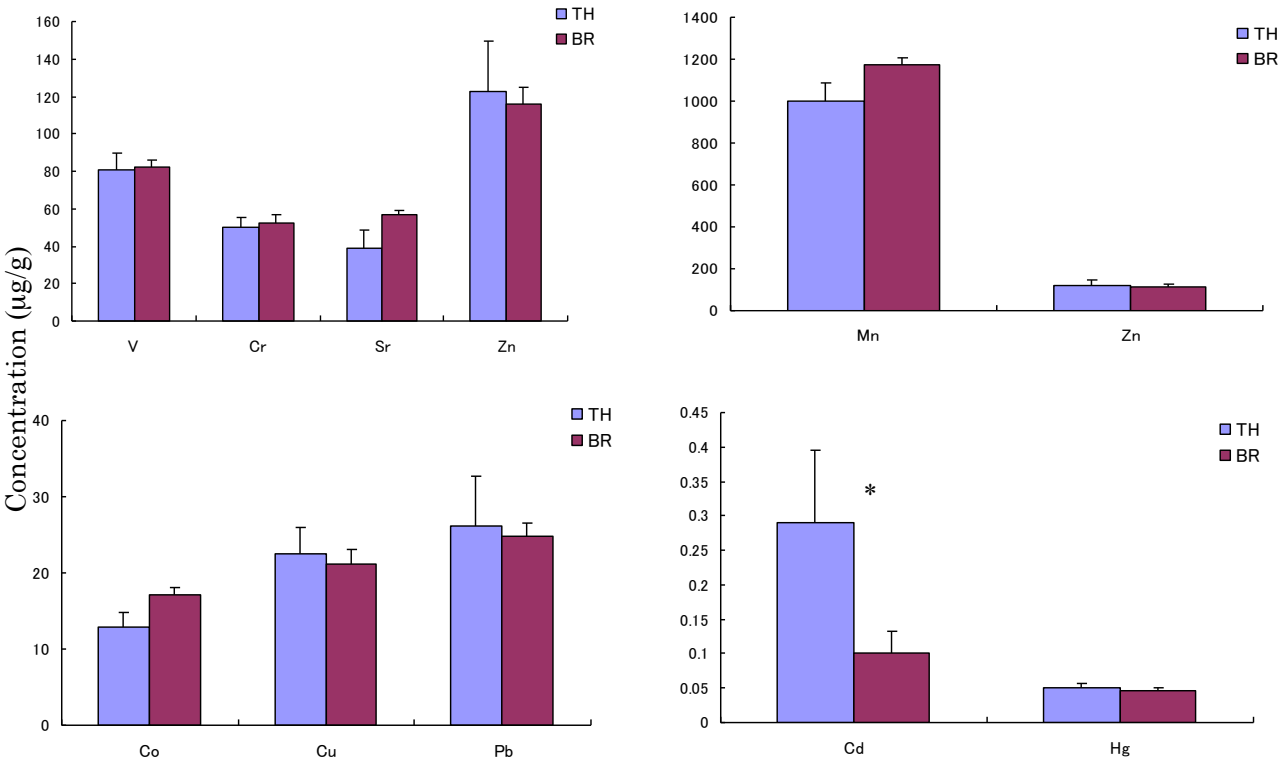
[a] Mean crust: Bowen (1979), *: The normal concentrations of the metals in soil (Merian, 1991)

[b] Back.: background concentration (Buchman, 2008)

[c] PEC: Probable effect concentration (McDonald et al. 2000)

[d] ng: No guideline

Figure 2.2. Comparing trace metal levels (µg/g) in sediment between two rivers



Note: TH: Thach Han River; BR: Ba River; *: significant difference

2.4. Discussion

In the Ba River, the metal levels were similar among sites and there were no iron mining or industrial manufacturing facilities along the river. Additionally, the levels of Cr, Cu, Zn and Pb in sediment of the Ba River were comparable to the less polluted river in central part of Vietnam such as Cude River in DaNang-Hoian area where these levels are supposed to close to background values (Thuy et al., 2000). However, the concentration of Pb in sediment was slightly higher than in the background levels of Bunchman (2008), which might relate to the geochemical characters of the location. Because the source of the river waters is the mountains and highlands of the central part of Vietnam, it is likely that the river is enriched with natural Pb contained in granulite rocks (Tran et al. 2001) of the region. The toxic metals, such as Cd and Hg, in the river were low levels when compared to the polluted rivers in Vietnam (Nguyen et al., 2007; Nguyen and Ohtsubo, 2007) and these levels did not exceed the probable effect concentration (PEC) for aquatic organism (MacDonald et al. 2000). However, it should be note that the levels of Hg in sediment reflected the presence of this metal at the high background concentration, as reported by Buchman (2008). For Mn and V, there are no data available of these metals in the previous studies of the studied and adjacent areas. While V level was comparable with the background level of Bunchman (2008), the extremely high concentration of Mn in sediment might related to the igneous rocks that contain the most abundance of natural Mn element derived from the Truong Son mountains (Turekian et al., 1961; Nguyen and Egashira, 2008). After weathering processes, the element was run-off to the river by rainfall, it was incorporated into the aquatic system and precipitates as oxides/hydroxides (Duzgoren-Aydin and Aydin, 2009). Similar in the Thach Han River, due to the river also originates from Truong Son Mountains and the geochemical characteristics of this river is similar to the Ba River. Therefore the almost all trace metal levels in the river are quite similar to the Ba River. However, the variation of levels of Pb in the river among sites might relate to anthropogenic sources, especially in site TH 1, because the collected site is nearby a bridge of busy traffic and it

closed to the urban area of Quang Tri city. The elevated levels of Pb in the site could be due to run-off from traffic activities and urban waste to the river. The increase of other metals, such as Cd, Cu, Zn, Co, V, and Cr, in the site TH1 might come from urban activities. Although Zn level in the river was slightly higher than that in Cude River (less polluted river), but lower than Han River and Hoian River (polluted rivers) from Danang-Hoian area (Thuy et al., 2000), Zn concentrations of non-contaminated soils was known to fall in a range from 10 – 300 μg/g (Merian, 1991) and the Zn levels in the present study were far below PEC found by MacDonald (2000).

Furthermore, both rivers, Thach Han and Ba Rivers, were important drainage for the large agricultural areas, the intensively agricultural activities have been occurred on both sides of the rivers for many decades. This might be the main sources of Cd in the rivers derived from anthropogenic source, because the commercial phosphate fertilizers which contained small amount of trace-metal contaminations were potential sources of Cd and were delivered to the rivers from up-stream areas by run-off during trace rains (Ngo et al., 2005). Like the Ba River, Hg in sediment of the Thach Han River existed at low levels and was comparable to the unpolluted rivers in Vietnam (Nguyen et al., 2007; Nguyen and Ohtsubo, 2007).

In case of Sr, the differences of Sr levels deposited in sediment of river might relate to the influence of seawater due to the higher level of Sr in seawater than that in freshwater and Sr was usually immobile in the environment, because of rapid precipitation as strontium carbonate (Rosenthal et al., 1970). Both rivers showed the same increasing trend of Sr levels from upper to lower parts. However, the seaward trend of Sr elevated levels in Thach Han River was more clearly than that in Ba River, which is related to the different dynamic character of the rivers.

Since previous studies in the study areas as well as contiguous areas were quite scarce, local or regional background values were unavailable, therefore, in order to assess the quality of sediment in Ba River, the metal accumulations were evaluated with Geo-accumulation index. The results showed that the $\boldsymbol{I}_{geo}$ values of trace metals from Ba River all were negative, while Zn, Cd and Pb in

upper part (site TH 1) and Zn in lower part (site TH5) from Thach Han River showed $\boldsymbol{I}_{geo}$ values in range from 0 to 1 (Table 2.3). This clearly indicated that the Ba River is not polluted, while Thach Han River is from unpolluted to moderately polluted trace metals but it is depended on the sites in the river.

Table 2.3. Geoaccumulation index values in the Thach Han and Ba Rivers

Sites	V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg
TH1	-1.11	-1.26	-0.30	-0.81	-1.31	**0.06**	-4.13	**0.03**	**0.30**	-3.87
TH2	-1.45	-1.62	-0.47	-1.39	-1.85	-0.59	-3.71	-0.74	-0.57	-3.67
TH3	-1.41	-1.58	-0.19	-1.28	-1.81	-0.27	-3.57	-1.03	-0.33	-3.47
TH4	-1.26	-1.43	-0.50	-1.14	-1.48	-0.41	-3.28	-0.58	-0.42	-3.61
TH5	-1.14	-1.32	-0.33	-1.22	-1.54	**0.13**	-3.17	-1.24	-0.11	-3.42
BR1	-0.75	-1.31	-0.15	-0.68	-1.98	-0.47	-2.19	-1.49	-0.29	-4.10
BR2	-0.70	-1.57	-0.07	-0.85	-2.02	-0.30	-2.16	-0.74	-0.41	-3.64
BR3	-0.68	-1.45	-0.11	-0.89	-1.92	-0.21	-2.15	-1.24	-0.23	-3.58
BR4	-0.86	-1.40	-0.13	-0.87	-2.37	-0.24	-2.04	-1.24	-0.18	-3.71

Chapter 3

Distribution of trace metals and methylmercury in soft tissues of the freshwater eel *Anguilla marmorata* in Vietnam

3.1. Introduction

Carnivorous fish species can be considered top consumers in aquatic ecosystems. As a consequence, pollutants discharged in the aquatic environment are likely to accumulate in fish and represent a potential risk not only to the fish themselves, but also to other fish consumers, particularly humans. The catadromous eel *Anguilla marmorata* widely distributes in the tropical Indo-West Pacific (Tesch, 2003; Minegishi et al., 2008) and is a top predator in freshwater ecosystems. It is abundant in Vietnam, where it is a commercial fish for both local consumption and exportation. However, the eel populations have declined dramatically in recent years, with the causes being attributed to overfishing, construction such as hydro-plant, pollution, climate change and other environmental factors (Tatsukawa, 2003; McKinnon, 2006). Recently, environmental pollution has been a cause for great concern because the sublethal effects of exposure to chemical compounds may cause lipid storage and reproductive problems in matured eels when they migrate a long distance to spawning areas (Robinet and Feunteun, 2002; Pierron et al., 2008). As eels are long-lived and habit in small range for a long time during yellow stage, they may accumulate much toxic substances in even less polluted waters especially mercury and methylmercury (MeHg) (Edwards et al, 1999). Additionally, because silver eels (maturing eels) do not eat when they leave the freshwater system, the transoceanic migration forces them to constitute the energy reserves needed for successful spawning. During migration and gonadal maturation, these energy reserves are depleted to provide energy. The rapid mobilization of energy reserves not only redistributes the essential substances (Fletcher and King, 1978; Miramand et al., 1991), but also causes a rapid release of stored pollutants into the bloodstream (Pierron et al., 2007, 2008). These substances then concentrated in fish organs, particularly in the gonad and liver at the crucial time of gametogenesis

(Robinet and Feunteun, 2002). Therefore, knowledge of metal tissue distribution provides information on specific organs that may be particularly selective and sensitive to trace metal accumulation. The differences in tissue metabolism and fish physiology play an important role in metal accumulation. Additionally, understanding metal distribution in fish organs can help in gaining an understanding of the mechanism of uptake in fish and assessing their physiological health, especially the reproductive biology of matured eels (Pierron et al., 2008). However, there is a little information available on trace metal accumulation in the matured freshwater eel in Vietnam (Le et al., 2009).

The purpose of this study was therefore to determine the distribution of the accumulation of trace metals and MeHg in various organs of the freshwater eel *Anguilla marmorata* in Vietnam; and to evaluate whether these levels are safe for human consumption.

3.2. Materials and Methods

3.2.1. Study site

The Ba River is one of the biggest rivers in the central part of Vietnam (Figure 3.1). The river has a length of 374 km, derives from Ngon Ro Mountain at 1 549 m height in the south west of Kon Tum province. It passes though some provinces before entering Phu Yen province and ultimately ends in South China Sea. In upper area of the Ba River, the water flow quickly runs though the Son Hoa highland and its flow is slow down when reaching to Tuy Hoa in downstream area. Although Tuy Hoa is 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 dynamic of water flows in flooding season once a year, the sediment dominants coarse sand or sandy mud covered by a thin unstable layer of silt on the surface. The Ba River is also natural habitat for anguillid eels and their exploitation constitutes in basis of economy of local professional fishermen.

Figure 3.1. Sampling site in the Ba River, Vietnam

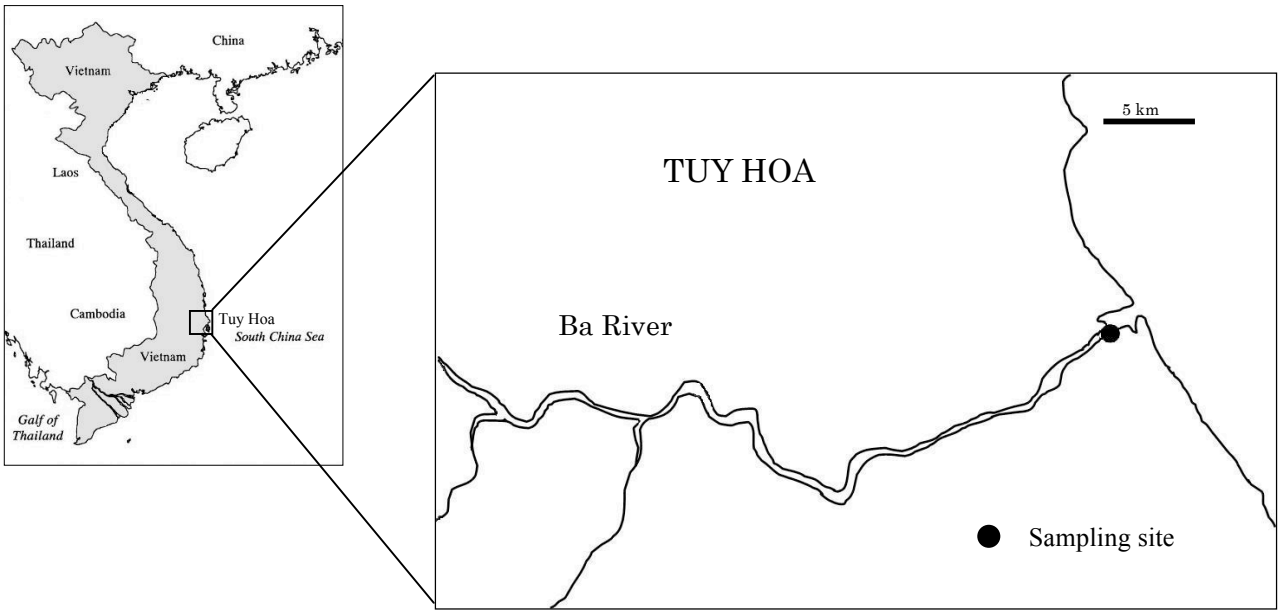


Table 3.1. The physical data of eel specimens collected in the Ba River, Vietnam

Sample No.	TL (mm)	Body W (g)	Liver W(g)	Gonad W (g)	GSI
1	1140	4005.7	43.67	187.5	4.7
2	1148	5342.6	57.86	154.6	2.9
3	1245	5850	62.90	215.1	3.7
4	1219.5	7160	111.46	306.6	4.3
5	1385	7615	86.83	352.5	4.6
6	1073.5	4985	46.63	147.5	3.0
7	1285	7695	81.99	184.8	2.4
8	1247	6084.5	72.48	261.8	4.3
9	1229	6886.5	70.25	283.2	4.1
10	1298.5	8115	57.5800	302.0	3.7
Mean±SD	1227.1±84.6	6373.9±1271.1	69.2±19.3	239.6±67.5	3.8±0.7

3.2.2. Sampling

A total of 10 wild *Anguilla marmorata* were collected by electric shockers from October to November 2008 at the downstream area of Ba River in Phu Yen Province, Vietnam (Fig. 3.1). The sampling site is about 2 km from the river mouth. The total length (to the nearest mm) and body weight (to the nearest gram) were measured (Table 3.1). The color of skin and fins was further observed to classify each specimen as either a yellow or silver stage, and sex was determined by examining the gonads. The gonad-somatic index (GSI) was estimated to determine the maturity of the eels using the following equation:

$$GSI = [\text{Gonad weight(g)}/\text{Body weight(g)}]\times 100 \qquad (1)$$

The eels were considered as yellow eels if the GSIs were below 1.0 and silver eels if the GSIs were above 1.0 (Utoh et al., 2004). Soft tissue from nine organs, the dorsal muscle tissue, heart, liver, kidney, gills, stomach, spleen, intestine, and gonad were dissected. All organs were put in clean polyethylene bags and stored at -20^{0}C in Vietnam before transportation to Japan for further chemical analyses.

3.2.3. Chemical analysis

All organ tissues were dried to constant weight before chemical analysis. The moisture in muscles and liver were estimated to determine the dry/wet-weight ratios. The dried tissue samples were digested in a Teflon bomb by a microwave oven as descried by Yang et al. (2004) (Fig. 3.2). Briefly, 3-5 g of muscle or liver tissues were cut by ceramic knife and dried for 12h at 80^{0}C, then ground with an almina motor. Approximately 0.1 g of powder of the sample was weighed in Teflon PTFE tubes, and 1.5 ml of purified HNO_3 was added. After predigestion at room temperature overnight, the Teflon tubes were tightly capped and the sample was digested in a microwave oven for 10 minutes at 200W, and this procedure was repeated three times. After letting the samples cool to room temperature, the tubes were opened and the digested sample was diluted with 50 ml Milli-Q water (Milli-Pore Company) into polyethylene tubes. The digested solutions were diluted

with 45 ml Milli-Q water (Milli-Pore Company) into polyethylene and stored at 4^0C until metal analysis.

Before measuring the eight trace metals (V, Cr, Mn, Co, Cu, Zn, Cd, and Pb) in organ tissue samples by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent-7500cs, Agilent Technologies, USA), internal standard solution including Scandium (Sc), Indium (In), and Bismuth (Bi) was added to each sample to correct the matrix effects and instrumental drift in ICP-MS during measurement. Hg was determined with a cold vapor atomic absorption spectrometer (Model MA-2000, Nippon Instruments Corporation, Japan).

Detection limits of V, Cr, Mn, Co, Cu, Zn, Cd, Pb and Hg were estimated to be 0.0001, 0.001, 0.001, 0.0001, 0.001, 0.01, 0.002, 0.0001, 0.0001 and 0.001 μg/g, respectively.

Liver and muscle were chosen for MeHg analysis because these organs are targets for Hg accumulation (Barak and Mason, 1990). MeHg was analyzed following the flow chart in Figure 3.3 as described by Hight and Corcoran (1987). In brief, approximately 1.0 g of wet homogenized tissue was put into a 50ml centrifuge tube, and 2.5ml of 9N HCl was then added. After shaking for 1-2 min, 20 ml of toluene was added and the tube was shaken again for 5 min. The mixture was then centrifuged at 2500 rpm for 10 min and the combined solution was put into a 100 ml separated funnel. The solution (toluene and acid) was then washed 3 times with 20 ml of NaCl (20%). Six ml of cysteine – acetate solution were added to the solution and shaken for 5 min. After standing for 10 to 30 min, the upper layer was put into a 10 ml test tube. After centrifugation, the upper layer was then put in a 20 ml test tube. After adding 1 ml of HCl and 4 ml of toluene, the mixture was shaken for 5 min and left standing for 10 to 30 min. The combined toluene was dehydrated by sodium sulfate anhydrous. The final solution was then concentrated by nitrogen gas into 1 ml before analyzing by gas chromatography/electron capture detection (GC/ECD) (Agilent 6890N, Agilent Technologies, USA). The detection limit of MeHg was estimated to be 0.025 μg/g wet weight for the biological samples.

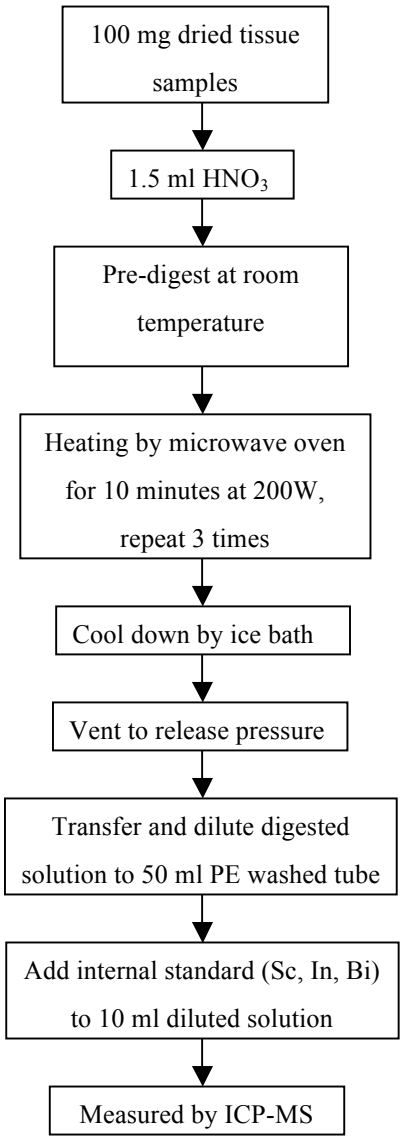


Figure 3.2. Microwave digestion method procedure for analysis of trace metals in soft tissues

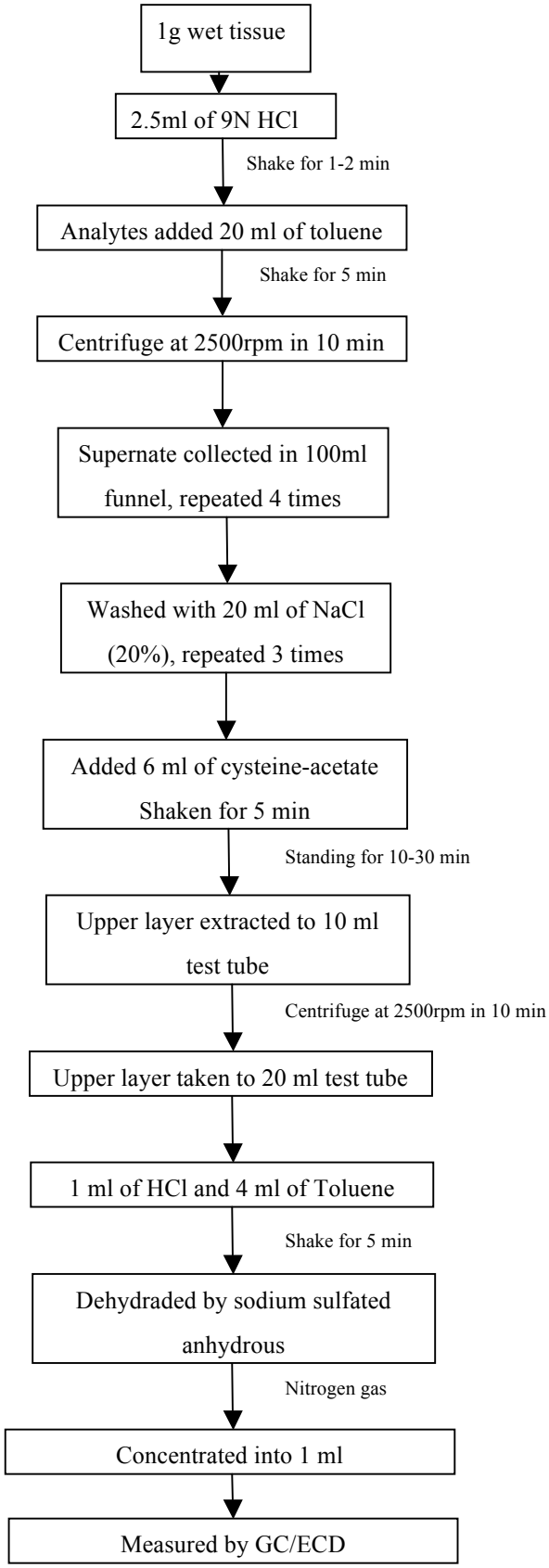


Figure 3.3. Analytical method procedure for methylmercury in soft tissues

Reference materials DORM 2 (dogfish, National Research Council, Ottawa, Canada), and SRM1577b (bovine liver, National Institute of Standards and Technology, USA) were also prepared in the same way. Recoveries of all trace metals and Hg in environmental and eel samples ranged from 96 – 105 %, while a range of 88 - 110% was the recoveries of MeHg analysis.

3.2.3. Estimation of chemical doses

The standard U.S. EPA (1992) (U.S. Environmental Protection Agency 1992) method was used to estimate the risk for human consumption due to ingestion of fish. The daily intake doses were determined using the equation below:

$$D_{ij} = (C_i \times I_j) \div W \quad (2)$$

Where:

D_{ij}: estimated dose ($\mu g.kg^{-1}.day^{-1}$) for chemical i at ingestion rate j

C_i: concentration of chemical i on fish

I_j: ingestion rate for j^{th} percentile of population.

W: assumed human body weight (50kg)

3.2.4. Statistical analysis

The results are expressed as mean ± SD. ANOVA and Tukey tests were performed to reveal any significant differences in metal concentrations between organs. Correlation coefficients were estimated between the metal concentrations and biological characteristics (total length (TL), body weight (BW), liver weight (LW) and gonad weight (GW). The statistical analyses were performed using STATISTICA 5.5 for Windows (Statsoft, Inc., USA).

3.3. Results

3.3.1. Physical characteristics

The size and weight (mean±SD) of the tropical eel *Anguilla marmorata* were 1227.1 ±84.6 mm and 6373.9 ±1271.1 g, respectively (Table 3.1). Liver and gonad weight were 69.2±19.3 (g) and 239.6±67.5 (g), respectively. All eel samples were female and classified as silver maturing stage based on the fin color and GSI. The GSI of eel samples ranged from 2.4 to 4.7 and the mean±SD was 3.8±0.7.

3.3.2. Metal accumulations in various organs

The distribution of trace metals in fish organs are given in Table 3.2 and Figure 3.4. The obtained results showed that the mean concentrations of Hg were highest in the liver, followed by the muscle and kidney. In contrast, the lowest concentrations of Hg were recorded in the gonad. The Hg concentrations in the liver, muscle and kidney were significantly higher than those in the intestine, stomach, gills, and gonad (Tukey HSD test, $p<0.01$); and Hg concentrations in the liver were significantly higher than in the spleen and heart (Tukey HSD test, $p<0.01$).

Though not statistically significant, the levels of total Hg (THg) in the liver were higher than in the muscle, while the levels of MeHg in the liver and muscle showed the opposite trend (Table 3.2). The moisture contained in the muscle and liver were estimated to be 72.2% and 71.2%, respectively. The result showed that MeHg/THg ratio in dry weight base ranged from 87.4 to 100% in the muscle and from 17.7 – 28.8% in the liver.

For other metals, the results indicated that the liver and kidney were dominant sites for almost all metal accumulations, except for Sr. The highest concentrations of V, Cd and Pb were observed in the kidney and liver; these concentrations were also significantly higher than those in the other organs (Tukey HSD test, $p<0.01$). The low concentrations of V were found in the gonad, muscle and gills, while the concentrations of Cd tended to decrease from the intestine, liver, spleen, gill, gonad and heart, with the lowest Cd concentration in the muscle. The lowest concentrations of Pb

were found in muscle and gonad, while the identical levels of Pb observed in heart, intestine, stomach and gill were significantly lower than those found in spleen (Tukey HSD test, $p<0.01$).

It was clearly indicated that higher concentrations of the essential metals such as Mn, Cu and Zn were accumulated in the liver than in other organs. The Cu and Mn concentrations were lowest in muscle and gonad, but, interestingly, a high accumulation of Zn was found in the gonad, while a higher concentration of Mn was observed in the stomach when compared to other organs such as the intestine, spleen, gills, muscle and heart (Tukey HSD test, $p<0.01$). Unlike the above essential metals, the Co and Cr accumulations were at the highest level in the kidney and the lowest concentrations were found in the muscle for Co and in the gills and intestine for Cr.

Unlike other metals, Sr did not accumulate in the liver and kidney. The highest concentrations were observed in the gills and intestine, followed by the heart, stomach, kidney, spleen, liver, and muscle. The lowest concentrations were found in the gonad. The concentrations of Sr in both the gills and intestine were significantly higher than in the gonad and muscle, and the Sr accumulations in the gills were also significantly higher than in the liver (Tukey HSD test, $p<0.01$).

3.3.3. Metal burdens in the organ tissues

The metal burden calculated by the mass of metal within whole or part of an organism is usually expressed as concentration. The metal burden percentages of various organs are depicted in Figure 3.5. Unlike the metal concentrations in organ tissues, trace metal burdens in muscle showed most abundant, especially Cr, Zn, Sr and Hg. Although liver and kidney were target organs for almost metal accumulation, metal burden in liver was largely in V, Cu, Cd, Pb, Mn, and Co and kidney specifically contained Cd. Surprisingly, gonad was the highest Cd burden, instead of liver or kidney. Stomach was principally in Mn, Co Pb and V, while other organs such as heart, spleen and intestine the metal burden were minor.

Table 3.2. Trace metal concentrations (μg/g dry wt.) in various organs of freshwater female eel *Anguilla mamorrata* collected in the Ba River, Vietnam.

Organs (n=9)	V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg	MeHg
Liver	0.85±0.56	0.36±0.23	9.13±3.20	1.35±0.95	73.08±33.75	239.7±89.6	0.85±0.37	0.37±0.42	0.34±0.34	2.21±1.40	0.16*
Kidney	1.04±0.71	0.95±0.33	8.14±2.26	2.34±1.27	11.25±5.34	102.6±32.3	1.21±0.41	4.94±6.33	0.33±0.29	1.74±1.25	
Gills	0.05±0.03	0.15±0.04	2.06±1.28	0.73±0.56	3.32±0.75	42.1±6.1	3.68±4.79	0.10±0.13	0.05±0.04	0.45±0.36	
Heart	0.09±0.06	0.30±0.14	1.89±0.67	1.67±1.04	16.01±8.58	100.8±35.9	1.61±0.49	0.03±0.03	0.08±0.10	1.05±0.51	
Gonad	0.03±0.01	0.46±0.14	0.55±0.26	0.26±0.13	3.25±1.40	159.3±40.0	0.18±0.07	0.07±0.12	0.02±0.01	0.09±0.11	
Muscle	0.03±0.01	0.58±0.24	0.69±0.22	0.17±0.07	1.32±0.70	76.8±25.1	0.42±0.29	0.01±0.004	0.02±0.01	1.82±0.42	0.51*
Spleen	0.10±0.05	0.28±0.06	2.11±0.73	0.95±0.51	14.55±14.76	87.2±19.8	1.01±0.28	0.13±0.05	0.16±0.21	0.90±0.63	
Stomach	0.09±0.05	0.28±0.06	13.30±12.03	1.15±0.58	6.59±0.72	71.1±11.5	1.57±0.76	0.05±0.04	0.07±0.1	0.48±0.25	
Intestine	0.13±0.06	0.27±0.06	2.97±1.1	0.99±0.36	8.08±2.62	99.4±41.5	3.38±2.21	0.40±0.32	0.08±0.04	0.64±0.38	

* The concentration in wet weight (μg/g).

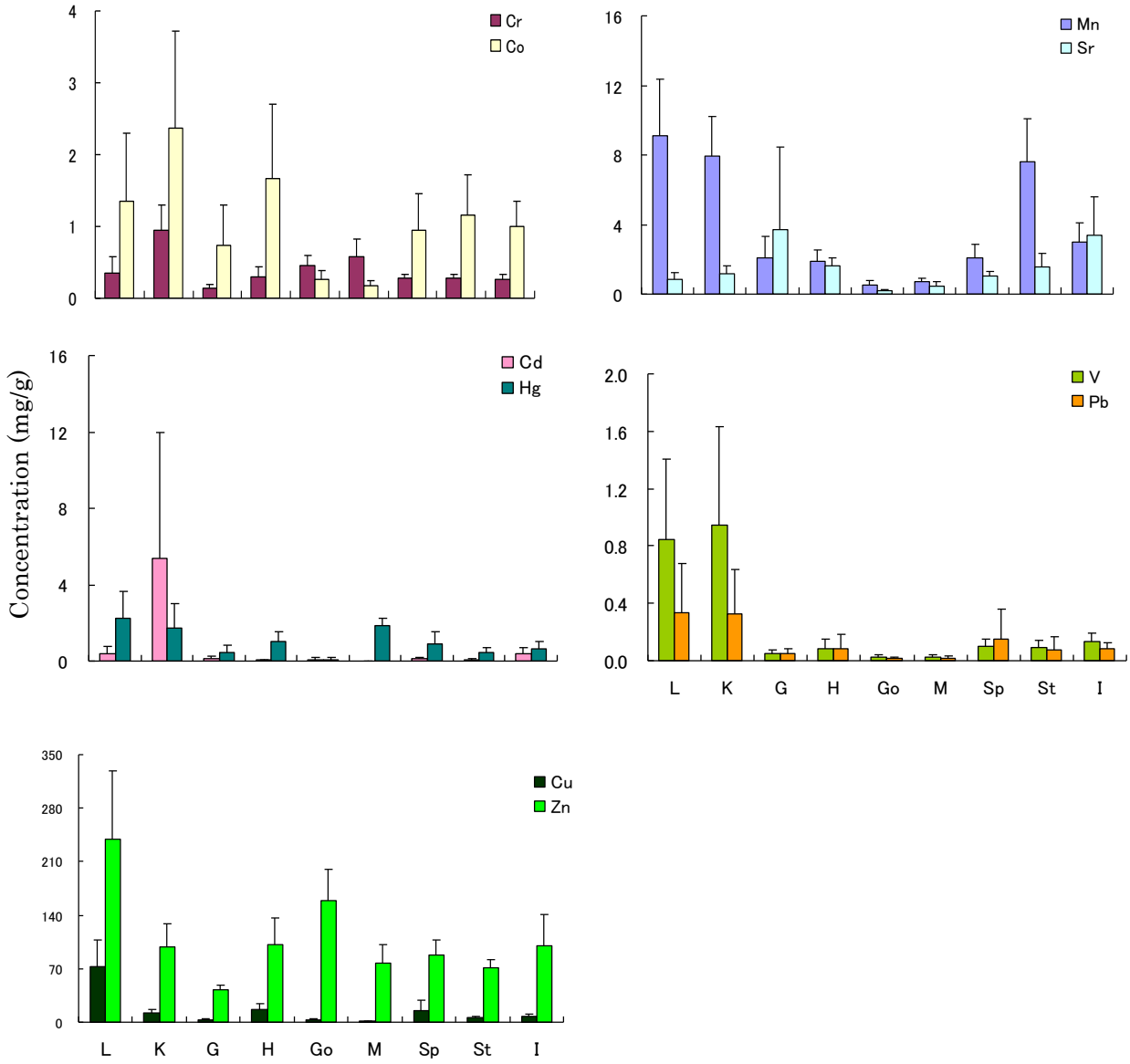


Figure 3.4. Trace metal distribution in various organs in *A. marmorata*

Note: I: Intestine, St: Stomach, Sp: Spleen, M: Muscle, G: Gill, Go: Gonad, H: Heart, K: Kidney, L: Liver

3.3.4. Relationships between the metal accumulations in organs and biological features

The correlations between the levels of accumulated metals in the organs and biological features (TL, BW, LW, and GW) were examined. Although weak correlations exist between Hg and MeHg levels in muscle with both TL and BW, they were not significant. Inversely, there were different correlations between the other trace metal levels and biological features in each organ. In the spleen, three essential metals, Mn, Cu, and Zn, negatively correlated with TL (r = -0.9, -0.6, and -0.8, respectively) (Figure 3.6), BW (r = -0.9, -0.9, and -0.9, respectively) (Figure 3.7), LW (r = -0.9, -0.8, and -0.8, respectively) (Figure 3.8), and GW (-0.8, -0.6, and -0.7, respectively) (Figure 3.9), while only a non-essential metal, Pb, showed a negative relationship with TL (r = -0.7) (Figure 3.10). A positive correlation between Zn concentrations and LW was observed in the liver (r = 0.7) (Figure 3.11). No further relationships between these metals with biological features in the intestine, heart, gill, gonad and stomach were found.

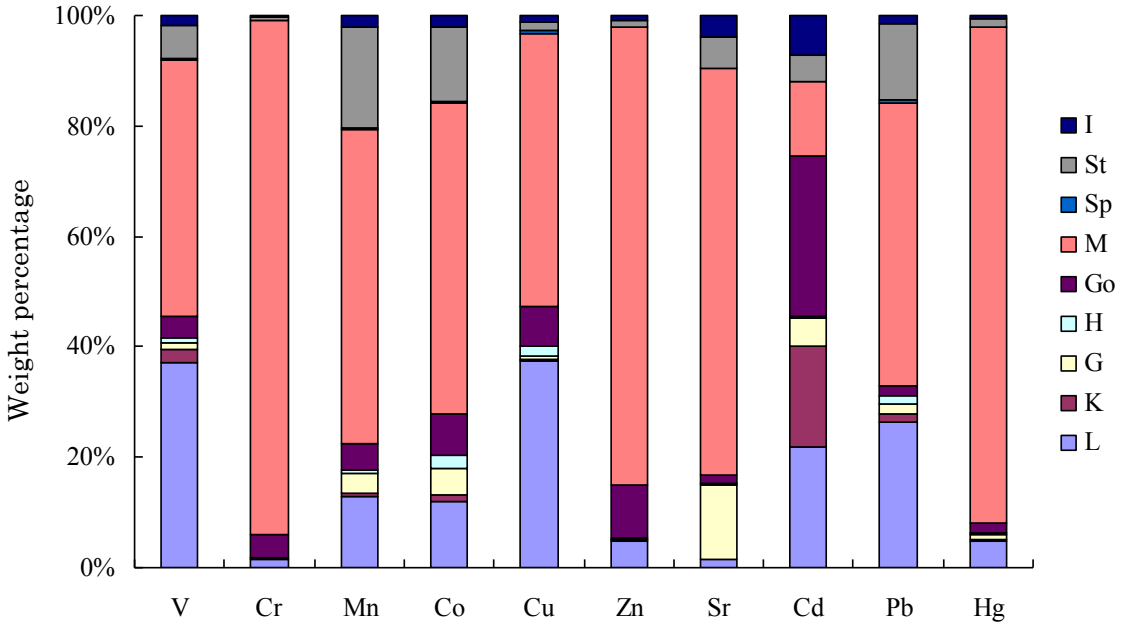
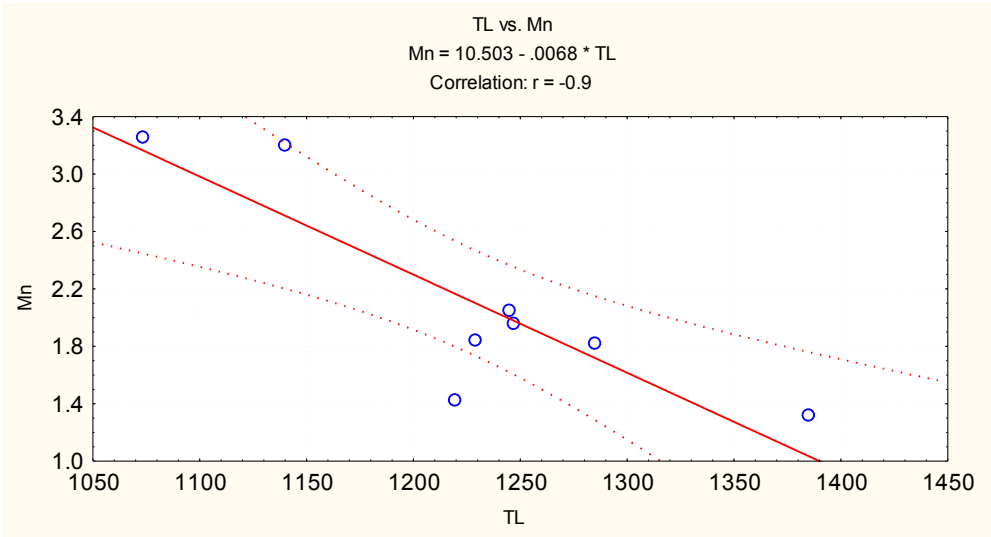


Figure 3.5. Metal burdens in the various organs of *A. marmorata*

Note: I: Intestine, St: Stomach, Sp: Spleen, M: Muscle, G: Gill, Go: Gonad, H: Heart, K: Kidney, L: Liver

a



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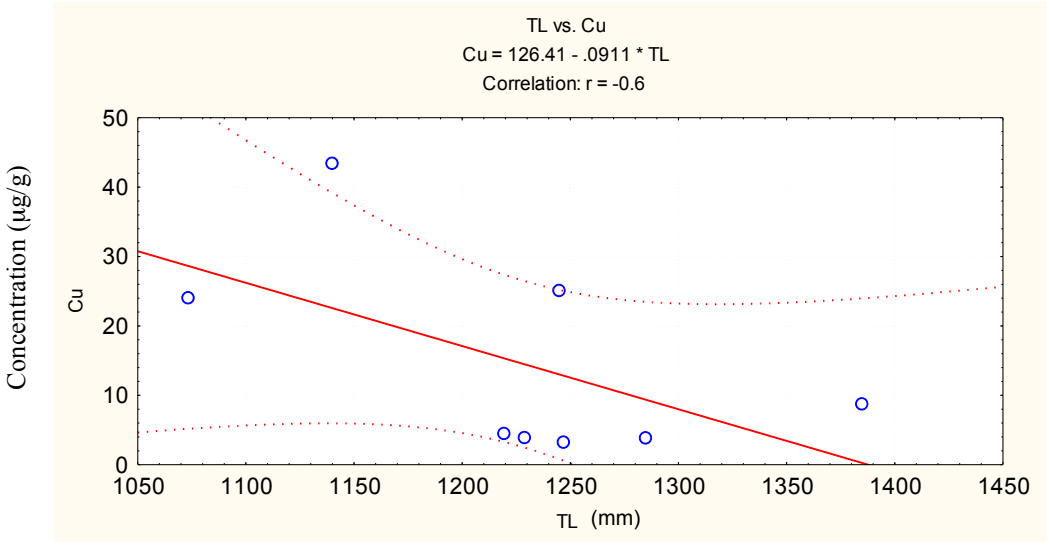

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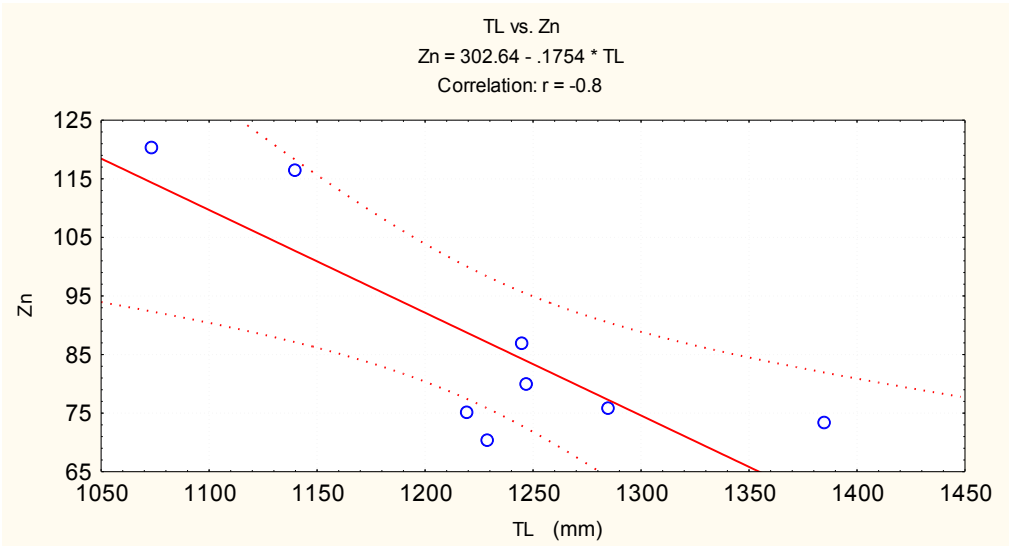


Figure 3.6. Relationship between metal accumulations in spleen and body length (TL - mm)

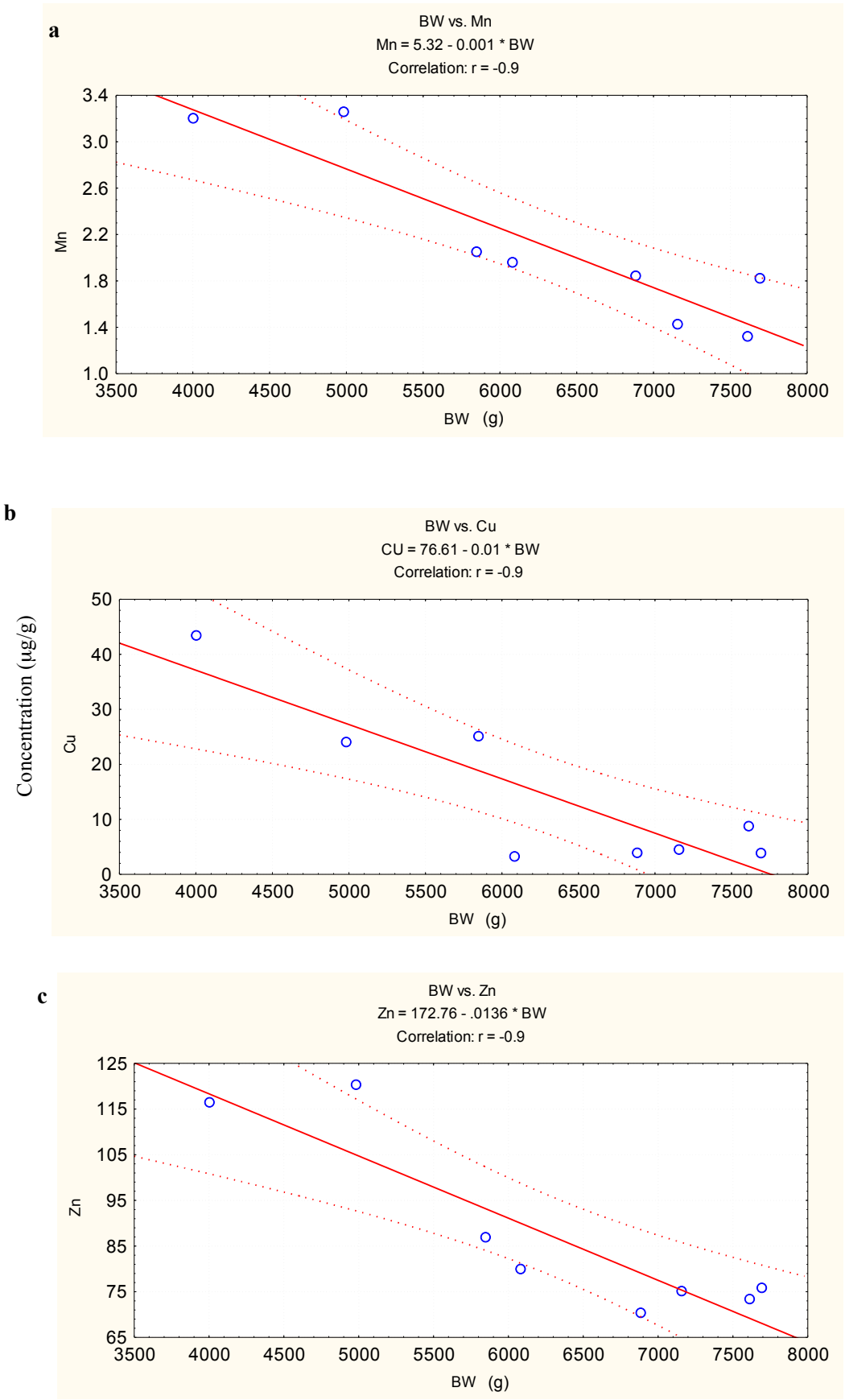


Figure 3.7. Relationship between metal accumulation in spleen and body weight (BW).

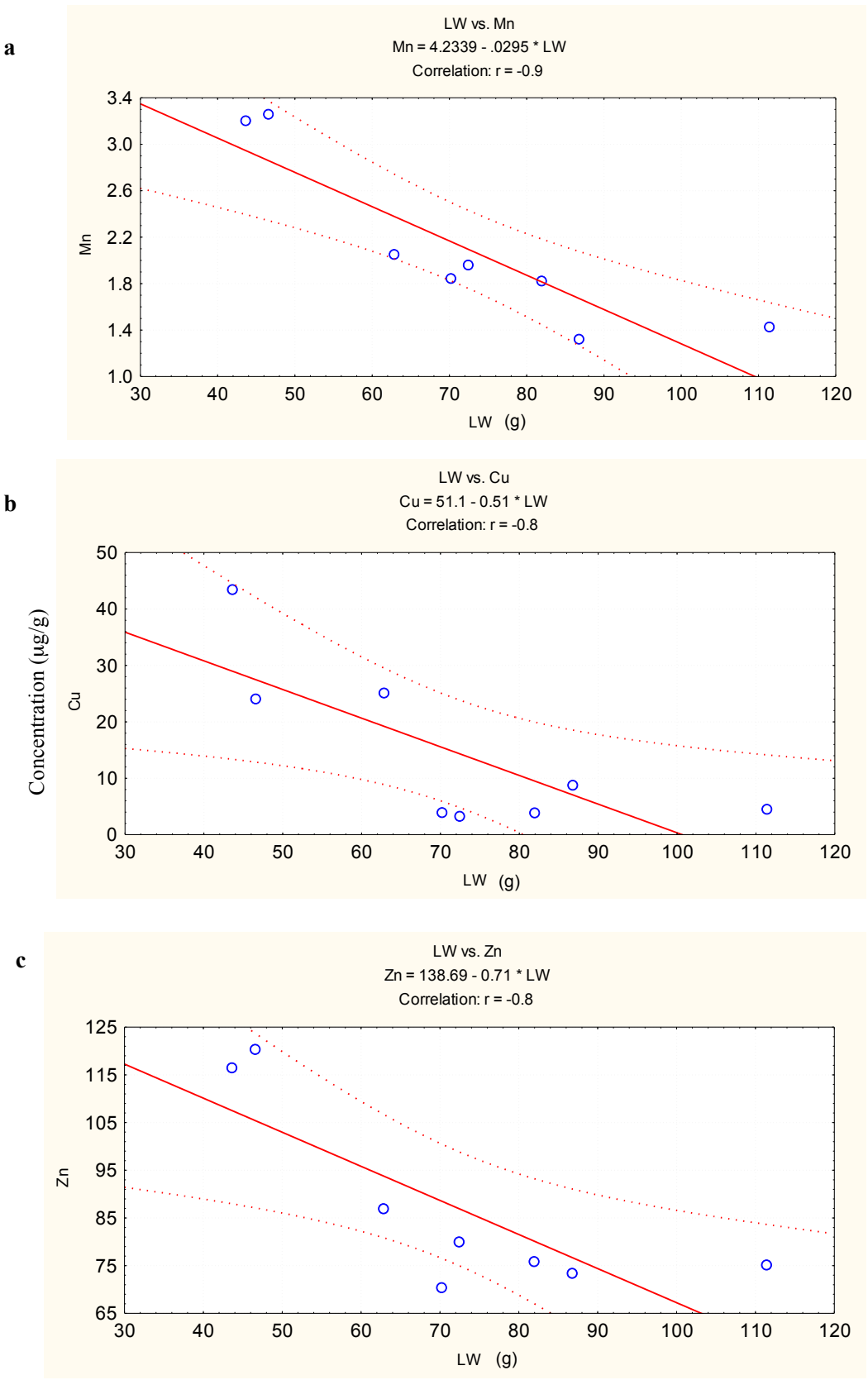


Figure 3.8. Relationship between metal accumulation in spleen and liver weight (LW).

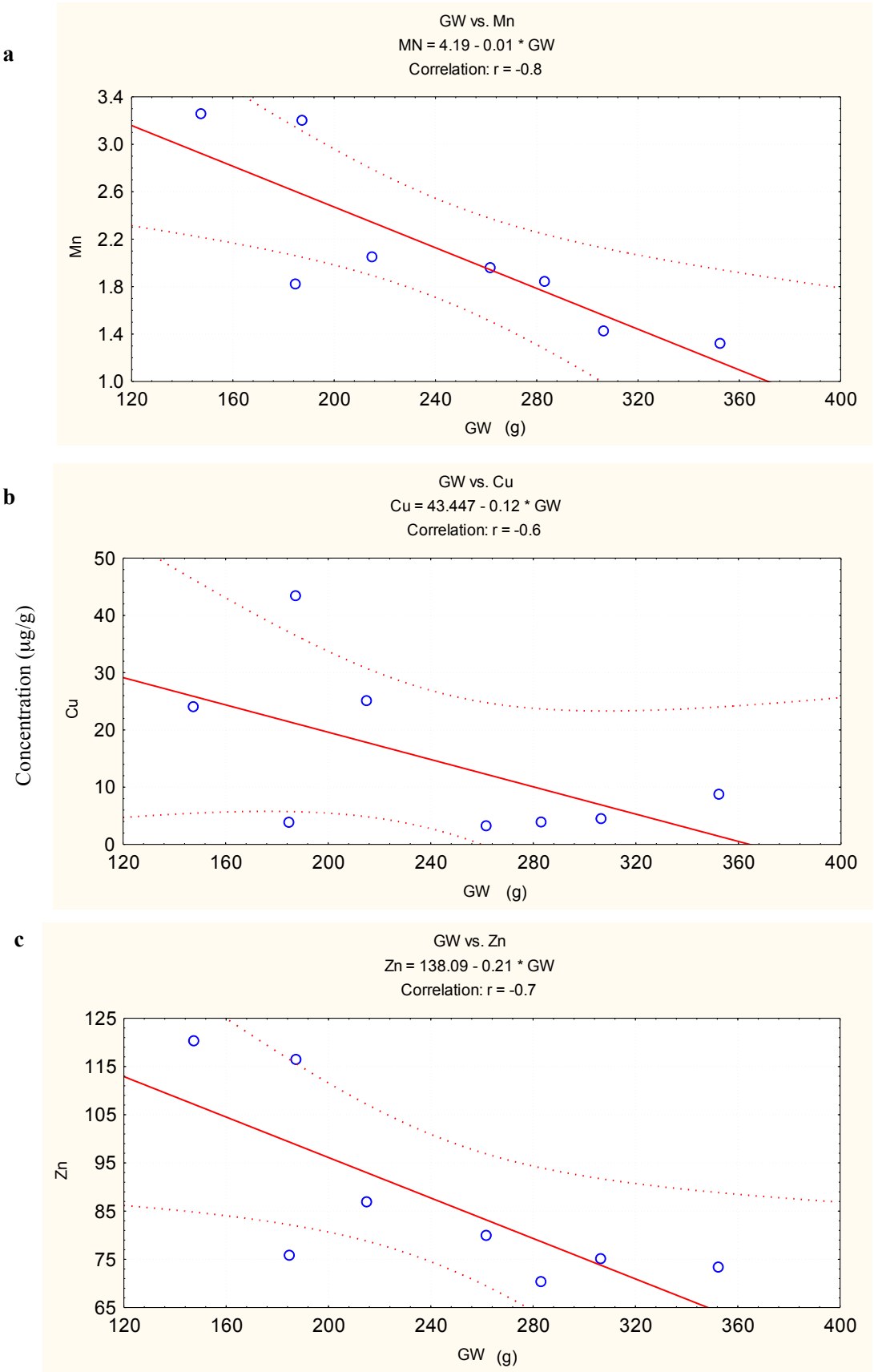


Figure 3.9. Relationship between metal accumulation in spleen and gonad weight (GW).

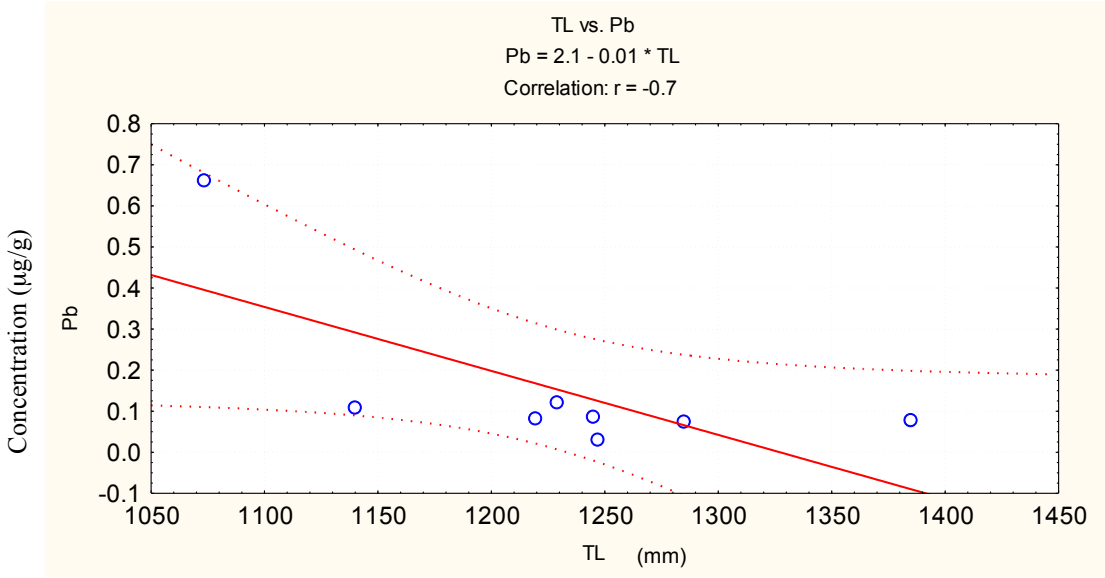


Figure 3.10. Relationship between Pb level in spleen and body length (TL).

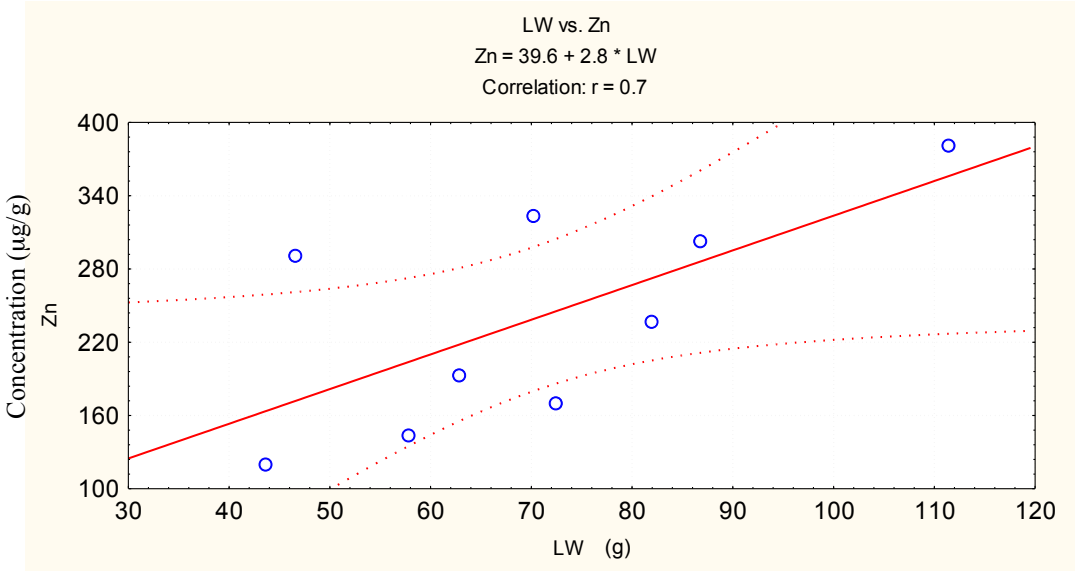


Figure 3.11. Relationship between Zn level in spleen and body length (TL).

3.4. Discussion

The investigation showed the target organs for the specific metal accumulations. Almost all metal highly accumulated in liver and kidney, inversely the elevated levels of Hg in muscle reflect the affinity of Hg accumulation. Since eels are long-lived and at the top of the freshwater food chain, they are susceptible to the biomagnification and bioaccumulation of Hg from where they forage and reside their entire lives (Edwards et al., 1999; Redmayne et al., 2000). When Hg was accumulated in the eel, it was excreted very slowly, with an excretion half-time ranging from 910 to 1030 days (Brusle, 1990). The elevated levels of Hg in eels generally related to the high accumulation rate of Hg from their prey and eels can accumulate high concentrations of Hg even in less polluted waters (Brusle, 1990; Redmayne et al., 2000). Therefore, positive correlation between the Hg levels and body size were often found in previous studies (Brusle, 1990; Szefer et al., 2003), however, there were weak correlations between Hg level and body size in this study, which was perhaps the fish collected in unpolluted area. The investigation of MeHg in liver and muscle was agreement with other studies indicated that the liver mainly accumulated inorganic Hg, which might be related to the detoxification and excretion procedure in the liver, while muscle had a good affinity for organic Hg (MeHg) (Brusle, 1990; Edward et al, 1999; Szefer et al, 2003). Moreover, the liver/muscle ratio of MeHg has been used to estimate Hg accumulation status for fish (Cizdziel et al. 2003), ratios of 1, or greater, indicate an increase in Hg uptake, while ratios below 0.5 indicate that Hg was being lost from fish. In this study, the ratios was around 0.3, indicate that Hg ceased uptake and start loosing in fish, this might relate to the fast of silver eel when they migrate to downstream area. Additionally, the starvation of the fishes may evenly result Hg distribution in inner organs such as liver, kidney, heart, spleen and intestine when blood vehicles for internal Hg distribution during the depletion of glycogen reserves (Cizdziel et al. 2003). Thus, the fasting migration may be a factor caused increasing Hg level in organ tissues, possibly including the brain, the ultimate site for toxic effects.

Alternatively, many studies have shown numerous adverse effects of Hg exposure on fish, including on growth, development, hematology, appetite, and behaviors (Verma and Tonk, 1982; Kirubagaran and Joy, 1995; Fiedmann et al, 2003; Raldua et al., 2007). According to Verma and Tonk (1982), Hg exposure decreased phospholipid content in the ovarian tissue of the fish *Notopterus notopterus*, because of the inhibition of vitellogenin synthesis in the liver; this may potentially affect the egg production (Kirubagaran and Joy, 1995). Vitellogenin is a female-specific glycolipoprotein yolk precursor produced by all oviparous animals, and is expressed specifically in the liver and transported to the gonads in females during egg development (Bun and Idler, 1993). However, the levels of Hg in the eel of this study were comparable with the low and moderate levels reported by Friedmann et al. (2002); these levels of Hg in fish potentially altered androgen profiles, but did not substantially decrease other indicators of general and reproductive health. Hence, the normal physiology of silver maturing eels in the Ba River might not be seriously affected by the Hg levels.

With respect to other trace metals, the liver generally had the highest essential metal concentrations (Mn, Co, Cu, and Zn); this is due to the major roles in metabolism and numerous other functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production and detoxification (Health, 1995; Hylland et al, 2003). The liver of maturing fishes even induces the synthesis of egg yolk phosphoproteins, and this mechanism stimulates the accumulation of essential metals such as Mn, Cu and especially Zn in the liver at the onset of sexual maturation (Thompson et al., 2003). Interestingly, a positive correlation between Zn concentrations and liver weight was observed. Therefore, a large amount of essential metals stored in the liver might relate to the growth stage of fish, especially maturing fish (Miramand et al., 1991). This indicates Zn might play some key roles in biological mechanisms in organisms compared to other trace metals, because over 300 proteins have been identified that need Zn for their functions and the number is increasing to date (Hogstrand and Wood, 1996). The elevated Zn concentrations in the gonad might relate to hepatic Zn, because the gonad of maturing

fish received Zn from the liver by vitellogenin protein during gonadal maturation (Hogstrand and Wood, 1996). Besides storing essential metals, the fish liver highly accumulated nonessential metals such as Cd, Pb and Hg in order to perform its detoxification mechanisms by the induction of antioxidant enzymes such as Cu,Zn-superoxide dismutase (Cajaraville et al., 2003); or the induction of metal-binding protein (metallothionein) synthesis (Pelgrom et al., 1995; Langston et al., 2002). In contrast, muscle showed less accumulated metals compared to other organs (except for Hg), because the fish continuously grew and increased in size during their entire lives, which might alleviate the burdens by the metal on the body by a growth dilution effect.

The other fish organs, the kidney, gill, intestine, and spleen, are the predominant barriers controlling the mechanism of absorption of bioavailable metals from external mediums (water and sediment) and from contaminated prey (Dallinger et al., 1987; Health, 1995; Andres et al, 2000; Hylland et al, 2003). The gills mostly uptake trace metals from water, and also play a significant role in metal storage and transfer to the internal compartments via the blood stream (Dallinger et al., 1987; Hylland et al, 2003). The kidney and intestine mainly uptake metals from food, sediment, and water (Dallinger et al., 1987; Brusle, 1990; Andres et al, 2000).

The kidney, however, is also a target organ of accumulation for many metals, due to its strong irrigation and excretion function, especially of toxic metals like Cd, Pb, and Hg (Dallinger et al., 1997; Andres et al., 2000). Therefore, these metals, particularly Cd, were accumulated in large concentrations in the kidney rather than in the other organs. The concentration of Cd in the kidney was very high, about 800 times higher than in muscle. The elevated Cd in the kidney can lead to an increase of Cu and Zn in the kidney because these metals compete the metal binding sites in metallothionein during the detoxifying mechanism (Pelgrom et al., 1995; Langston et al., 2002). Additionally, the trophic route seemed to play an important role in Cd contamination of wild eels (Pierron et al. 2007). The dietary Cd absorption was characterized by a high level of cadmium in the intestinal mucosa and its slow transfer to other internal organs (Baldisserotto et al., 2005;

Roesijadi et al., 2009). Like Cd, Pb that is a toxic metal to living organisms mainly distributes in the liver, kidney and spleen in this study. The high levels of Pb in the liver, kidney, and spleen can be justified by the fact that the liver and kidney are the main metallothionein-synthesizing organs in eels (Smet et al., 2001). The spleen plays important roles in regard to red blood cells and the immune system, and the blood is the best indicator of Pb exposure and accumulation (Merian, 1991).

Unlike Cd and Pb, Co is an essential metal as a component of vitamin B12 and plays a part in the physiological mechanisms in the body. Co aids in maintaining healthy nerve cells and red blood cells, and binds to iron transport proteins more strongly than iron itself (Merian, 1991). Additionally, Co also aids in binding of protein in food. During the digestion process, hydrochloric acid in the stomach discharges vitamin B12 from the protein contents found in food (Merian, 1991). Therefore, Co could evenly distribute in internal organs such as the stomach and intestine in this study. Although vitamin B12 is mainly stored in the liver and is excreted primarily through the bile (Merian, 1991), the highest accumulated level of total Co was in the kidney in this study. This might relate to the slow renal excretion of Co as inorganic compounds, because Co was slowly excreted with a half-time of 10 days (Merian, 1991).

Cr was considered to be essential to a part of the living organisms, and has been found primarily in two forms: trivalent Cr (III), which is biologically active and found in food, and hexavalent Cr (VI), a toxic form resulting from industrial pollution (Merian, 1991). Chromium transported by blood is distributed to tissues and organs that have a different retention capacity (Guthrie, 1982; Weber, 1983; Merian, 1991). The liver, kidney and spleen are the organs generally showing the highest and most stable content of Cr after exposure (Weber, 1983). In the liver, Cr is linked to proteins and smaller peptides, while in the spleen it accumulates debris of red blood cells (Weber, 1983; Merian, 1991). In this study, the accumulation of Cr was, however, the high level in the kidney and muscle and it evenly distributed to other organs such as the liver, spleen, heart, intestine,

stomach and gonad. In addition, the Cr levels in the environment were comparable to the concentration in the mean crust. Therefore, the concentrations of Cr in various organs of the eel were assumed to be within the normal physiological range.

Mn functions as a cofactor for some kinds of enzymes in living beings, including arginase, oxidoreductases, transferases, and hydrolases (Merian, 1991). It is especially stored in organs, which are rich in mitochondria, such as the liver and kidney (Merian, 1991). However, in this study, the high accumulation of Mn in the kidney and liver was accompanied by high concentrations of Mn in the stomach. While silver maturing eels were thought that they do not eat after the beginning of downstream migration (Tesch, 2003). Therefore, the high Mn retention in the stomachs of the eels seemed to be influenced from food content residues in this study. Additionally, the Mn concentrations in sediment in the river were extremely high. This might also be one reason why Mn was high in the stomach, because eel indirectly or directly ingest Mn from sediment when they feed on benthic invertebrates.

There is little information available on the distribution of V in wild fish organs, as almost all V retention in tissue organs results from exposure of fish to V in water or food (Bell et al., 1980; 1981; Ray et al. 1990). Nevertheless, the present study showed the same accumulation trend of V in fish organs. Ray et al. (1990) reported the V retention was found to be the highest in the kidney, followed by the liver, gill and intestine of catfish *Clarias batrachus* after exposure to vanadium. They indicated that the uptake of V from water by the fish was affected by both duration of exposure and the concentration of the metal. Additionally, Bell et al. (1980, 1981) also reported that the kidney, liver and bone of the European eel *Anguilla anguilla* were target organs for V retention, and the rate of entry of vanadate into eels was directly proportional to its environmental concentration.

Unlike other metals, Sr did not highly accumulate in the liver; the concentrations of Sr insignificantly varied in the organs. This is because Sr is known to accumulate in hard tissues like

the bones, fins, head, scales, or even the otolith instead of being retained in soft tissue organs (Outola et al. 2009).

While metal concentrations were measured to determine the target fish organ for specific metal accumulation and distribution, the metal body burden was important for evaluation of exposure and risk. The examination of body burden of metals revealed that the highest metal amounts in muscle, which comprised on average 60-65% of body weight, followed by liver because of physiological function of this organ. However, surprisingly the highest burden of Cd was in gonad, but not in kidney or liver that were the target organs for Cd accumulation. The elevated Cd burden in gonad might relate to interference of Ca uptake to gonad from previous stored organs, especially liver. Because vitellogenin is, a phosphoglycolipid - protein enriched Ca, synthesized in liver, Ca plays important roles during gonad maturation. Ca and Cd are chemically very similar elements as they present similar load and ionic radius (Simkiss and Taylor, 1989). Alternatively, Cd was known as the toxic metal caused potential impact on the reproductive capacity of European eels (Pierron et al., 2008). Pierron et al. (2008) found that Cd pre-exposure strongly stimulate the pituitary-gonad-liver axis of maturing female silver eels leading to early and enhanced vitellogenesis. This was followed by a strong phenomenon of oocyte atresia and eel mortality. Thus, liver and gonad have the correlations (Figure 3.5) in transferring not only essential metals but also nonessential metals. When compared to the present study, almost Cd levels of liver and kidney in the silvering eel specimens was lower than in the study of Pierron et al. (2008). This suggests that Cd has no adverse effects on reproductive capacity of *A. marmortata*. However, one specimen showed the Cd hepatic (1.46 μg/g) and rental (21.3 μg/g) levels were the similar or higher affected levels in the study of Pierron et al. (2008). This might present a risk for the eel when it migrates to the spawning area.

Finally, the study determined the trace metal distribution in various organs and the correlation of metal accumulation among organs. There was a strict link of metal accumulations between liver

and gonad in view of cluster analysis. The study suggests that hepatic trace metals, both essential and nonessential, may have a significant contribution to the fish biological mechanisms during gonadal maturation. Eels are vulnerable to pollution, they accumulate large quantity of persistent contaminants such as Hg and Cd, even in less polluted areas. Although toxic metals in the study site might not affect most maturing silver eels, the high levels of Hg in muscle of eels might pose a risk for human consumption.

3.5. Estimation of potential health risk

The daily intake doses of all trace metals were estimated and compared with reference doses (RfDo) of the risk-based concentration table for chemical contamination (U.S. EPA 2008), except for Pb (Table 3.3). Because Pb was not on the RfDo list, the studies of Agusa (2007) were referred to for comparison. The daily ingestion of fish in Vietnam was supposed to be 150 g/day wet wt. and the average body weight of the Vietnamese to be 50 kg (FAO 2005). The ratio of dry weight/wet weight in eel muscle was estimated to be approximately 27.8% in this study. The intake doses of trace metals for fish consumption have been estimated and presented in Table 3.3. Hg and MeHg were compared with the recommended values (0.3 μg/g wet wt.) for safe human consumption (U.S.EPA, 2008). The maximum metal levels (except for Hg) in the muscle of the tropical eel *Anguilla marmorata* were found to be below the RfDo guideline for fish consumption. However, approximately 80% of silver maturing eels from the Ba River contained Hg exceeding the levels recommended (0.300 μg/g) by the U.S.EPA (2008), and thus might present a risk for human consumption.

Table 3.3. Daily intake (μg/day) of trace metals for person (50kg) consumed freshwater eel

Intake	V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg
Mean	0.02	0.49	0.57	0.14	1.10	64.05	0.35	0.01	0.02	0.48
Min	0.01	0.21	0.30	0.08	0.42	39.31	0.12	0.00	0.00	0.21
Max	0.04	0.87	0.82	0.24	2.30	103.03	0.88	0.01	0.03	1.12
RfDo value μg/kg day	**5**	**1.5**	**140**	**20**	**40**	**300**	**600**	**1**	**3.57***	**0.3****

* Reference dose for Pb were referred from Agusa et al. (2007).

** The recommend values of USEPA (2009).

Chapter 4

Trace metals in a tropical eel *Anguilla marmorata* from the central part of Vietnam

4.1. Introduction

Anguillid eels often live in freshwater until maturation, migrate offshore in the sea for spawning and then die. The yellow eels (immature eels) are territorial and live in rivers and estuaries for several years to a few decades. Ultimately, they undergo metamorphoses into silver eels and at that time they are ready to go downstream to the spawning areas in the ocean (Tesch 2003). Among anguillid eel species, the tropical eel *Anguilla marmorata* is known to have the widest distribution (Tesch 2003; Minegishi et al., 2008). It distributes in the tropical Indo-West Pacific, from South Africa to the Society Islands (French Polynesia) north to southern Japan and in Africa: inland Mozambique and lower Zambezi River. It is also abundant in the central part of Vietnam, where it has been exploited intensively throughout its life cycle for multiple purposes, including aquaculture and human consumption. Like other species belonging to anguillid eel, *A. marmorata* is a long-lived fish. Their migration is mysterious since they move from the marine environment to brackish waters to freshwater within their lives; unfortunately, however, their wide range means that the eels can easily be exposed to various pollutants throughout its migration route. Among the various pollutants, trace metals, in particular, are widespread contaminants released into aquatic systems from numerous anthropogenic sources. Some metals are known to be toxic even at low concentrations, including As, Cd, Hg, Pb etc. Others, such as Cu, Zn and Co, are known to be essential elements and play important roles in biological metabolism at very low concentrations. These metals can enter the eel, which is a top predator, through the food web and become poisonous when their concentrations exceed the metabolic requirements of the eel. This exposure to metals can lead to mortality, as has occurred in the Rhine River (Anonymous 1987), but it can also adversely affect animals (Batty and Deporah 1996) and even humans (Tsuchiya et al. 1969) that

consume them. In previous studies, eels have been used as bioindicators of metal pollution since their tissue concentration reflects the increased concentrations of metals and metalloids in the aquatic environment (Edwards et al. 1999; Maes et al. 2008). However, there is little information available regarding the status of metal pollution in eels in Vietnam.

Therefore, the aim of this study was to determine whether the residues of trace metals in edible muscle tissue of eels is safe for human consumption, and to explore the relationship between metal accumulation and body size of the tropical eel *A. marmorata*, in order to evaluate the potential risk of metal pollution in eel populations in the central part of Vietnam.

4.2. Materials and methods

4.2.1. Sampling

The wild eel samples were collected by angling and electrical shocks from the rivers in four provinces in the central part of Vietnam, Quang Tri (QT), Quang Ngai (QN), Binh Dinh (BD), and Phu Yen (PY) (Figure 4.1). These locations are chosen because tropical eels are abundant and they also have been potentially affected by human activities such as marine ports, industrial zones, and agriculture. Eels were collected from river in QT (n=10) in September 2007, and from rivers in QN (n=10), BD (n=30), and PY (n=10) during February and March 2008 (Table 4.1). Among the 30 specimens in BD, 10 specimens (537.6 ± 31.5mm body length; mean±SD) were selected for comparing metal concentrations in the muscle of eels with the other provinces (Table 4.1) and all specimens (n=30) were grouped into body size classes to determine the relationship between metal concentrations in muscle and the body size of eels as described in sub-section 4.2.3. All eels collected were in the yellow stage of development, and no silver eels were observed. The total length (mm) and body weight (g) were measured for each fish. Muscle and liver tissues were dissected out, weighed, put in clean polyethylene bags, and stored at -20^{0}C in Vietnam. All samples were then transported to Japan in cool boxes with dry ice for further chemical analyses.

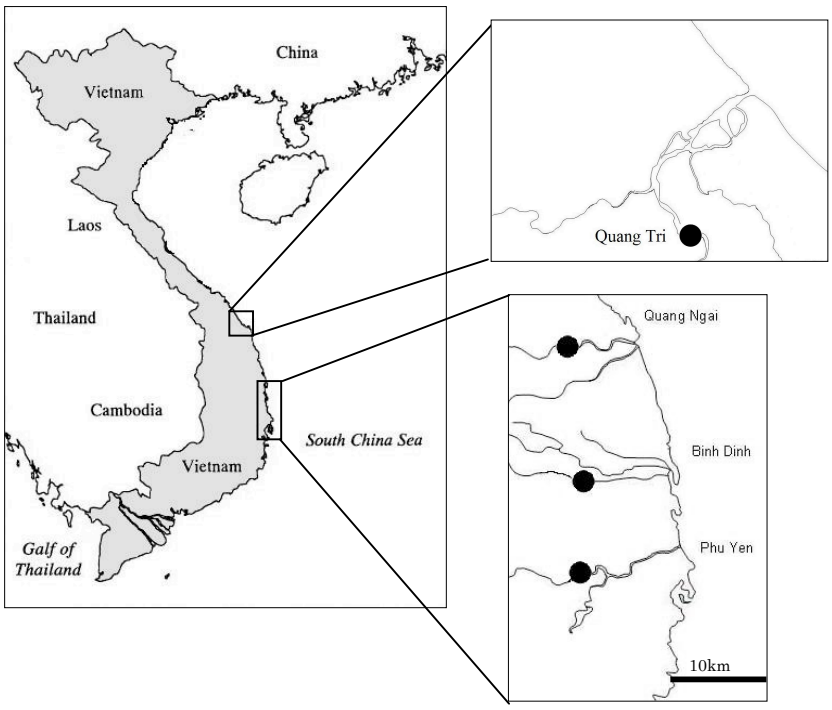


Figure 4.1 Sampling locations of *Anguilla marmorata* in the central part of Vietnam

Table 4.1. Total length and body weight of *Anguilla marmorata* collected by sampling locations

Location	n	Length (mm)	Weight (g)
Quang Tri (QT)	10	503.0 ±110.4	315.4 ± 186.7
Quang Ngai (QN)	10	399.2 ± 40.6	144 ± 50.0
Binh Dinh (BD)	10	537.6 ± 31.5	329.92 ± 84.6
Phu Yen (PY)	10	827.1 ± 95.0	1623.6 ± 626.8
Relationship analysis of samples from BD	30	620.6±214.8	803.4±989.2

4.2.2. Chemical analyses

The chemical analyses were conducted as the methods in Chapter 3.

4.2.3. Relationship analyses

A larger number of eel samples collected from BD (n=30) was used to explore the relationship between metal residue in the muscle of eels and body size. Since eel growth was quite uninterrupted in tropical areas, the mean growth rate for yellow eel is approximately 100 mm/year (Aoyama, 2009), and the age of tropical anguillid eels was difficult to accurately determine because there are no clear annual growth zones in otolith (Jellyman 1991; Chino and Arai 2010). Otherwise, there was a relationship between body length and age (Moriarty 2003), which suggests that eels in similar size classes are of similar age (Mallawa et al. 1992). Therefore the eels with similar size were grouped and the various ranges of total length for *A. marmorata* were classified into 5 body size classes: (mm) 400-460 (S1); 475-535 (S2); 560-620 (S3); 760-820 (S4); >1000 (S5). A logarithmic transformation of the data was applied, and the data were plotted on the linear scale and lines drawn following the equation below:

$$\log_{10} C = \log_{10} a + b \times \log_{10} L \quad (1)$$

Where: C: the dry weight metal concentration (μg/g)

L: the total length (mm) of the fish.

4.2.4. Estimation of chemical doses

Estimation of chemical doses was conducted as the method in Chapter 3.

4.2.5. Statistical analyses

All values are presented as mean ± SD. One-way ANOVA was used to compare eel samples between the different locations. Where ANOVA showed differences, Tukey HSD was used as a post hoc test for comparison among groups. Linear correlation analysis was conducted between the metal concentrations in muscle and the body size of the eels. The analyses were performed using

STATISTICA for Windows, Release 5.5.

4.3. Results

4.3.1. Biological information

The size and weight (mean±SD) of the tropical eel *Anguilla marmorata* from QT were 503.0 ±110.4 mm and 315.4 ± 186.7, respectively; samples from QN, BD and PY were 399.2 ± 40.6 mm and 144 ± 50.0 g, 537.6 ± 31.5 mm and 329.92 ± 84.6 g, and 827.1 ± 95.0 mm and 1623.6 ± 626.8 g, respectively (Table 4.1). The eel samples collected from the river in Phu Yen (PY) were significantly bigger (Turkey HSD test, $p<0.01$) than those from the rivers in Binh Dinh (BD), Quang Ngai (QN), and Quang Tri (QT), but no significant differences in body size were found between the last three locations.

4.3.2. Differences in metal concentrations in eel muscle between locations

Trace metal levels in eel muscle were shown in Table 4.2 and Figure 4.2. The Zn concentrations were highest in QT (Tukey HSD test, $p<0.05$) compared to all other locations, but there were no significant differences in Zn levels among other locations (QN, BD, and PY). Cu and Sr concentrations in eels in QT were also significantly higher than those in BD and PY (Tukey HSD test, $p<0.05$) and slightly higher than those in QN, but not significantly. The concentrations of Cr in fish were lowest in QN, but similar levels were found in the other locations. The V level in eel muscle was found to be highest in QT and lowest in QN, however no significant differences were found between QT and PY as well as QN and BD (Tukey HSD test, $p<0.05$). Unlike Zn and Cu, Co concentrations in eels were found to be the lowest in QT (Tukey HSD test, $p<0.05$) among all locations, but there was no significant difference between QN and BD. Cd concentrations in eel's muscle from QT and QN were higher than those in BD and PY (Tukey HSD test, $p<0.05$). The amounts of Mn, Pb and Hg did not appear to differ significantly between locations.

Table 4.2. Mean concentrations (µg/g dry wt.) of trace metal in muscle tissue of *Anguilla marmorata* in locations

Elements	Metal concentration (µg/g dry weight)			
	Quang Tri (n=10)	Quang Ngai (n=10)	Binh Dinh (n=10)	Phu Yen (n=10)
V	0.09±0.05	0.02±0.02	0.05±0.02	0.06±0.01
Cr	0.56±0.38	0.28±0.10	0.62±0.31	0.42±0.11
Mn	1.67±1.41	1.31±0.64	0.85±0.38	0.98±0.69
Co	0.11±0.07	0.18±0.09	0.18±0.16	0.22±0.09
Cu	2.18±1.19	1.91±0.99	1.23±0.28	1.21±0.42
Zn	82.08±18.26	49.44±10.31	45.62±18.27	42.28±12.82
Sr	4.06±1.94	2.52±1.75	1.79±2.06	1.34±0.83
Cd	0.13±0.10	0.09±0.07	0.05±0.04	0.02±0.03
Pb	0.13±0.05	0.11±0.07	0.18±0.074	0.14±0.09
Hg	0.49±0.13	0.50±0.13	0.56±0.14	0.51±0.09

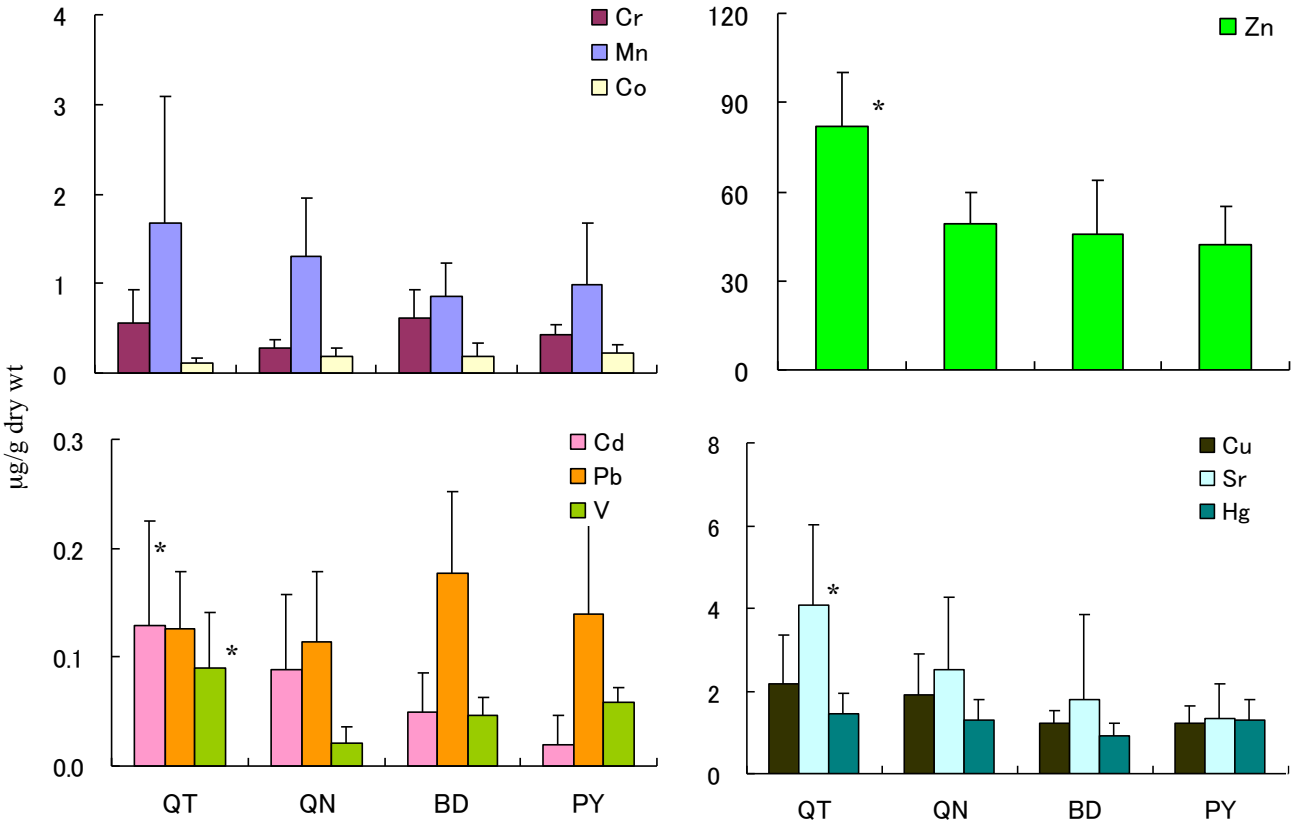


Figure 4.2. Mean concentrations (µg/g dry wt.) of trace metal in muscle tissue of *Anguilla marmorata* in locations

4.3.3. Differences in metal concentrations in eel liver among locations

Trace metal levels in eel liver were shown in Table 4.3 and Figure 4.3. All trace metal levels in liver, except for Cr, was higher than those in muscles. Like muscle, metal levels of Cr, Mn, Cu, Zn, Sr, Cd and Hg in QT were higher than those in other location, however significant differences of specific metal levels were found between the certain locations. For instance, the Zn and Cd levels in QT were significant higher than those in PY while Mn level in QT was higher than that in BD, however there was no difference between other locations (Tukey HSD test, $p<0.05$). The Cr level in both BD and QT were significantly higher than that in PY and QN, however there was no difference between BD and QT as well as PY and QN (Tukey HSD test, $p<0.05$). Although Cu, Sr, and Hg were found to be the highest levels in QT, the significant differences were found between QT and QN (Tukey HSD test, $p<0.05$). Unlike other metals, Co level in PY was the significant higher than that in QT (Tukey HSD test, $p<0.05$), but there was no significant difference between QN and BD. Meanwhile, V level in QT and Pb level in BD was slight higher than other sites, however no significant difference were found among sites.

4.3.4. Relationships between trace metal concentrations and body size

The mean ± SD of total length and body weight of eels in BD (n=30) were 620.6±214.8 mm and 803.4±989.2 g, respectively (Table 4.1). A significant positive correlation between total length and body weight was found ($r^2= 0.98$, $p<0.0001$). Thus, the relationship was just made between metal concentration in muscle tissue and the total length of the eel (Fig. 4.4).

Based on the body size classes classified in the method section, concentration of the ten metals in *A. marmorata* of 5 size classes is presented in Table 4.4. Only a positive correlation between Hg levels and body size was found (Figure 4.5), in contrast negative correlations between concentration and sizes were noted between Mn, Zn, Sr, and Pb, in which the highest coefficient values for Sr and Mn were -0.9 and -0.9, respectively (Figure 4.6 a, b). Pb and Zn levels showed the weaker correlations with body sizes (Figure 4.6 c, d). Moreover, no significant correlations

between V, Co, Cd, Cr, and Cu with body size were found, however the trend of metal levels such as Co, Cd was decreased with increasing of TL (Figure 4.7 a, b).

Table 4.3. Mean concentrations (µg/g dry wt.) of trace metal in liver tissue of *Anguilla marmorata* in locations

Elements	Metal concentration (µg/g dry weight)			
	Quang Tri (n=10)	Quang Ngai (n=10)	Binh Dinh (n=10)	Phu Yen (n=10)
V	1.15±0.44	0.71±0.34	0.80±0.54	0.93±0.69
Cr	0.58±0.28	0.30±0.07	0.45±0.15	0.31±0.06
Mn	8.15±1.50	6.71±1.46	6.02±0.95	7.59±2.19
Co	0.59±0.39	0.91±0.33	1.32±0.95	1.53±0.76
Cu	53.82±28.73	22.91±4.61	31.22±15.65	49.30±27.17
Zn	125.9±32.5	82.9±17.7	72.2±14.0	89.2±33.6
Sr	4.72±1.35	2.82±1.05	2.059±1.46	1.53±0.93
Cd	3.57±2.42	1.95±1.00	1.72±1.05	0.35±0.47
Pb	0.81±0.63	0.82±0.50	0.95±0.51	0.44±0.39
Hg	1.08±0.35	0.65±0.25	0.76±0.10	0.98±0.44

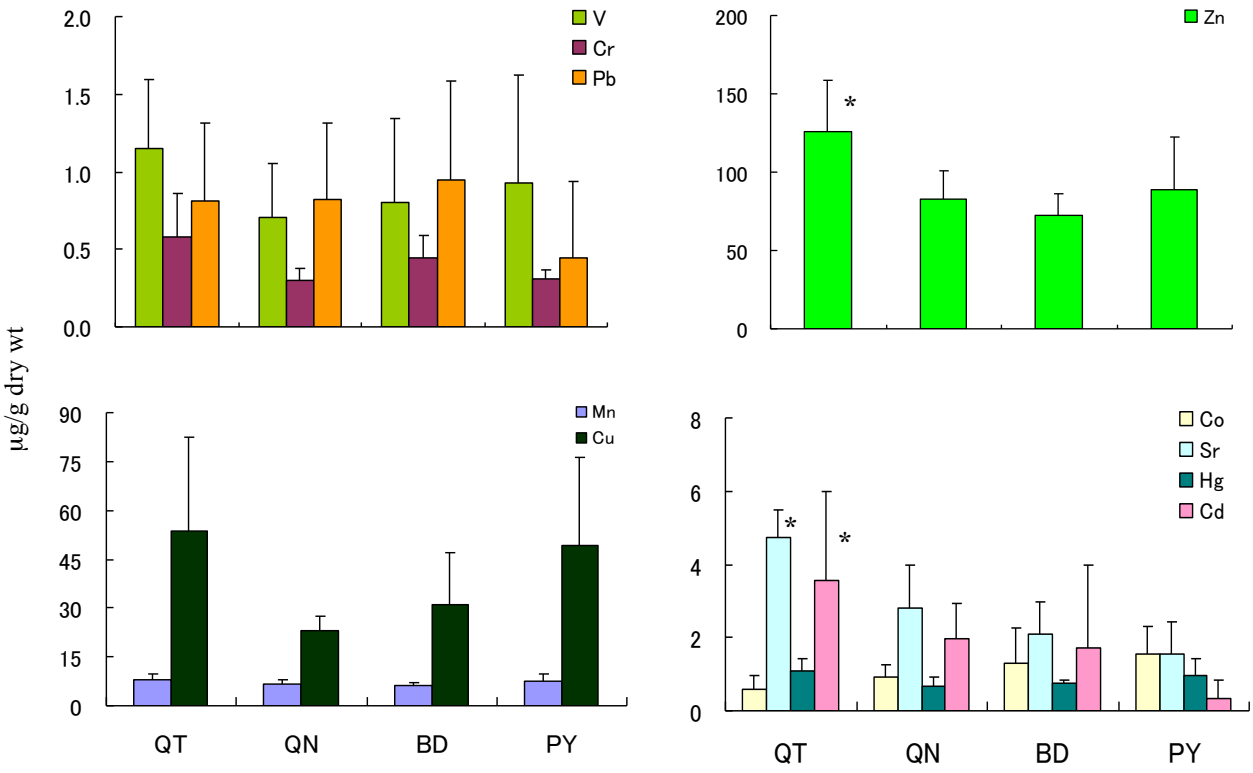


Figure 4.3. Mean concentrations (µg/g dry wt.) of trace metal in liver tissue of *Anguilla marmorata* in locations

Table 4.4. Concentrations of eight metals (µg/g dry wt.) in *A. marmorata* specimens from BD of 5 size classes from BD

Size class (mm)		V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg
S1	400-460 (n=8)	0.032	0.27	1.35	0.13	2.11	58.0	3.17	0.08	0.10	0.34
S2	475-535 (n=6)	0.042	0.59	0.77	0.25	1.62	53.1	1.70	0.04	0.15	0.34
S3	560-620 (n=7)	0.049	0.52	0.78	0.13	1.37	37.0	1.59	0.06	0.14	0.59
S4	760-820 (n=5)	0.050	0.48	0.47	0.20	1.37	44.9	1.21	0.07	0.06	0.58
S5	>1000 (n=4)	0.040	0.42	0.49	0.11	2.03	41.8	0.95	0.04	0.06	0.83

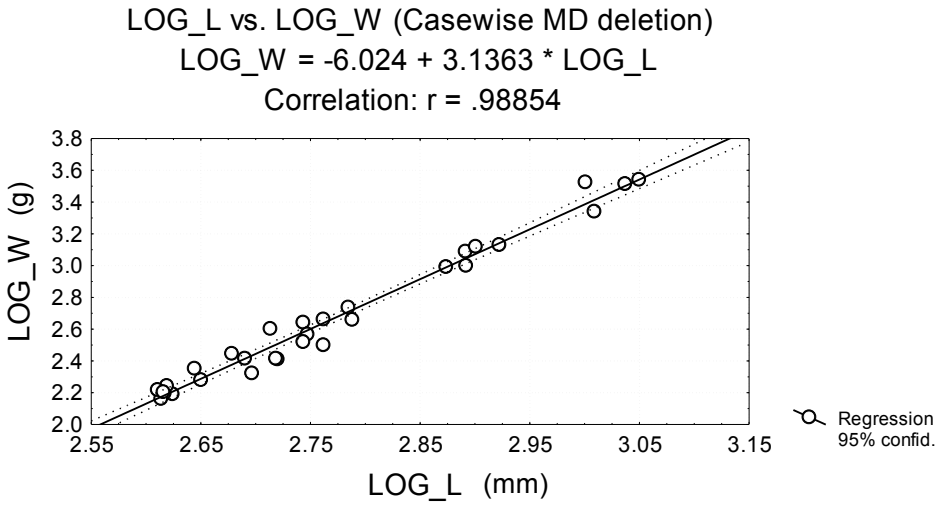


Figure 4.4 Correlation between total length and body weight of *Anguilla marmorata*

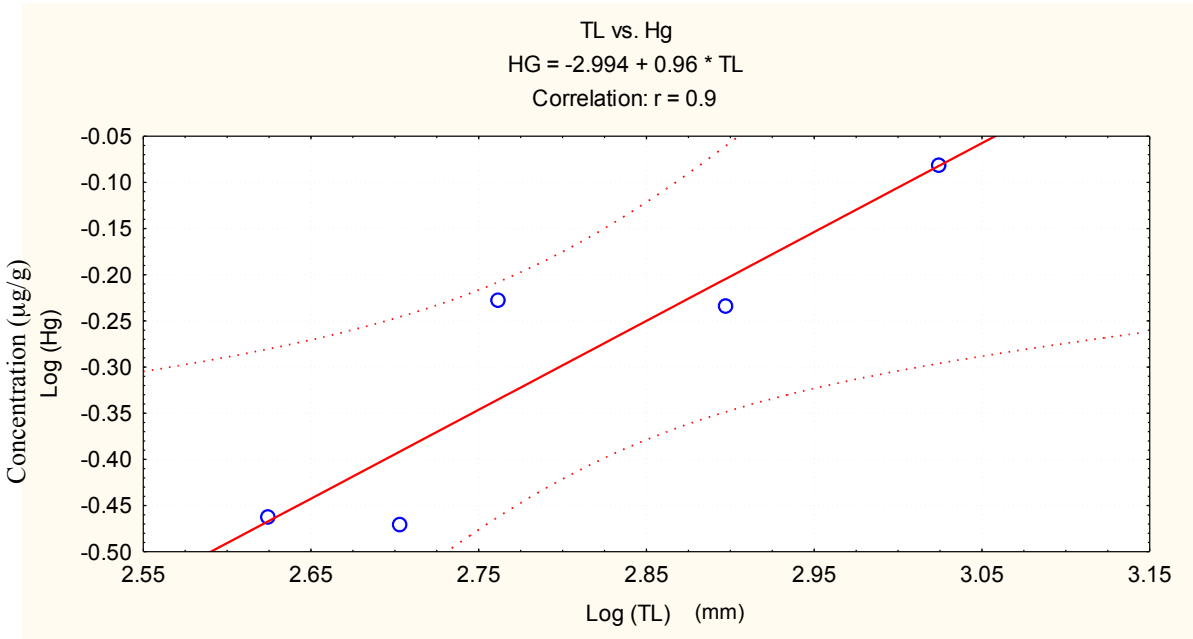


Figure 4.5 Correlation between Hg level in muscle and total length of *Anguilla marmorata*

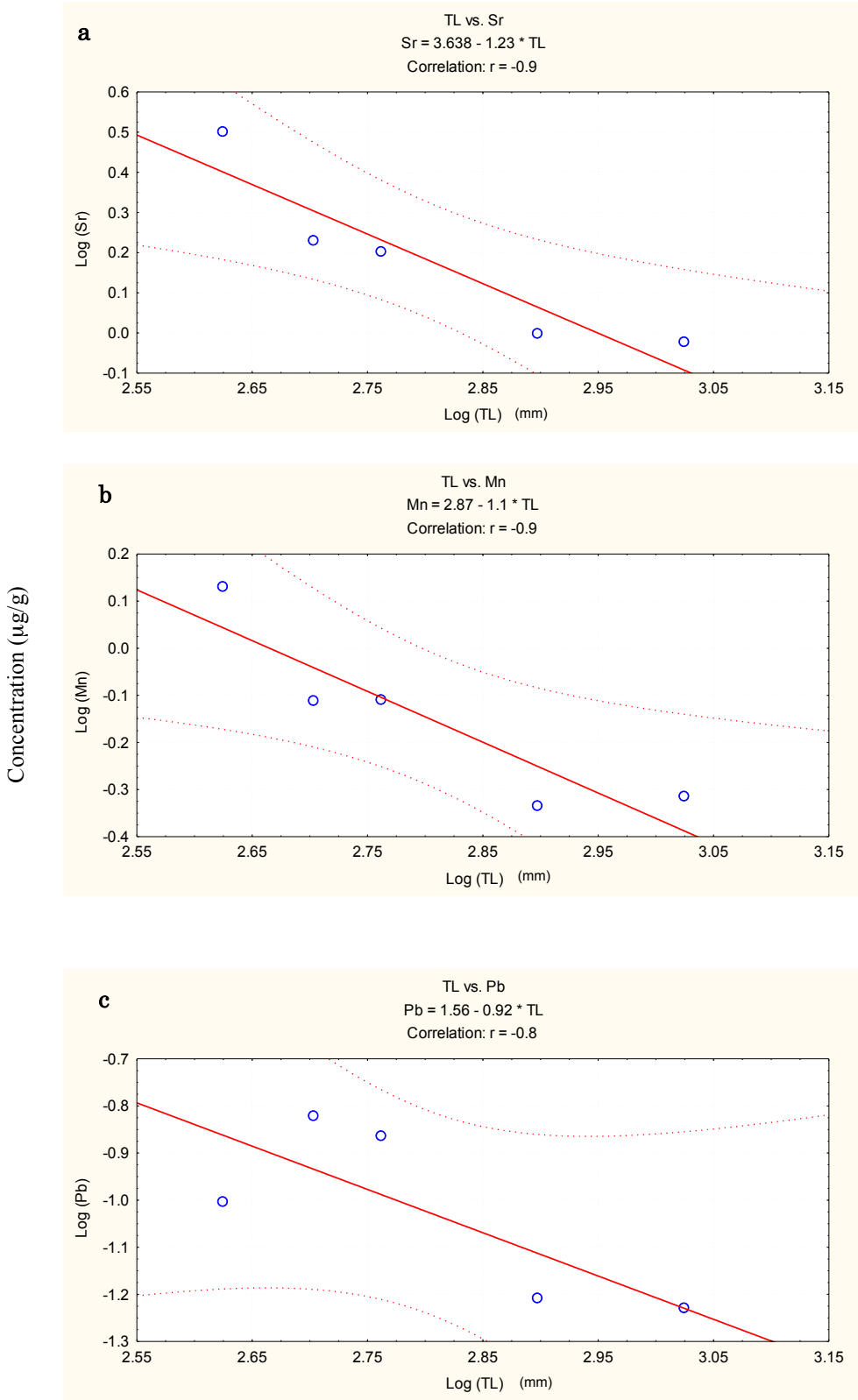


Figure 4.6. Negative relationships between metal levels in muscle and total length of *Anguilla marmorata*

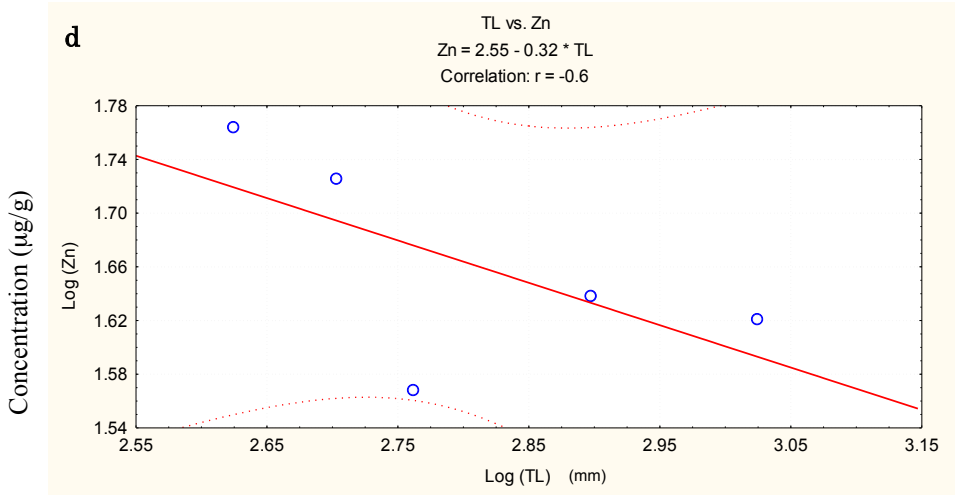
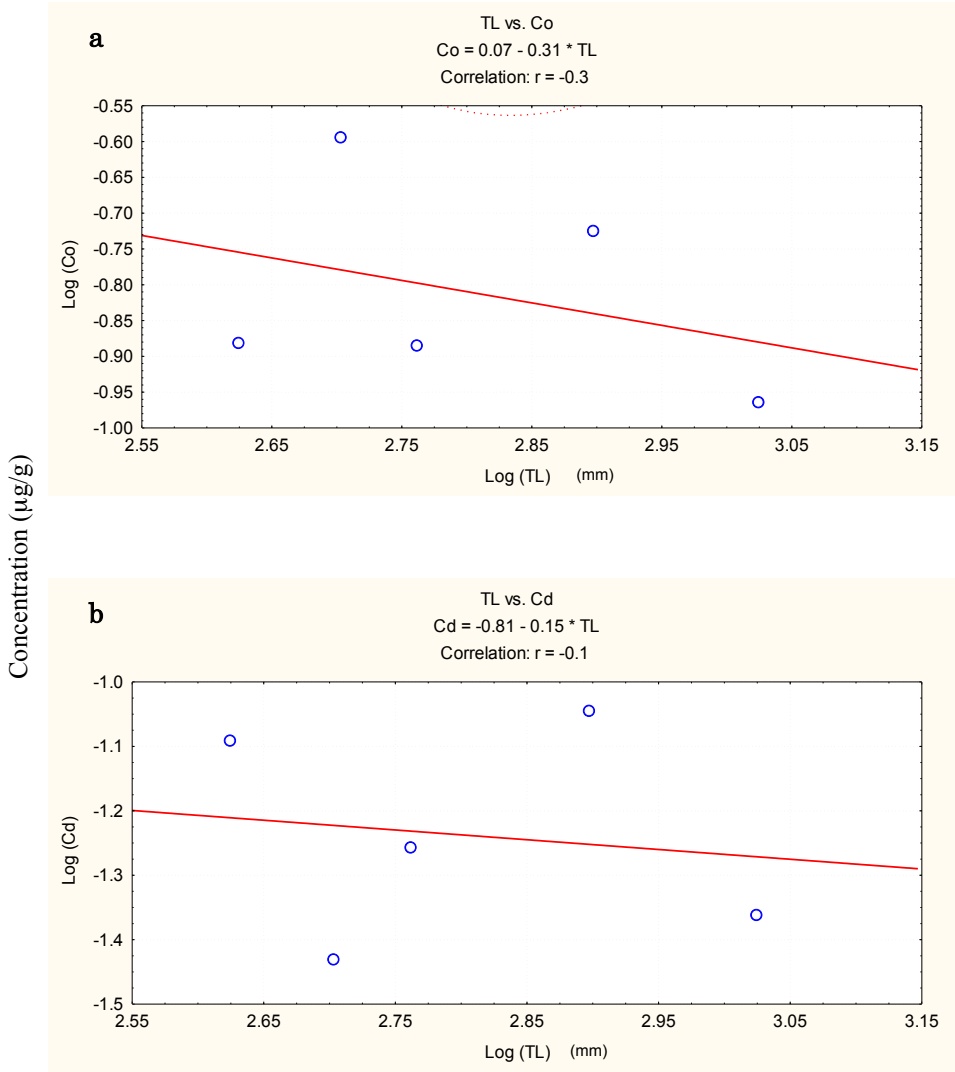

d
TL vs. Zn
Zn = 2.55 - 0.32 * TL
Correlation: r = -0.6
Concentration (µg/g)
Log (Zn)
1.78
1.74
1.70
1.66
1.62
1.58
1.54
2.55
2.65
2.75
2.85
2.95
3.05
3.15
Log (TL) (mm)

Figure 4.7. The trend of Co and Cd levels decreased with increasing of TL of *Anguilla marmorata*



4.3.5. The levels of metal burden in eel' muscles

All metal burdens in eel elevated with the increase in body sizes and were showed the same trends of metal accumulation in muscle (Figure 4.8). The metal burdens in body size classes S1, S2 and S3 showed similar levels, by contrast the elevated levels of metals were found in S4 and S5. There were no significant differences of all metal burdens among body size classes of S1, S2 and S3. The significantly higher levels of V, Cr, Mn and Hg in S4 and S5 than those in S1, S2 or S3 (Tukey HSD test, $p<0.05$), while the burden concentration of Co, Cu, Zn and Sr in S5 were highest when compared with other body size classes (Tukey HSD test, $p<0.05$). Although the levels of Cd and Pb in S4 and S5 showed slightly higher than those in S1, S2, and S3, no significant differences were found.

4.3.6. Estimation of potential health risk

Daily intake doses of all trace metals were estimated and compared with reference doses (RfDo) of the risk-based concentration table for chemical contamination (U. S. EPA 2008), except for Pb (Table 4.6). Because Pb was not in the list of RfDo, the studies of Agusa (2007) were referred to for comparison. In addition, the daily ingestion of fish in Vietnam was assumed to be 49 g wet wt./day (FAO 2005) and the average body weight of Vietnamese to be 50 kg. The ratio of dry weight/wet weight in eel muscle was estimated to approximately 20% in this study. The intake doses of trace metals for fish consumption are estimated and presented in Table 4.5.

Figure 4.8. Metal burden levels in eel' muscle in five body size classes

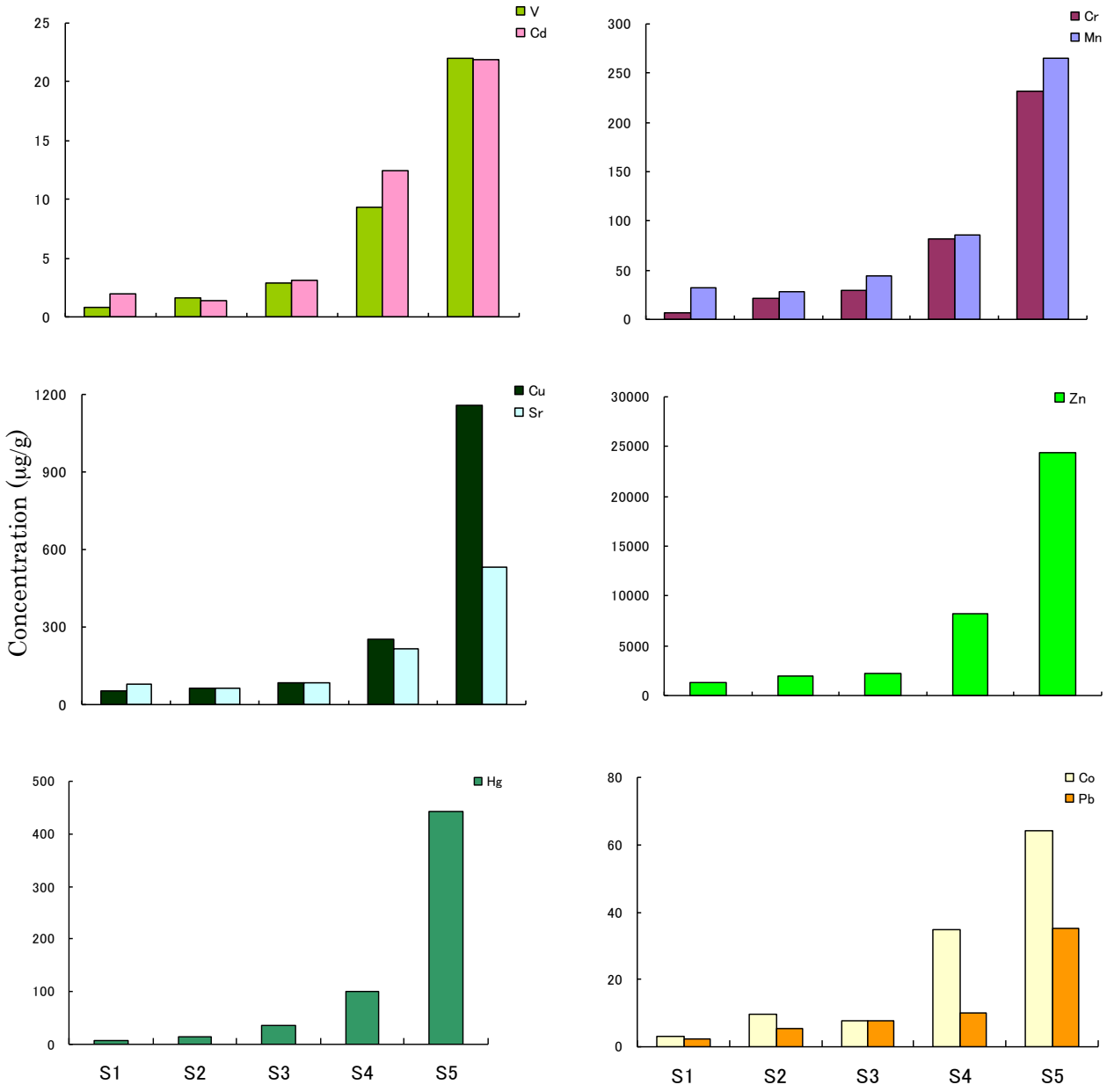


Table 4.5. Daily intake dose ($\mu g.kg^{-1}.day^{-1}$) of trace metals for a person (50kg) consuming eel flesh in Vietnam

Location	Intake	V	Cr	Mn	Co	Cu	Zn	Sr	Cd	Pb	Hg
Quang Tri	Mean	0.014	0.123	0.327	0.021	0.44	16.1	0.80	0.025	0.025	0.096
(n=10)	Min	0.006	0.067	0.106	0.008	0.20	12.2	0.35	0.004	0.011	0.061
	Max	0.018	0.227	0.991	0.054	0.85	24.9	1.66	0.057	0.041	0.137
Quang Ngai	Mean	0.003	0.054	0.256	0.036	0.37	9.7	0.49	0.017	0.022	0.098
(n=10)	Min	0.001	0.034	0.093	0.014	0.18	5.5	0.05	0.004	0.006	0.063
	Max	0.010	0.091	0.452	0.078	0.82	13.5	1.37	0.046	0.047	0.132
Binh Dinh	Mean	0.008	0.088	0.165	0.032	0.33	9.4	0.37	0.012	0.021	0.104
(n=30)	Min	0.004	0.031	0.051	0.008	0.11	3.0	0.03	0.001	0.003	0.048
	Max	0.013	0.224	0.427	0.117	0.76	21.8	1.37	0.042	0.061	0.172
Phu Yen	Mean	0.011	0.083	0.191	0.044	0.24	8.3	0.26	0.004	0.027	0.100
(n=10)	Min	0.009	0.061	0.068	0.024	0.18	4.1	0.07	0.001	0.001	0.133
	Max	0.016	0.132	0.549	0.073	0.45	12.5	0.54	0.019	0.053	0.075
RfDo value $\mu g.kg^{-1}.day^{-1}$		**5**	**1.5**	**140**	**20**	**40**	**300**	**600**	**1**	**3.57***	**0.3****

* Reference doses for Pb were referred from Agusa (2007).

** The recommend values of USEPA (2009).

4.4. Discussion

4.4.1. Concentrations of trace metals in tissues of eels

Almost all metals in liver were found to be higher than those in muscle, this indicated the target organ of metal accumulation. Furthermore, metal levels in both muscle and liver tissues showed the elevated level in QT when compared to other sites. Therefore, the metal levels in eels obviously reflected the environmental status where they inhibit. The differences in the metal contents of eel tissues among the rivers might result from natural or anthropogenic sources of locations. While metal accumulations of eels in BD, QN and PY were similar levels, this reflects the same metal load in aquatic environment of the locations. The elevated concentrations of Cd in QT might derive from many anthropogenic sources such as agricultural and urban activities. The presence of Cd in this location was primarily due to the intensive use of fertilizers in agricultural areas (Ngo et al. 2005). They suggested that commercial phosphate fertilizers which contain small amount of trace-metal contaminations are potential sources of Cd which are delivered to the rivers from up-stream areas by run off during trace rains. Cd is a nonessential and highly toxic metal, and can induce adverse effects on eel physiology (Lionetto et al. 1998, 2000), especially on reproduction (Pierron et al. 2008). Moreover, the increased concentrations of Zn in QT may be related to the high concentrations of Cd, because in animals, Zn protects against Cd injury to animals by competition at the uptake sites and induction of metallothionein synthesis (Hamilton and Mehrle 1986). The weak tolerance of Cd in animals has been found in deficient Zn nutrition (Merian 1991). In some studies, the accumulation of Cd in aquatic organisms has been observed to decrease in the presence of Zn (Elliot et al. 1986). However, exposure to high concentrations of Cd in toxicity experiments has been observed to result in increased accumulations of Zn in tissue of the freshwater fish *Tilapia nilotica* (Kargin 1999). These results suggest that potential pollution of Cd has existed in QT. Alternatively, the appearance of elevated concentrations of Cd in QT has also influenced the uptake of Cu (essential element) in fish, because Cd induces redistribution of Cu,

resulting in a deficiency of this metal in animals (Merian 1991; Pelgrom et al. 1994), and these trace metals often occur together in polluted areas (Pelgrom et al. 1995). However, in this study, the interaction between Cd and Cu was not clear, and the Cu in the liver and muscle of *A. mamorata* might be taken up from anthropogenic sources that run off in the river from urban activities in Quang Tri and Dong Ha cities.

Vanadium is involved in many physiological systems, although not considered an essential element (Nechay et al., 1986; Soares et al., 2008). The finding of the differences of V among the study sites might relate to the metal loads in environment, however there is little information on V contamination in wildlife (Merian 1991; Mochizuki et al., 1999; Soares et al., 2008). At higher concentrations, V becomes toxic to the cells inducing several injury effects at specific target organs, such as liver and kidney (Soares et al., 2008).

For other metals such as Cr, Mn, Pb and Hg, although the concentrations of these metals in muscle did not differ in all locations, conversely their levels in liver did. This suggests metal accumulation in liver clearly discriminated metal pollution status among sites. Therefore, liver tissue appears to be suitable indicator of inorganic pollution in the environment, and muscle plays a vital role in evaluating the possible existence of risk to human health of fish consumption. Cr was considered to be essential to a part of the living organisms, and has been found primarily in two forms: trivalent Cr (III), which is biologically active and found in food, and hexavalent Cr (VI), a toxic form resulting from industrial pollution (Merian, 1991). Mn is an essential element in nature and functions as a cofactor for some kinds of enzymes in living beings such as arginase, oxidoreductases, transferases, hydrolases etc. (Merian 1991), though it becomes toxic at very high concentrations. In contrast, Pb and Hg are nonessential elements; they are poisonous and can damage nervous connections and cause blood and brain disorders (Merian 1991). In fish, Pb is distributed by the blood system (Debus 1987) and accumulates mostly in bones, but also in the liver, kidneys, and gills (Hagner 2002). The Hg contamination in fish is of concern because of the

risks to human health posed by methylmercury (MeHg) associated with fish consumption (Clarkson, 1990). In the eel, the target organ tissues for Hg accumulation were the liver and muscle (Barak and Mason, 1990), however most of the Hg in eel muscle tissue was found to be MeHg (>84%) (Redmayne et al. 2000) and liver mainly accumulated inorganic Hg (Brusle, 1990; Edward et al, 1999; Szefer et al, 2003).

Co is an essential element for all higher animals and humans. Although Co concentrations are low in the environment, the increase in use of cobalt in agricultural practices, natural cobalt transport processes, and mining activities will likely become a problem in the future (Merian 1991).

4.4.2. Correlation between metal concentrations and metal burden in eel muscle with body size

In this study, the positive correlation between the Hg accumulation levels and body sizes found in this study was in agreement with other studies (Brusle, 1990; Szefer et al., 2003; Redmayne et al., 2007). Because the Hg was slowly eliminated in eel muscle with a half-time excretion rate ranging from 910 to 1030 days (Brusle, 1990). Eels are susceptible to the biomagnification and bioaccumulation of Hg from where they forage and reside (Edwards et al., 1999). In contrast, it is not surprising to find none or negative correlation between other metal contents and total length in the fish, because metal levels in the muscle of eel in BD was at the low or moderate levels and were assumed to be within the normal physiological range. Other studies also indicated that effects related to size or age were negative or nonexistent (Faskar et al. 2000; Bird et al. 2008). Size effects might vary depending on the metal contaminant. In fact, Mn, Zn, Sr and Pb concentrations tended to decline with increasing total length may relate to the somatic growth dilution (Marks et al. 1980; Ward et al. 2010). While inorganic toxic metals such as Cd tend to accumulate in the liver and kidney in correlation to metallothionein levels (Bird et al. 2008; Linde et al. 1999), the muscle tissue usually contains low concentrations of Cd. The correlation between the metal concentrations and body size usually relate to the status of polluted areas or time of exposure to toxic elements in toxicity tests (Yang et al.1996) have often been demonstrated.

Unlike the correlations between studied trace metals and body size, the metal body burden in eel's muscle showed positively correlated with body sizes and the elevated levels were clearly observed from body size classes S4 and S5. Hence fish growth and size might influence body burdens of metal due to a growth dilution effect. The given trace elements accumulate largely from food and those can be uptaken slightly from water. The similar levels of metal body burden in the body size classes S1, S2 and S3 (<620 mm) of eels might relate to their same diet such as small crabs or other invertebrates, while the larger sizes of eels (S4 and S5) were progressively piscivorous and they required energy values of prey organisms not only to grow but also to store energy reserves for gonad development and spawning migration, especially in body size S5 (>1000 mm). Ryan (1986) studied on the stomach contents of freshwater eels *Anguilla autralis* in Lake Ellesmere, Australia, found that the diet of eels changed with increase in their body size. He indicated that the smaller eels (≤400mm) fed primarily on invertebrates, while the larger size of eels (>501 mm) became piscivorous and preferred to forage fish such as gobies or smelts, because they became piscivorous.

4.4.4. Potential health risk

No permissible trace metal daily intake levels from fish consumption have been established in Vietnam, while food safety levels for trace metals have been instituted at various levels by different countries. For instance, the permissible limits of Pb and Cd for seafood consumption in Malaysia are 2.00 and 1.00 μg/g dry wt.; in Hong Kong 6.00 and 2.00 μg/g wet wt.; and in Thailand 6.67 μg/g dry wt. for Pb, but with no recommendations for Cd (Yap et al. 2004b). In Brazil, permissible limits for trace metals are higher for Cd (5 μg/g dry wt.) (Yap et al. 2004a, b) while European countries have controlled the safety levels for food consumption by a Provisional Tolerable Weekly Intake (PTWI) that have been decided for Cd 7 μg/kg body wt (1 μg/kg/day). This is equal to 490 μg/week (or 70μg/day) for a person weighing 70 kg (average body weight of European people) (SCOOP - scientific co-operation on questions relating to food 2004). In the present study,

therefore, safety level for fish consumption was compared with RfDo for trace metals of EPA (2008) and the study of Agusa et al. (2007). The maximum metal levels in the muscle of tropical eel *Anguilla marmorata* were found to be far below RfDo guideline values and also lower than the guidelines mentioned above for fish consumption. The result leads to the conclusion that the muscle tissue of eel from the central part of Vietnam may not currently cause any serious health risk for human consumption.

Chapter 5

Differences of trace metal contents between growth and maturity stages of tropical anguillid eels in Vietnam

5.1. Introduction

Life history of diadromous eels is well-known. Being leptocephalus larva in ocean after the hatch, they drift toward the continents following ocean currents and recruit to estuaries at glass eel stage. After migrating upstream at elver, they, are yellow eels, colonize a variety of different habitats (rivers, lakes, marshes, estuaries) for several years to decades depending on the hydrosystem and species. Before eels 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 require sufficient energy reserves in their yellow stage in forms of lipid and protein (McKeown 1984). Thus, they can accumulate both essential elements for their biological mechanisms and incidentally gain non-essential ones in the stage. The quality and quantity of energy store in silver eels are very important for the success of gonad development and migration. For instance, silver eels accumulate high level of nonessential elements such as Cd, this can impair their reproductive capacity (Pierron et al., 2008) or they can not reach the spawning area if their energy store is not enough (Boëtius and Boëtius, 1980). Although silver eels are not fully mature when they leave the place where they grew, there was difference of lipid and protein contents between yellow and silver stages of eel (Boëtius and Boëtius 1980). This can influence the accumulation levels of trace metals in eels between these stages. However, information is insufficient about the difference in the levels of trace metal inclusing essential and non-essential metals, between the two stages of tropical anguillid eels. Most studies focused on temperate anguillid eel that were considered as bioindicator to assess the health of aquatic system (Barak and Mason, 1990; Knight, 1997; Usero et al. 2003; Has-Schön et al., 2006).

Therefore, the aim of this chapter is to evaluate whether differences of the metal accumulation related to maturity stages of two tropical anguillid eel species, *A. marmorata* and *A. bicolor pacifica*, in order to elucidate the metabolisms of trace metals in tropical eels.

5.2. Materials and Methods

5.2.1. Study site

The study site was described in Chapter 3.

5.2.2. Sampling

Total 60 wild eels were collected by electric shocks or angling from the two sites in the Ba River, Vietnam (Fig. 5.1). The first sampling site placed in lower part of the river was 1 km from river mouth where dominated by the brackish water (site A). The other situated in upper part of river was about 18-20 km from the first site where the freshwater was dominant (site B). The eels were collected in each site within 2-3km range along the river. The skin color was further observed to help in the categorization of each specimen as either a yellow (immature) or silver (maturing) stage, and sex was determined by examining the gonads. In the present study, female eels were used due to the limited number of male samples. Species identification was conducted by their morphological characters based on Ege (1939) and Watanabe (2003). *A. marmorata* was easily recognized basing on the skin color on its back, a brownish to black marbling on a greyish yellow background. Whereas *A. bicolor pacifica* was more difficult to identify because of similar characters with *A. bicolor bicolor*, however basing on the geographical distribution of these two species (Watanabe, 2003), the eel specimens collected in the Ba River were supposed to *A. bicolor pacifica*. In February 2008, a total 21 specimens were all identified as *A. marmorata*, in which 17 specimens collected from the upper part of the river were categorized as yellow stage, whereas 4 specimens collected from the lower part of the river were as the silver maturing eels. In November 2008, total 38 eel specimens collected from lower part of the river. Among them, 9 specimens were identified as *A. marmorata*, which were categorized to be at

silver stage. Twenty nine specimens were identified as *A. bicolor pacifica*, of which 15 and 14 specimens were categorized to be yellow and silver stages, respectively. The total length (to the nearest mm) and body weight (to the nearest gram) were measured (Table 5.1). 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 gonad-somatic index (GSI). 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. The digestive tracts were opened to take food content that will be analyzed to understand the trace metals from food source. All biological samples were put into clean polyethylene bags and stored at -20^{0}C until chemical analyses.

5.2.3. Chemical analysis

The liver, muscle tissues and food contains were dried in oven at 80^{0}C or in freeze-drier (FDU-2200, Japan) at 7.8 Pa and -86.7^{o}C to constant weight, respectively. The dried tissue samples were digested in a Teflon bomb by a microwave oven as described in Chapter 3. The digested solutions were diluted with 45 ml Milli-Q water (Milli-Pore Company) into polyethylene and stored at 4^{0}C until metal analysis. Hg was determined with a cold vapor atomic absorption spectrometer (Model MA-2000, Nippon Instruments Corporation, Japan).

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 %.

5.2.4. 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 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 TL or 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 were strong correlations, therefore TL, GW 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).

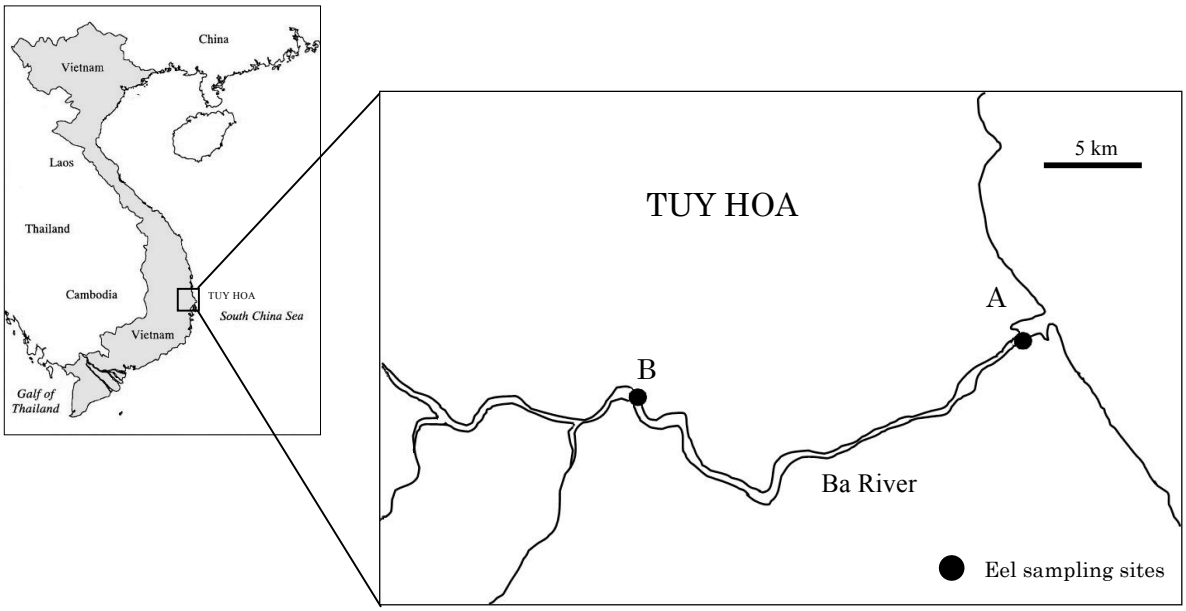


Figure 5.1. Sampling sites of anguillid eels from Ba River, Vietnam

Table 5.1. Biological information of anguillid eels collected from Ba River Vietnam

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

5.3. Results

5.3.1. Metal levels residue in organ tissues

Trace metal concentrations in liver and muscle of two eel species are shown in Table 5.2. Liver was main target site for almost trace metals such as V, Co, Mn, Cu, Zn, Cd, Pb and Hg, muscle was dominated by Zn, Cu, Mn and Hg. Although the metal levels contained in liver and muscle of two eel species were different, the levels of Mn, Cu and Zn were most dominant elements in both tissues and the accumulation trend of these metals was identical in 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) (Figure 5.2 a, b). The concentrations of Mn in muscle of *A. bicolor pacifica* were significantly higher than those in *A. marmorata*, whereas Mn levels in 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 no significant difference of Pb levels in muscle between two species was found. 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.

5.3.2. Differences of metal contents in tissues between the different maturity stage

Differences of trace metal levels in tissues between eel species are shown in Table 5.2. Almost of all trace metals in tissues tended to increase at higher levels in silver eel than those in yellow eels, excepted for Cd in muscle tissues. In *Anguilla bicolor pacifica*, the levels of Zn in muscles of silver eels were significantly higher those of yellow eels (Figure 5.3), while the levels of Cr, Mn, Cu and Zn in livers of silver eels were significantly higher than those of yellow eels (Tukey HSD test for unequal N, $p < 0.05$) (Figure 5.4). In *Anguilla marmorata*, the levels of Cr, Co and Zn in muscles of silver eels were significantly higher than those in yellow eels (Figure 5.5). Only Zn

levels in livers of silver eels was significantly higher than those in yellow eels (Tukey HSD test for unequal N, $p < 0.05$, with BW as covariate) (Figure 5.6). 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.

Table 5.2. Levels of trace metals (μg. g^{-1}) in liver and muscle of anguillid eels from Ba River, Vietnam

Organs	Species	Maturity stages	V	Cr	Mn	Co	Cu	Zn	Cd	Pb	Hg
Liver	*A. bicolor pacifica*	Yellow (n=15)	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 (n=14)	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 (n=17)	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 (n=14)	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 (n=15)	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 (n=14)	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 (n=17)	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 (n=14)	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

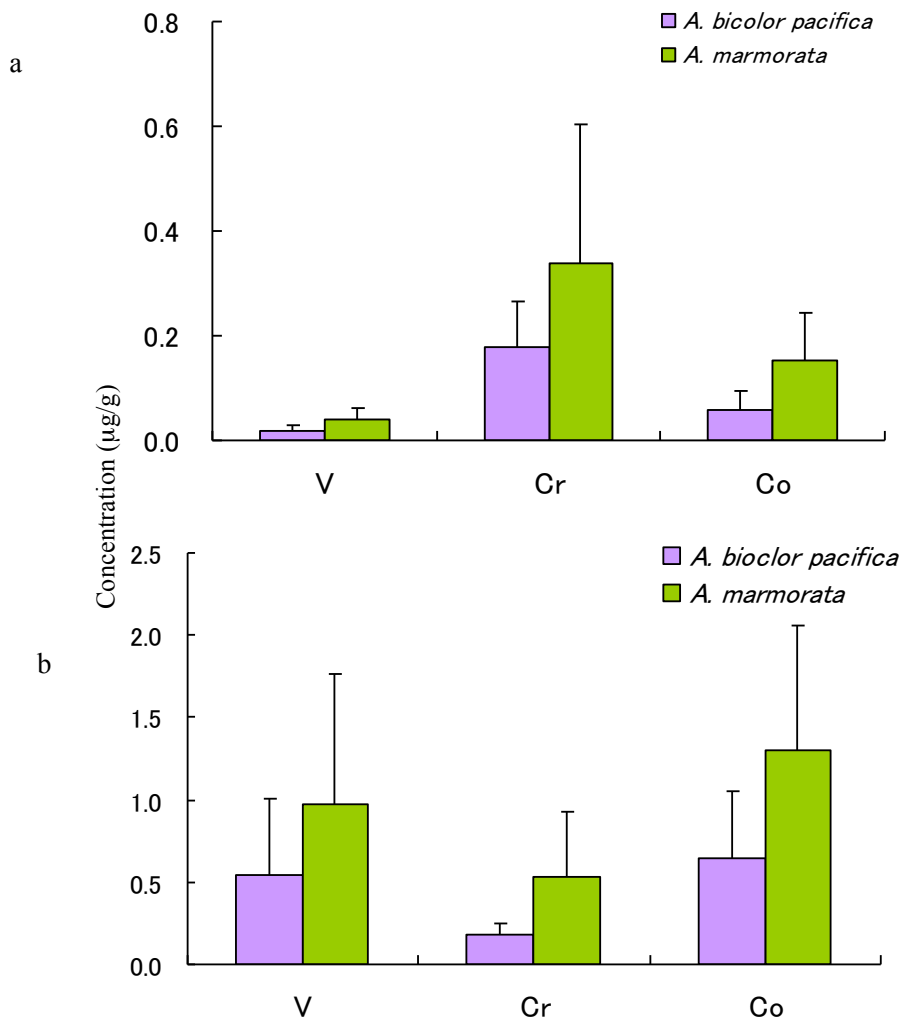


Figure 5.2. Difference in V, Cr and Co concentrations (μg/g) in muscle (a) and liver (b) between the two eel species

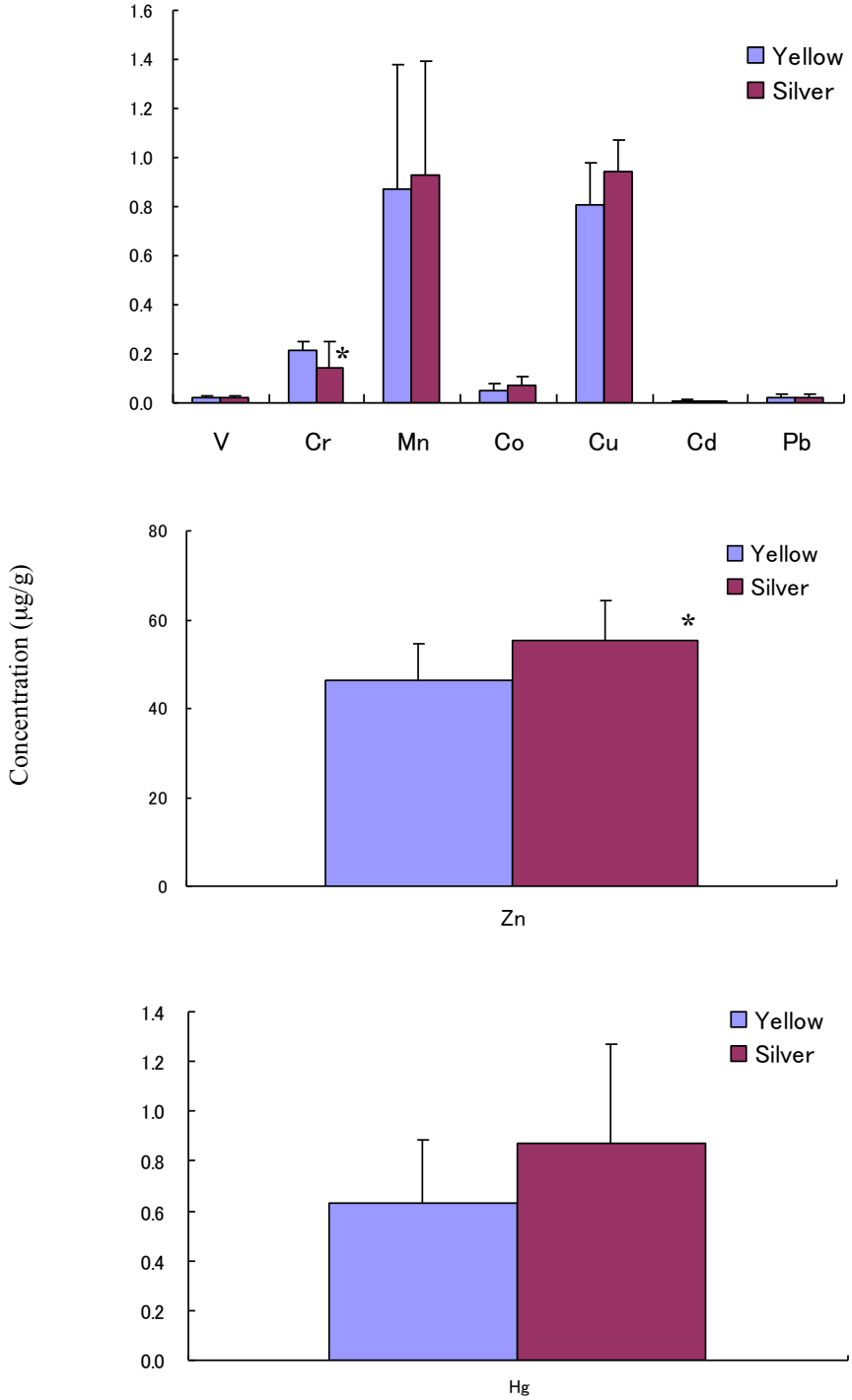


Figure 5.3. Levels of trace metals (μg/g) in muscle of yellow and silver stages of *Anguilla bicolor pacifica*

Note: (*) significant difference ($\alpha<0.05$)

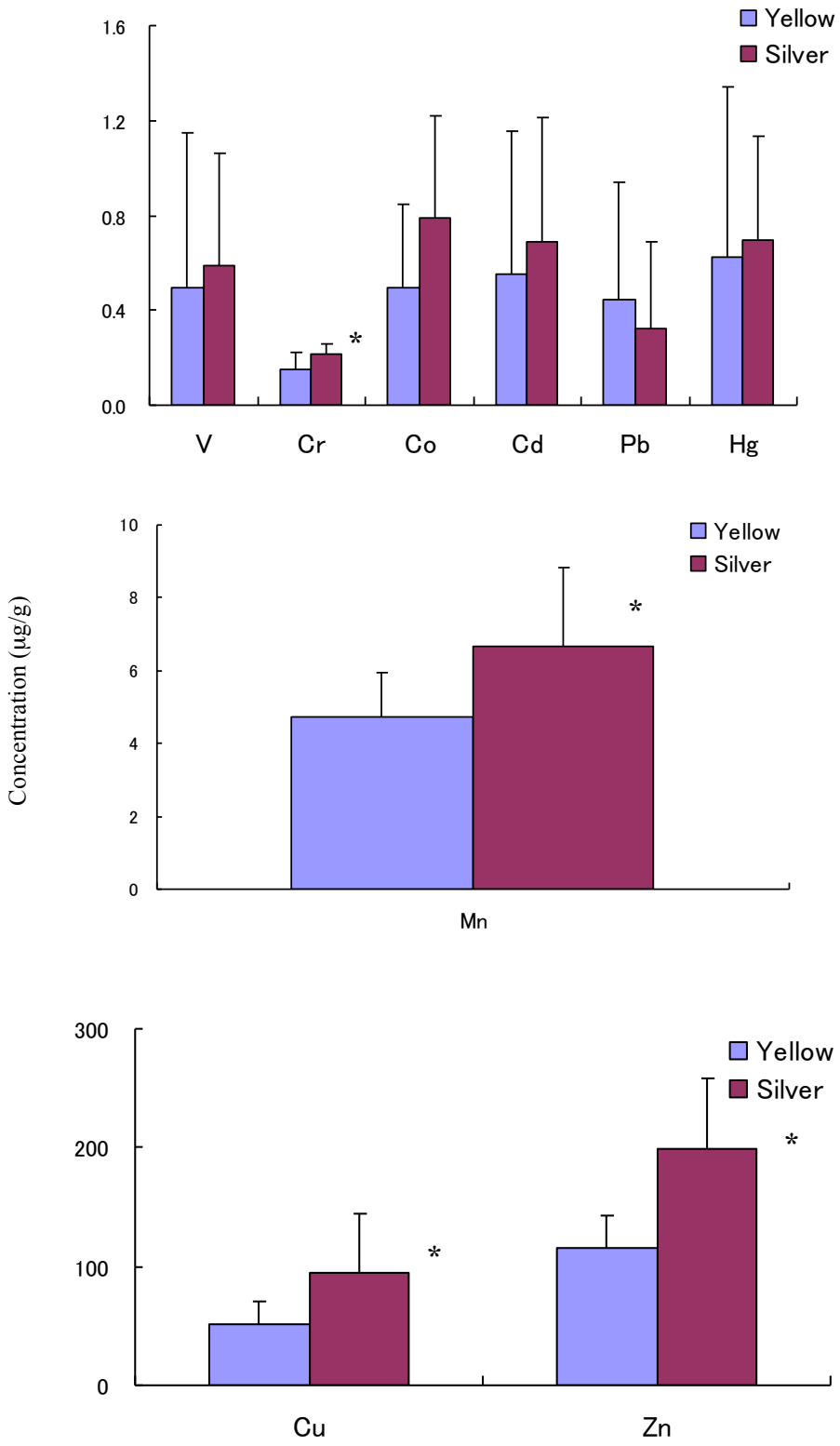


Figure 5.4. Levels of trace metals (μg/g) in liver of yellow and silver stages of *Anguilla bicolor pacifica*

Note: (*) significant difference ($\alpha<0.05$)

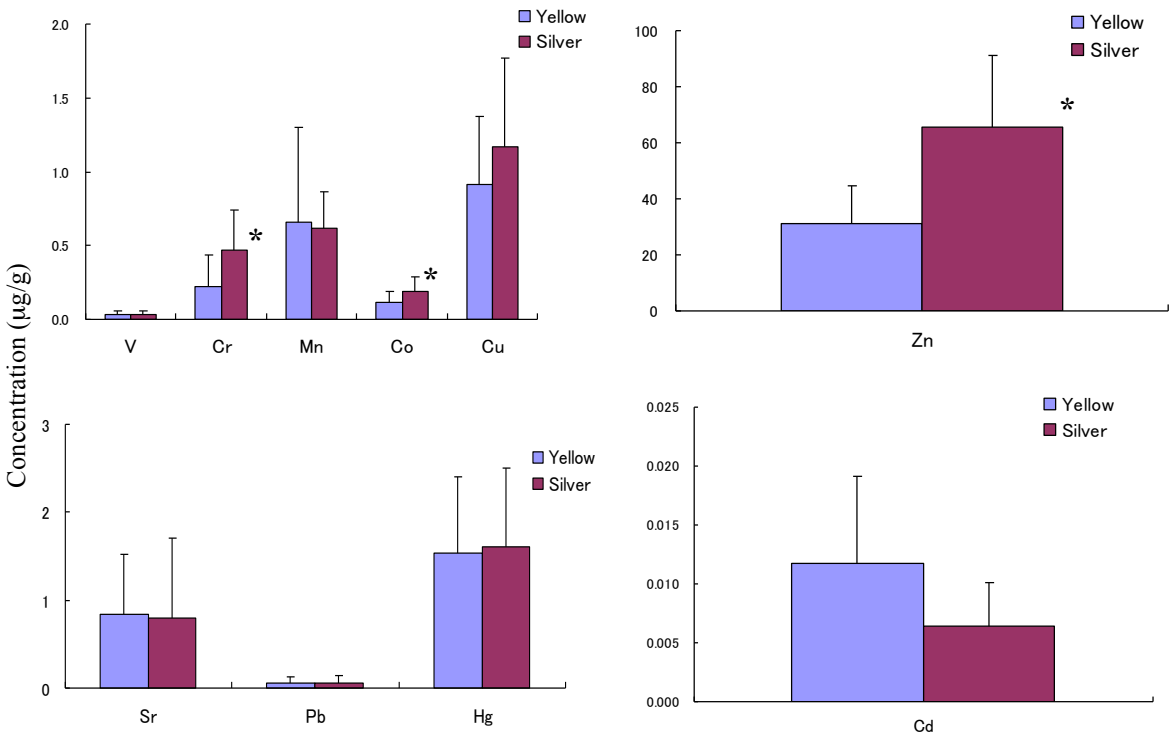


Figure 5.5. Levels of trace metals (µg/g) in muscle of yellow and silver stages of *Anguilla marmorata*

Note: (*) significant difference ($\alpha<0.05$)

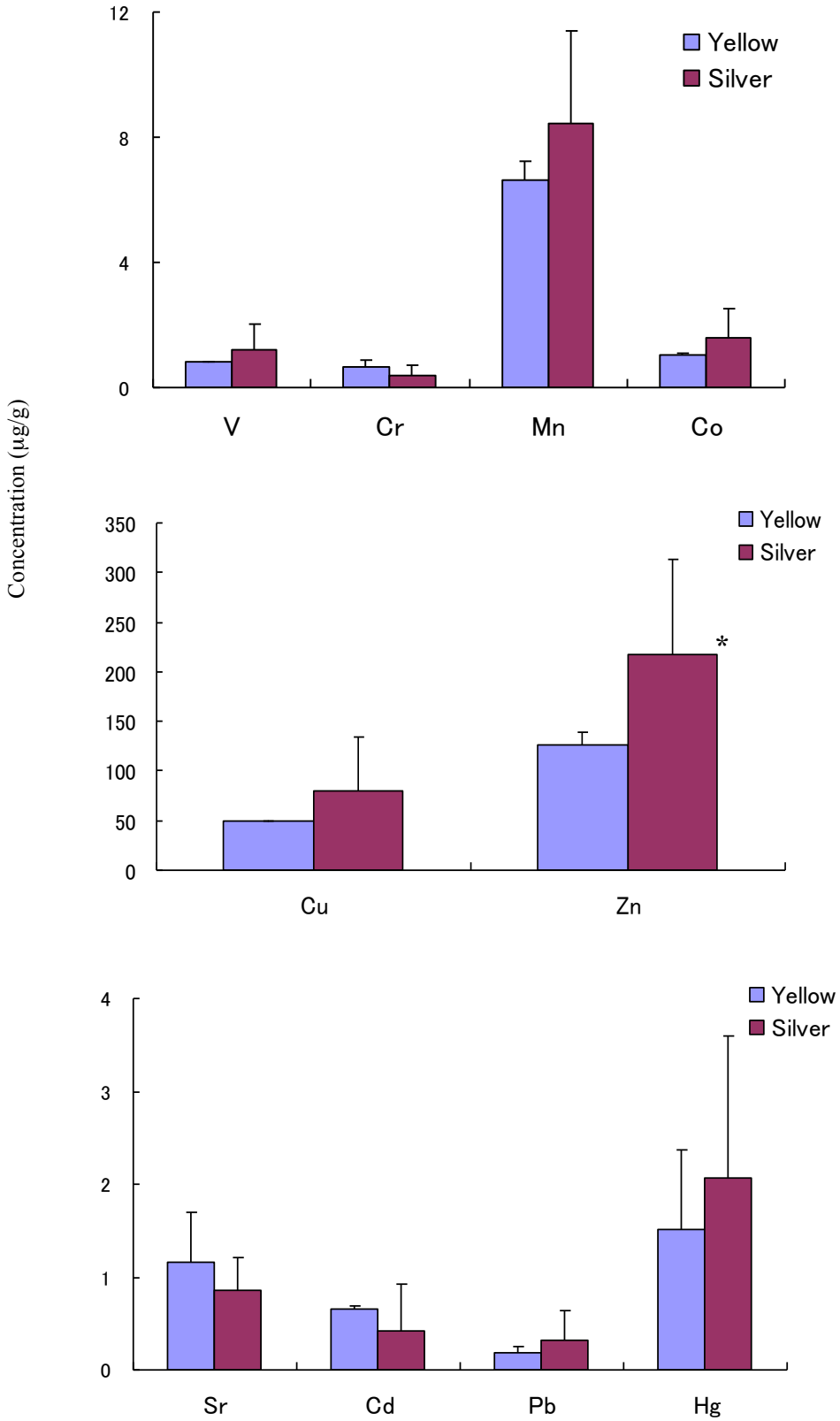


Figure 5.6. Levels of trace metals (µg/g) in liver of yellow and silver stages of *Anguilla marmorata*

Note: (*) significant difference ($\alpha<0.05$)

5.3.3. 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) (Figure 5.7 a, b) and GW (r = 0.6 and r = 0.6, respectively) (Figure 5.7 c, d), while only Mn levels in livers correlated with GSI (r = 0.6) (Figure 5.7 e). In muscles, there were correlations between Co levels and TL with r = 0.5 (Figure 5.8 a), and Zn levels and GSI with r = 0.5 (Figure 5.8 b).

In *A. marmorata*, Mn and Zn levels in livers positively correlated with GSI (r = 0.4, and r = 0.6, respectively) (Figure 5.9 a, b), while in muscles, Zn level showed correlations with TL, GW and GSI (r = 0.73, r = 0.7 and r = 0.8, respectively) (Figure 5.10 a, b, c) and Cr levels were correlative with GSI (r = 0.6) (Figure 5.10 d). It was not surprising that Hg levels in both liver and muscle tissues in *A. marmorata* were correlated with TL (r = 0.6, r =0.6) (Figure 5.11 a, b), however, no significant correlation of Hg levels in tissues and biological features of *A. bicolor pacifica* were found.

5.3.4. Metal levels in food contents

The food contents in digestive tracts were examined, there were no food remain in digestive tracts of both maturing silver eel species. Whilst small crabs were found in stomach of yellow stage of *A. bicolor pacifica*, there was no food item in yellow stage of *A. marmorata*. The food contain (n=5) were analyzed the metal levels. The result indicated that the domination of trace metals in food contain were Sr, Mn, Zn, and Cu. Among them, Sr concentrations were highest (2420.1 μg/g, in a range of 1731.2 – 2837.0 μg/g), followed by Mn (625.5 μg/g, 523.2 – 971.3 μg/g), Zn (64.2 μg/g, 39.1 – 96.0 μg/g), and Cu (9.84 μg/g, 6.37 – 13.35 μg/g). Among minor trace metals in food contain, Hg was the lowest levels, 0.05 mg/g (in a range of 0.04 – 0.06 mg/g), slight higher levels of Cr and Cd were observed 0.17 μg/g (0.13 – 0.22 μg/g) and 0.22 μg/g (0.11 – 0.53 μg/g), respectively. The similar levels of V and Co were found 0.59 μg/g (0.42 – 0.72 μg/g) and 0.57 μg/g (0.37 – 0.76 μg/g), respectively. Mean level of Pb were 1.62 μg/g (in a range of 1.12 – 2.40 μg/g).

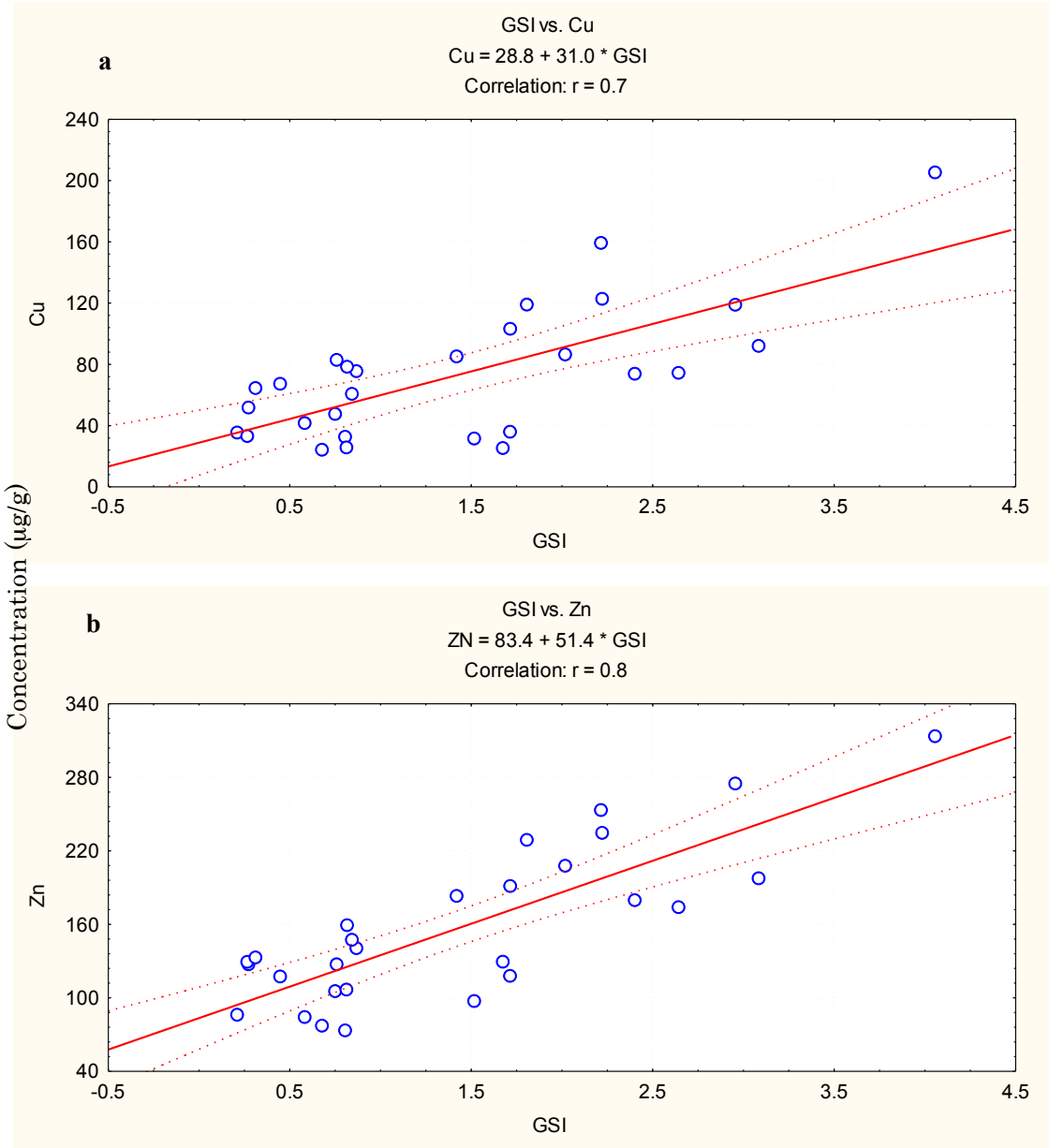
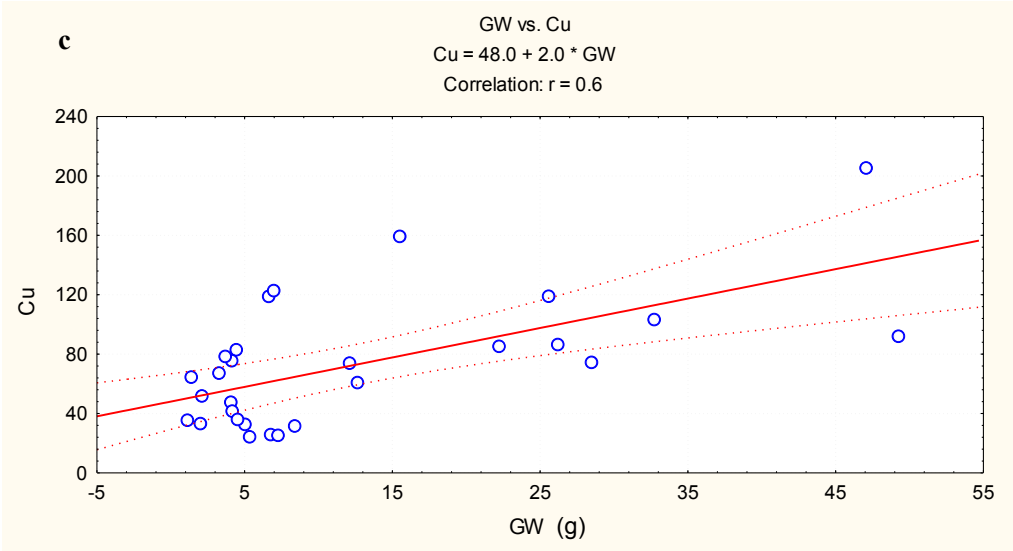
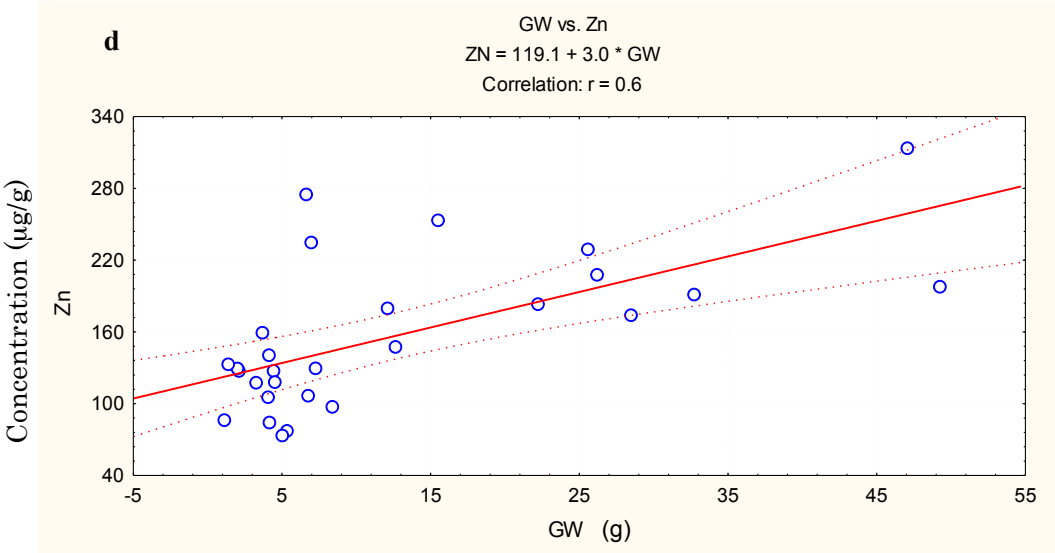
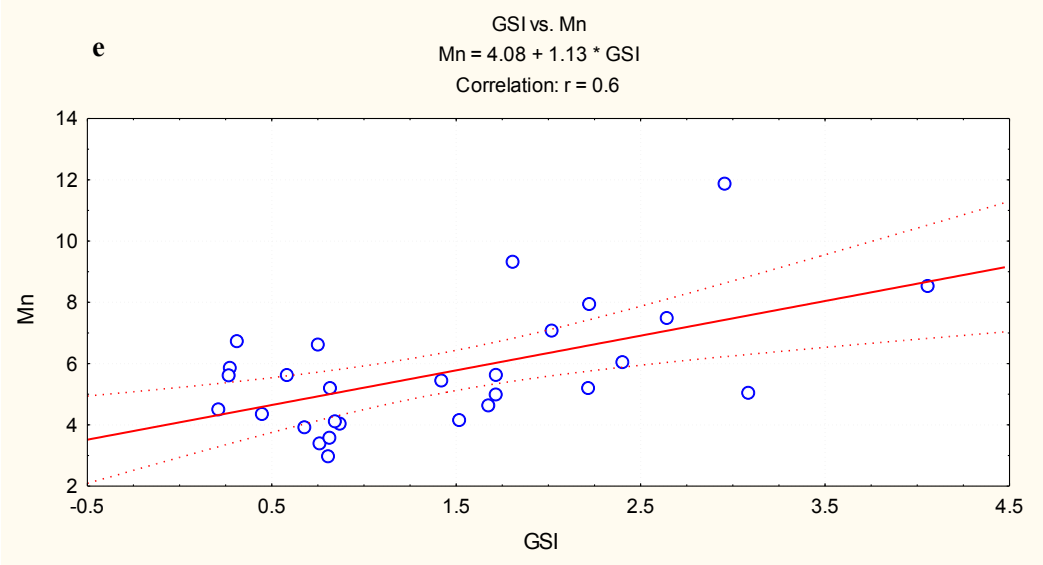


Figure 5.7. Relationship between metal levels in liver of *A. bicolor pacifica* and physical characteristics


c
GW vs. Cu
Cu = 48.0 + 2.0 * GW
Correlation: r = 0.6
Cu
GW (g)


d
GW vs. Zn
ZN = 119.1 + 3.0 * GW
Correlation: r = 0.6
Zn
GW (g)
Concentration (µg/g)


e
GSI vs. Mn
Mn = 4.08 + 1.13 * GSI
Correlation: r = 0.6
Mn
GSI

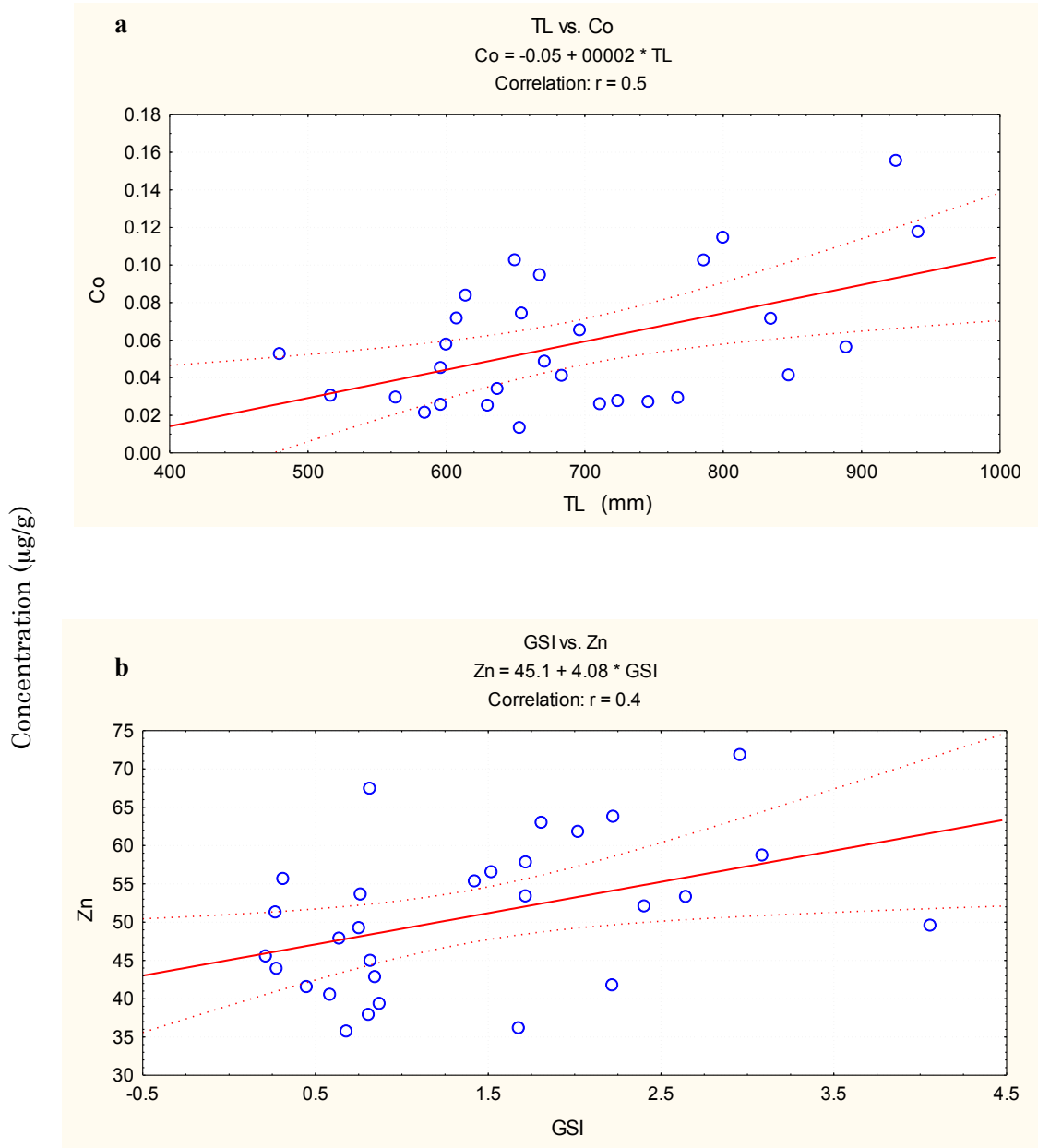


Figure 5.8. Relationship between metal levels in muscle of *A. bicolor pacifica* and physical characteristics

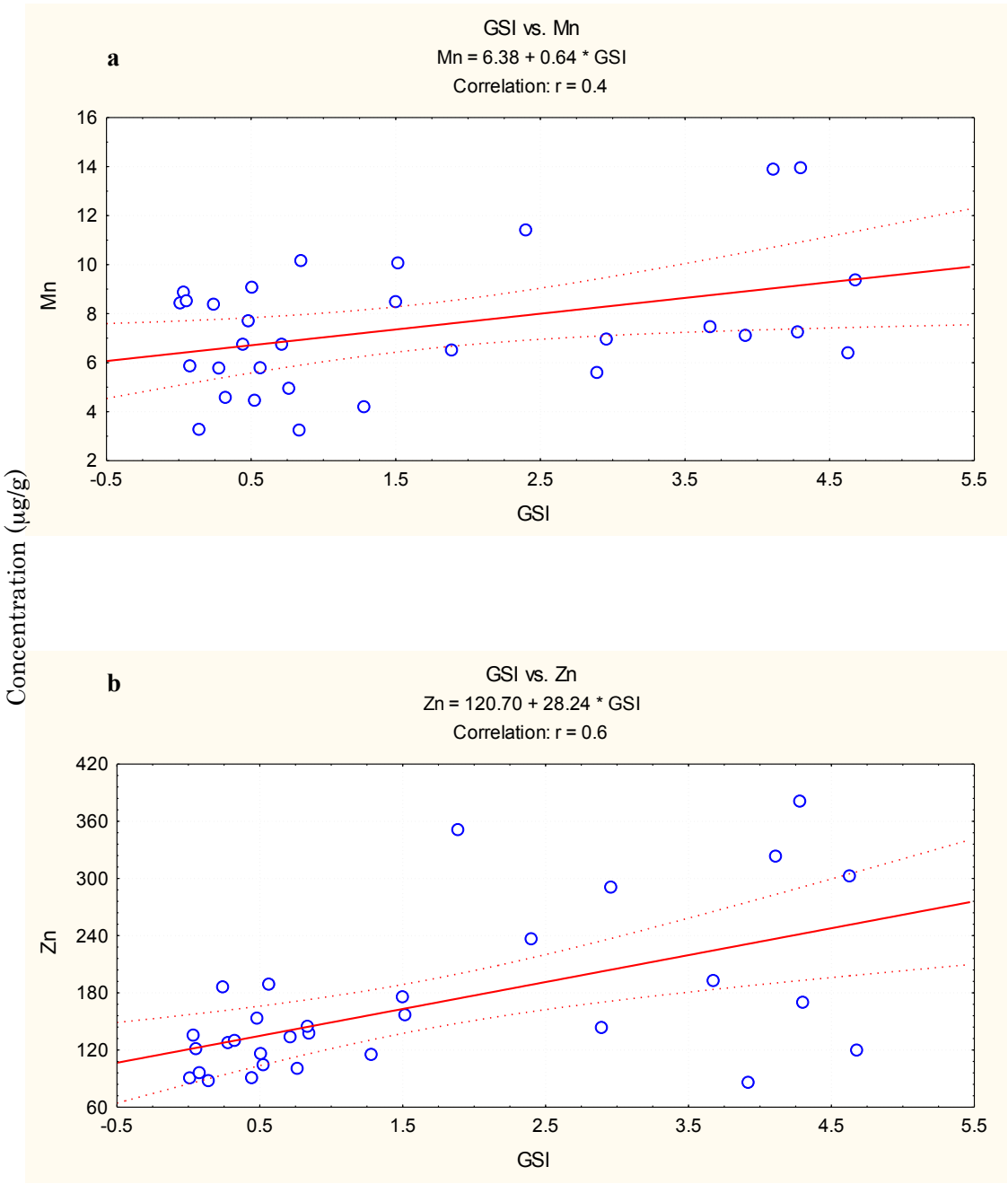


Figure 5.9. Relationship between metal levels in liver of *A. marmorata* and physical characteristics

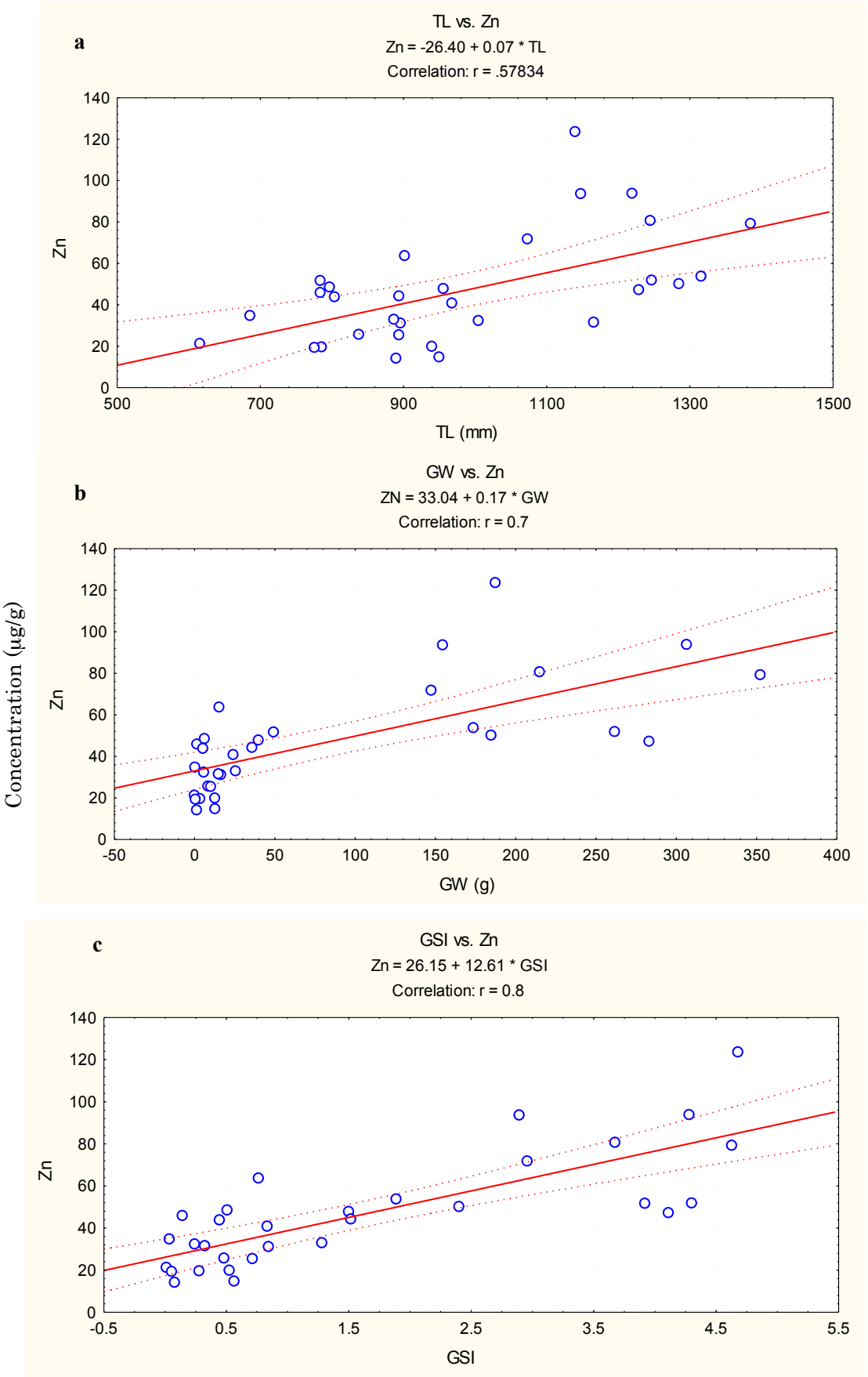
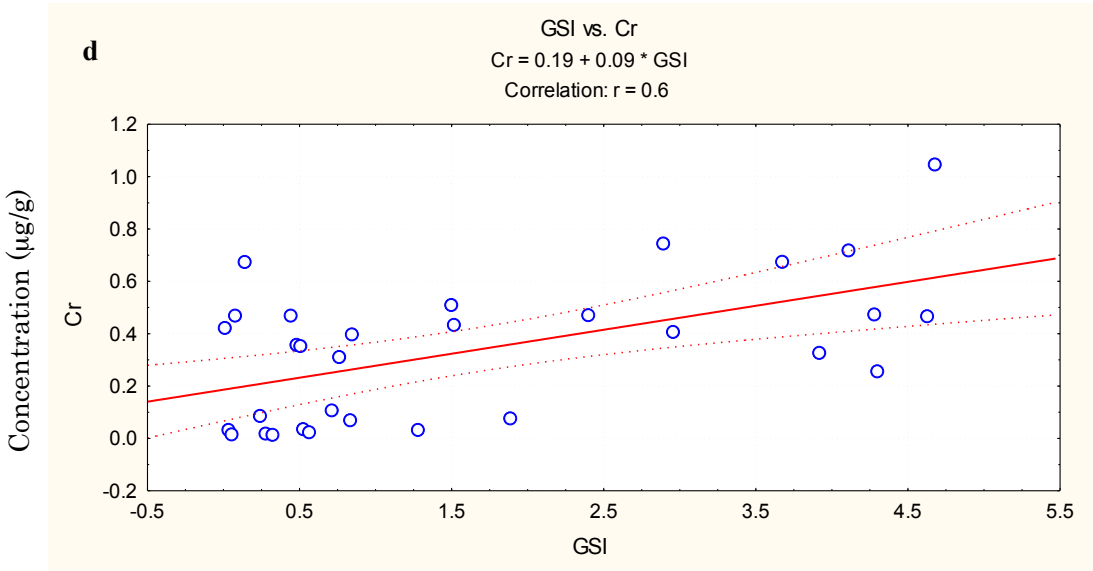


Figure 5.10. Relationship between metal levels in muscle of *A. marmorata* and physical characteristics


d
GSI vs. Cr
Cr = 0.19 + 0.09 * GSI
Correlation: r = 0.6
Concentration (µg/g)
Cr
GSI
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.5
0.5
1.5
2.5
3.5
4.5
5.5

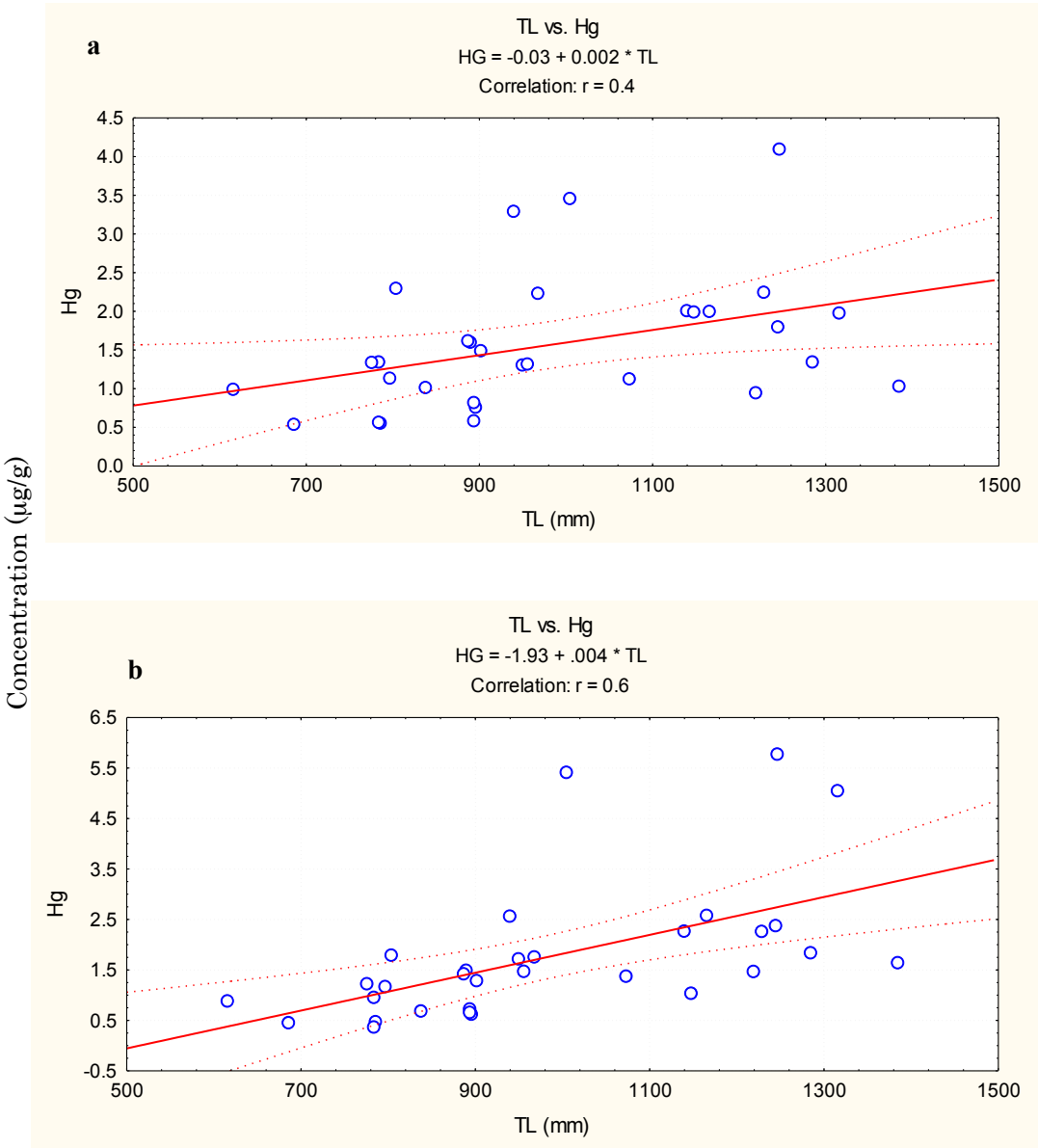


Figure 5.11. Relationship between Hg levels in muscle and liver of *A. marmorata* and body size

5.4. Discussion

5.4.1. Difference of metal accumulation between species

Since food items in gut of *A. marmorata* were not found, it was not possible to assess the metal uptake sources of the species clearly. 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 and preferred preys. The metal accumulations in tissues of the eel species might not be influenced by local metal pollution, because 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 (Chapter 2). In case of Hg, the higher Hg levels of *A. marmorata* compared to *A. bicolor pacifica* is supposed to be caused by the biomagnification of Hg via specific local food chain and to preferred preys (Brusle 1990; Hall et al. 1997). *A. bicolor pacifica* was dominant in the lower reach of the river and estuary (Chino and Arai 2010) and the food content in the stomach, which were mostly small crabs, contained low levels of Hg in this study. In contrast, *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 fish; especially larger eels are known to be piscivorous. These food items could be considered as higher potential Hg source, because they are at high trophic levels in the aquatic system (Wong et al. 1997; Kehrig et al. 2009). The difference in Hg levels between 2 eel species even may also 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 N.A-E. and Mason 1990). For the body size, *A. marmorata* was significantly larger than *A. bicolor pacifica* (Table 5.1), which can partly explain the difference of Hg levels between two species. However, it is known to be difficult to estimate the age of tropical eels from otolith rings, the ear-stone of fish that is usually used to estimate the fish's age of many studies (Jellyman 1991; Chino and Arai 2010). In fact, many incomplete rings were observed in

both eel species' otolith in this study and it was not possible to validate the annual formation of otolith zones (Figure 5.12). Thus, it is uncertain whether the difference of Hg levels between eel species relates to age. The significant correlations between the body sizes and Hg levels were only found in *A. marmorata* but not in *A. bicolor pacifica*. This may be due to the difference in biological mechanisms such as Hg uptake and elimination rate between the two species. Unlike Hg, the levels of Cd and Pb in tissues showed no significant difference between species, although the metal levels in *A. mamorata* tend to be slightly higher than those in *A. bicolor pacifica*. This may reflect the uniform distribution of these metals along river and the slight difference may relate to the feeding habits and habitats of eel species.

5.4.2. Difference in trace metal accumulation between maturity stages.

Not all trace metal levels in silver eel were significantly higher than those in yellow eels. The difference of metal levels between maturity stages of both species principally related to the essential metals such as Cr, Co, Mn, Cu, and especially Zn. These metals play important roles in metabolic mechanism in animals. However, the levels of Zn, Cu and Mn in the tissues of maturing silver eels were remarkably higher compared to yellow eels of both species. This may relate to demands of essential elements for gonad maturation and migration. In fish, the energy use for gonad formation originated not only directly from the food, but also from the energy reserve in muscle, mainly as fat, protein and carbohydrate deposits (McKeown 1984; Kemler. 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 liver of silver other than yellow eels of both species, especially Zn, Cu and Mn. 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 maturing 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 was well-known as a constituent of many molecules involved in protein, lipid and carbohydrate metabolism. In maturing fish, Zn can be transported from muscle to liver by blood serum to synthesize vitellogenin under the influence of estrogen, 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 were found in this study. Additionally, Danivol and Shechenko (1973) also found that during prolonged fasting, fish lose Zn from muscle. In present study silver eels were collected in estuary and they were at onset of downstream migration, thus, the muscle depletion of Zn did not occur 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 (Chapter 3), whereas the positive correlation between Zn and body size found in present study. The opposite results might be related to the accumulation of protein after lipid storage process in the muscle of mature fish (Kemler., 1992) and the elevation of the trace metals might associate to the increase of metalloproteins in tissues for their biological functions (Garcia et al. 2006; Dudev and Lim 2008). Metalloproteins, such as enzymes, transport and storage proteins, and signal transduction protein, have many different functions in cells. Additionally, Miramand et al (1991) also reported that the 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 related to the sex and reproductive cycle than to the ambient levels in marine environment.

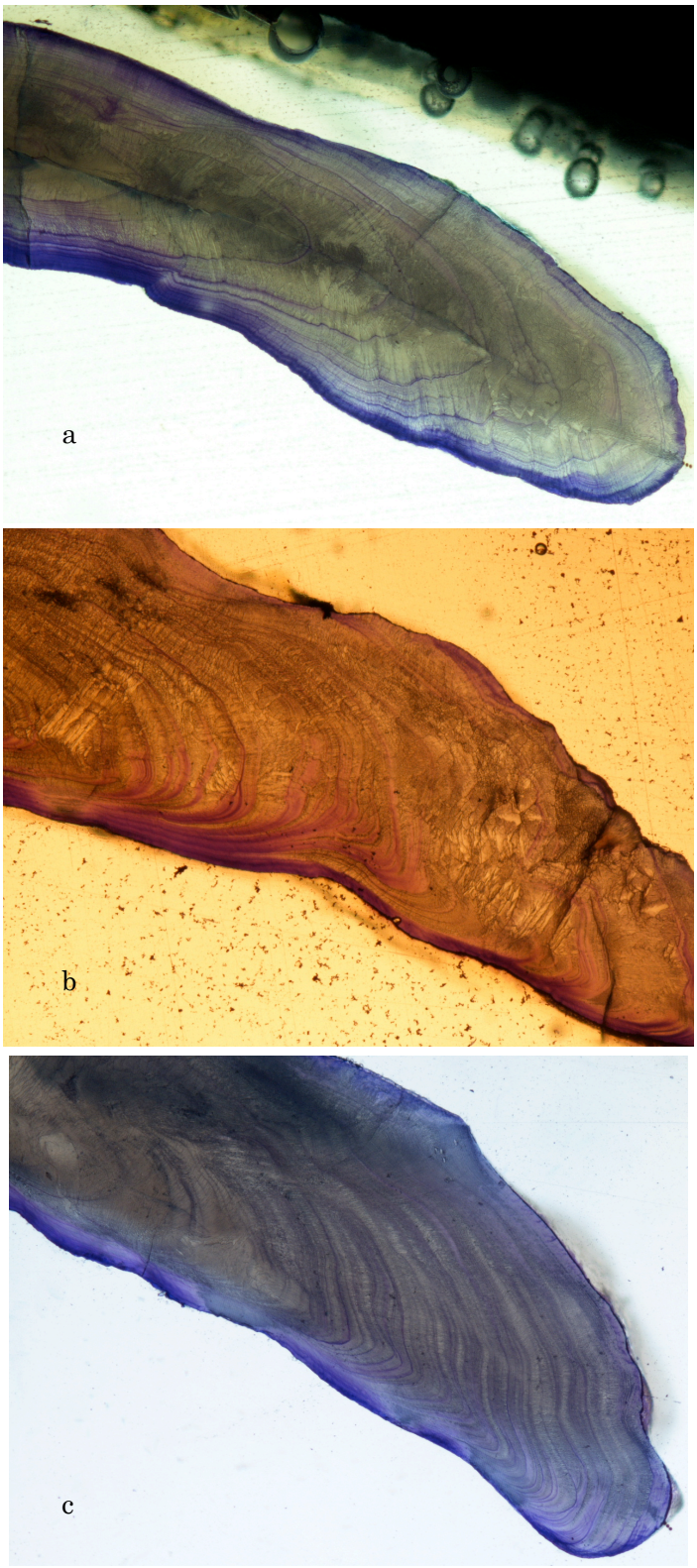


Figure 5.12. Comparing aging estimation by transect otolithes of Anguilllid eels: a – *Anguilla japonica;*

b – *Anguilla marmorata*; c – *Anguilla bicolor pacifica*

Chapter 6

Can migratory types of Japanese eel be useful tool to determine local metal pollution?

6.1. Introduction

The Japanese eel, *Anguilla japonica*, a diadromous fish, is widely distributed in East Asia, from Taiwan, through China, Korea, and Japan (Tesch, 1977). Japanese eel has a unique life cycle, being catadromous with a larval stage in the sea (leptocephalus, glass eel), a juvenile stage in freshwater (elver, yellow eel) and an adult spawning phase again in the sea (silver eel). A part of eels are known to reside in coastal area without freshwater lives (Tsukamoto et al., 1998; Tsukamota and Arai, 2001). In Japan, Japanese eel is not only an important freshwater aquaculture species, but also is used as a bioindicator of environmental pollution (Ohji et al. 2006). However, Japanese eel populations have declined dramatically in recent years (Tatsukawa, 2003) and several factors, such as overfishing, construction of hydroelectric plants, pollution, and climate change have been reported as the causes for the decline. Recently, environmental pollution, especially pollution involving trace metals such as arsenic, cadmium, mercury, lead, etc., has been greatly concerned, because they are widespread contaminants, persistence and toxicant that are released into aquatic systems from various anthropogenic sources. Since anguillid eels are top predators in their habitat and have a long-lived, they can incidentally accumulate high concentrations of pollutants via different uptake routs even in less polluted waters during the energy-storing phase of the yellow stage. Silver eels leaving their habitat to spawning areas often sufficiently store energy-reserves for migration and spawning, and is with accidental uptake of high level of pollutants in their tissues. Otherwise, the accumulated pollutants in target tissues of silver eels can be released to the blood stream when the energy reserves are optimally used for both swimming and gonadal maturation during the fasting transoceanic migration (Pierron et al., 2008). The previously accumulated

contaminants can be remobilized and redistributed, thus triggering potential toxic events that can affect eels' reproductive success (Robinet and Feunteun, 2002; Palstra et al. 2006; Pierron et al., 2008). Therefore, silver eels can face to higher ecological risk in aquatic system than yellow eels do.

Recently, using the Sr:Ca ratio in the otolith of the Japanese eel, many studies indicated that some eels never migrate into freshwater and spend their entire life in seawater or brackish water, while others flexibly migrate between freshwater and marine habitats (Arai et al., 1997, 2004, 2006; Tsukamoto and Arai, 2001). Indeed, the Sr:Ca ratio in the otoliths of fishes reflects their various habitats in the sea, estuaries or fresh water during their life. Additionally, Tsukamoto and Arai (2001) classified Japanese eels collected in Japanese coastal waters into three types, based on their migratory patterns: (1) "sea eels", which spend most of their life in the sea and do not enter freshwater, (2) "estuarine eels", which inhabit estuaries or switch between different habitats, and (3) "river eels", which enter and remain in freshwater river habitats after arrival in the river. While Japanese eels are known to be adaptable to the variation of environmental salinity (Tsukamoto and Arai, 2001; Tzeng et al., 2003; Arai et al., 2004), and they also are bio-indicator to assess the pollution status in environment (Ohji et al. 2006). Additionally, Arai (2006) found that Japanese eels could flexibly migrate between freshwater and marine habitats, as revealed by otolith microchemistry. These raise a doubt that anguillid eels can be incidentally collected in a specific location for the biomonitoring studies when they move from different habitats and salinities. Therefore, the level of metal accumulation in eels may not reflect accurately the environmental pollution status of the site where they are caught without historical migration examination. However, there has been no study to date on the relationship between metal accumulations and different migratory type in Japanese eel.

Therefore, the aim of this chapter was to examine whether levels of trace metals in the livers of eels vary among the migratory types and the ecological risk for silver eels.

6.2. Materials and Methods

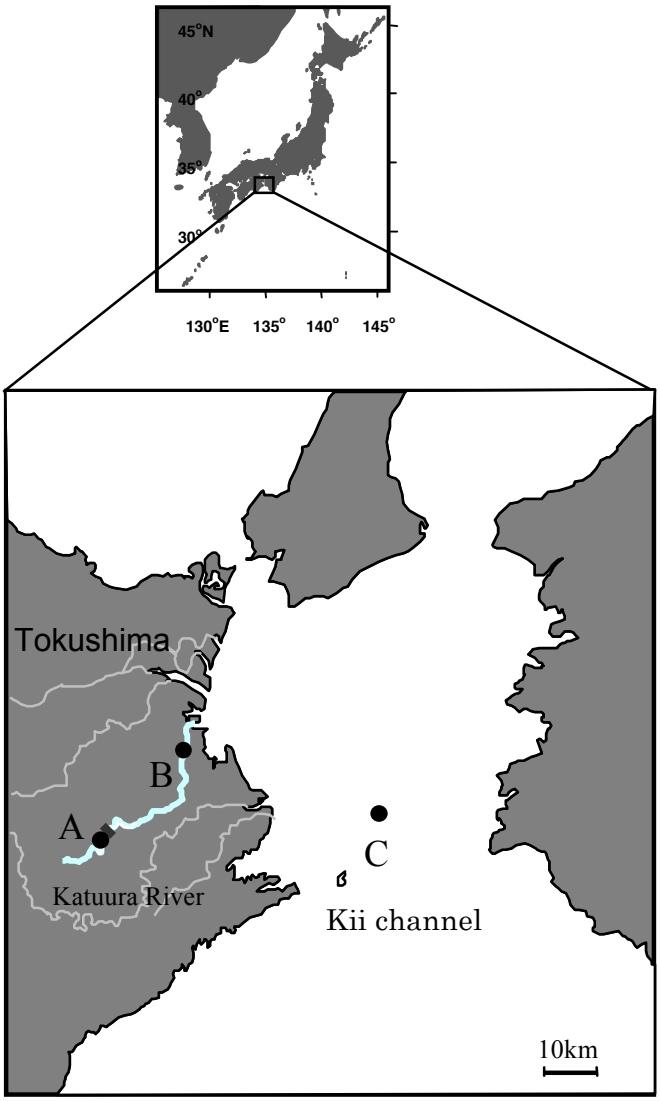
6.2.1. Sampling

The Japanese eels were collected by angling at two sites of the Katsuura River, Tokushima Prefecture; the one site (A) was at the upper reach of the river without influencing by tidal effect, and the other site (B) was 1-2 km from the river mouth in the intertidal zone. Eels were also collected by trawling fishing from Kii Channel (C) of Tokushima Prefecture. These three sites are located on the eastern part of Shikoku Island, western Japan (Figure 6.1). A total of 29 Japanese eel specimens were collected by set net or angling at three sites. Among the specimens, 11 were collected from the Katsuura River (5 from the site A, 6 from the site B) from July to August, 2005. The other 18 were collected from the site C from December, 2007 to January, 2008. The total length (to the nearest mm) and body weight (to the nearest gram) were measured. The color of skin and fins was further observed to categorize each specimen as either a yellow or silver stage, sex was determined by examining the gonads and all eel specimens were classified as female. The gonad-somatic index (GSI) was estimated to determine the mature condition of the eels using the equation: GSI = [Gonad weight (g)/Body weight (g)]×100. The eels were considered as yellow eels if the GSIs were below 1.0 and silver eels if the GSIs were above 1.0 (Utoh et al., 2004). Liver of the eel was chosen for the study because it was target for trace metal accumulation (Chapter 2). Livers of all specimens were dissected and stored in a freezer at -20 ºC until chemical analysis was performed. The sagittal otoliths were extracted for Sr:Ca ratio measurement and age determination.

6.2.2. Chemical analysis

The chemical analyses were conducted as the methods described in Chapter 3.

Figure 6.1. Sampling sites, (A) upstream of Katsuura River, (B) downstream (close to estuarine area) of and (C) offshore of Tokushima Prefecture



6.2.3. Otolith preparation and otolith X-ray microprobe analysis

The otolith preparation and analysis of Sr and Ca concentrations were conducted by the methods similar to those described by Arai et al. (1997, 2003, 2004). In brief, the sagittal otolith was extracted from each eel, embedded in epoxy resin (Struers, Epofix), and mounted on a glass slide. The otoliths were then ground to expose the core along the anterior-posterior direction in the frontal plane using a grinding machine equipped with a diamond cup-wheel (Struers, Discoplan-TS), and polished further with OP-S suspension on an automated polishing wheel (Struers, RotoPol-35) equipped with a automatic specimen mover (Struers, PdM-Force-20). Finally, they were cleaned using distilled water and ethanol, and dried in a 50 ºC oven prior to examination. The ground surfaces of the otoliths were examined at ×200 magnification with a light microscope, and photographs were taken to measure the 'radius' of the elver mark (the longest distance from the otolith core to the elver mark). For electron microprobe analyses, all otoliths were Pt-Pd coated by a high-vacuum evaporator. 'Life-history transect' analysis of the Sr and Ca concentrations in all specimens was performed by measuring along the longest axis of each otolith from the core to the edge using a wavelength dispersive X-ray electron microprobe (JEOL JXA- 8900R), as described by Arai et al. (1997, 2004). Wollastonite ($CaSiO_3$) and tausonite ($SrTiO_3$) were used as standards, and the accelerating voltage and beam current were 15 kV and 1.2 $\times 10^{-8}$ A, respectively. The electron beam was focused on a point 10 μm in diameter, with measurements spaced at 10 μm intervals.

The migratory types of Japanese eels were determined using otolith Sr:Ca ratios outside the 'high-Sr core' region, which was marked off by elver mark and was corresponded to the period of ocean life during the leptocephalus and early glass eel stages (Arai et al.,1997; Tsukamoto and Arai 2001). According to Tsukamoto and Arai (2001), the mean Sr:Ca ratio values outside the elver mark were used to categorize the specimens into 'sea eels' (CS) (Sr:Ca $\geq 6.0\times10^{-3}$), 'estuarine eels' (CE) ($2.5\times10^{-3} \leq$ Sr:Ca $< 6.0 \times10^{-3}$) and 'river eels' (CR) (Sr:Ca $< 2.5\times10^{-3}$). After electron

microprobe analysis, the otoliths were repolished to remove the coating, etched with 1% HCl and then stained with 1% toluidine blue. The age of each specimen was determined by counting the number of blue-stained transparent zones, outside the elver mark, as described by Arai et al. (2004).

6.2.4. Statistical analyses

The results are expressed as mean ± SD. One-way ANOVA and Tukey tests were performed to reveal any significant differences among migratory types and between the growth stages. The statistical analyses were performed using STATISTICA 5.5 for Windows.

6.3. Results

6.3.1. Migratory types of Japanese eels

The life history transects of otolith Sr:Ca ratio showed that all specimens generally had a high Sr core, which corresponded to the period of leptocephalus and early glass eel stages in the ocean (Arai et al., 1997), but there were a variety in change patterns of Sr concentration (Sr:Ca ratio) with concentric rings outside the elver mark in the otolith (Figure 6.2). The change of in the otolith Sr:Ca ratio indicated different migratory history: 'river eels', 'estuarine eels', and 'sea eels' (Tsukamoto and Arai, 2001).

The otolith Sr:Ca ratios outside the elver mark of Japanese eels collected from up and downstream sites (sites A and B) of the Katsuura River averaged 1.59±0.34 and 2.31±0.92, respectively. This means that all specimens in the site A resided in freshwater (river eels) throughout their lives, whereas all specimens from the site B distributed in rather brackish water. All specimens from Kii Channel (site C) were classified into three migratory types. Mean and standard deviation (SD) of otolith Sr:Ca ratio outside the elver mark in specimen classified into the sea, estuarine and river eels were 7.20±0.59, 4.63±1.21, and 1.32±0.30, respectively (Table 6.1).

Figure 6.2. Typical patterns in the otolith Sr:Ca ratio along line transects from the core (0 μm) to the edges (left) and the images of Sr concentration in the otolith frontal plane of the Japanese eel (right) classified into three migratory categories: river eel (a) estuarine eel (b), and sea eel (c).

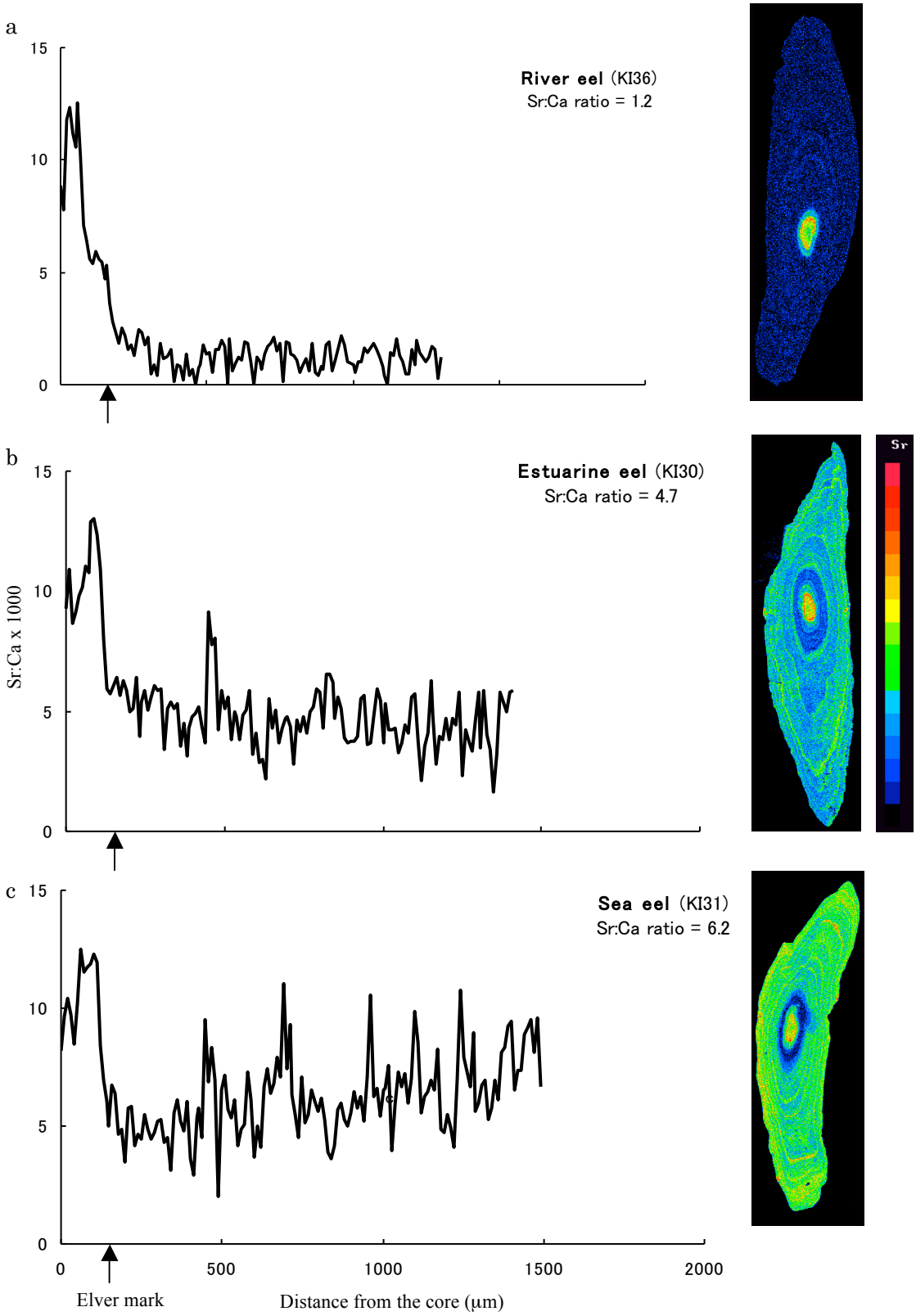


Table 6.1. Specimens (*Anguilla japonica*) used for trace metal and otolith microchemistry analysis. GSI (gonad-somatic index), BW (body weight), TL (total length)

Sites	Migration type	Specimens (n)	Growth stage	TL (mm)	BW (g)	Age (year)	GSI	Mean Sr:Ca×1000
A	Total	5	Yellow	544.6±54.3	226.5±70.5	8.6±0.9	0.36±0.08	1.59±0.34
	Sea eels	0						
	Estuarine eels	0						
	River eels	5	Yellow	544.6±54.3	226.5±70.5	8.6±0.9	0.36±0.08	1.59±0.34
B	Total	6	Yellow	522.0±23.9	208.2.0±39.2	8.2±1.0	0.36±0.18	2.31±0.92
	Sea eels	0						
	Estuarine eels	0						
	River eels	6	Yellow	522.0±23.9	208.2.0±39.2	8.2±1.0	0.36±0.18	2.31±0.92
C	Total	18	Silver	666.8±85.3	463.1±202.0	8.3±1.5	2.70±0.15	4.85±2.60
	Sea eels	8	Silver	701.9±84.4	538.1±252.1	8.4±1.1	2.78±0.54	7.20±0.59
	Estuarine eels	5	Silver	637.6±50.4	404.5±107.2	7.6±0.9	2.53±0.45	4.63±1.21
	River eels	5	Silver	640.0±107.0	401.5±174.4	8.8±2.5	2.77±0.45	1.32±0.30

6.3.2. Physical characteristics

Data of 29 eels from the sites A, B, and C are shown in Table 6.1. There was a significant difference in TL between sites B and C, but no significant difference was found in TL between sites A and C or between sites A and B. There were significant differences in BW between sites B and C and between sites A and C, but no significant difference was found in BW between A and B (Tukey HSD test for unequal N, $p<0.05$). These significant differences can be explained by the differences in the growth stages of fishes between the sites. The fishes collected from site C were in the silver stage with a GSI index of 2.70±0.15, whereas all specimens from sites A and B were in the yellow stage with GSIs of 0.36±0.08 and 0.36±0.18, respectively. None significant difference in GSI between the migratory patterns of the silver eels was found, with the highest GSI found in the sea eel, 2.78±0.54, followed by the river eel, 2.77±0.45, and the estuarine eel, 2.53±0.45 (Tukey HSD test for unequal N, $p<0.05$). Age determination showed that there were no significant differences among the three sites or among the migration patterns at site C. The ages (mean ± DS) of eel specimens from sites A, B, and C were 8.6 ± 0.9, 8.2 ± 1.0 and 8.3± 1.5, respectively.

6.3.3. Metal accumulation in liver tissues between growth stages

The means and SD of the concentrations of nine elements in the livers of eels are shown in Table 6.2. The results indicated that the concentrations of V, Mn, Cu, Zn, and Pb in the silver eels were significantly higher than those in the yellow eels (Tukey HSD test for unequal N, $p<0.05$ with BW and TL as covariates). The concentrations of Zn and Pb in the silver eels were found to be 2 times higher than those in the yellow eels; the levels of Cu and V in the silver eels were from 3.5 to 5 times higher than those in the yellow eels; the Mn levels in the silver eels were 10 times higher than those in the yellow eels; the Cr levels in the silver eels were 2.3 times lower than those in the yellow eels (Tukey HSD test for unequal N, $p<0.05$ with BW and TL as covariates). The levels of Co, Cd, and Hg in silver eels slightly higher than those in yellow eels but there were not significant differences between these stages (Tukey HSD test for unequal N, $p<0.05$ with BW and TL as

covariates).

6.3.4. Differences in trace metal levels among sites and migratory patterns

Obvious differences in levels of trace metals and body sizes were found between the yellow and silver eels and between silver eels with different migratory patterns at site C. Therefore, a comparison of metal accumulation was performed between sites A and B and among different migratory patterns of the silver eels at site C.

The mean ± SD concentrations of site A and B are shown in Table 6.3. Almost all metal levels of sites A and B in the yellow stage were similar, except for those of Zn and Cd. The concentrations of Zn and Cd at site B (166.03 ± 21.57 μg/g and 0.684 ± 0.300 μg/g, respectively) were significantly higher than those at site A (122.25 ± 23.06 μg/g and 0.323 ± 0.148 μg/g, respectively) (Tukey HSD test for unequal N, $p<0.05$).

Comparisons of the metal accumulation among 3 migratory patterns of the silver eels in site C showed that the V concentration in sea eels was significantly higher than that in river eels, but there were no significant differences in V levels between sea and estuarine eels, and between the estuarine and river eels (Tukey HSD test for unequal N, $p<0.05$). The Pb levels were found to be highest in the sea eels compared to river and estuarine eels; however, there was no significant difference between the river and estuarine eels (Tukey HSD test for unequal N, $p<0.05$). Though not statistically significant, the concentrations of Co, Mn, Cu, Zn, Cd and Hg in sea eels were higher than those in estuarine and river eels, whereas Cr was higher in river eels compared with sea and estuarine eels (Table 6.4 and Figure 6.3).

Table 6.2. Mean concentration and standard deviation (μg/g) of nine elements in liver of yellow and silver eels

Elements	Metal concentration (μg/g dry wt.)		
	Yellow eel	Silver eel	
V	0.211 ± 0.103	1.078 ± 1.155	*
Cr	0.656 ± 0.250	0.288 ± 0.091	
Co	0.373 ± 0.177	0.261 ± 0.167	
Mn	1.270 ± 1.876	15.238 ± 4.492	*
Cu	39.59 ± 17.72	139.59 ± 61.89	*
Zn	143.95 ± 29.62	374.92 ± 77.02	*
Cd	0.520 ± 0.299	0.889 ± 0.908	
Pb	0.311 ± 0.260	0.730 ± 0.496	*
Hg	0.928 ± 0.280	0.844 ± 0.189	

Note: (*) significant diffirence (α <0.05)

Table 6.3. Metal levels (μg/g dry wt.) in liver of Japanese eel *A. japonica* between site A and B in Katsuura River

Elements	Mean concentration ± SD		
	Site A (n=5)	Site B (n=6)	
V	0.273 ± 0.054	0.160 ± 0.109	
Cr	0.540 ± 0.299	0.753 ± 0.170	
Co	0.482 ± 0.195	0.282 ± 0.102	
Mn	1.991 ± 3.601	1.337 ± 2.097	
Cu	32.54 ± 7.35	45.46 ± 22.22	
Zn	122.25 ± 23.06	162.03 ± 21.57	*
Cd	0.323 ± 0.148	0.684 ± 0.300	*
Pb	0.248 ± 0.174	0.363 ± 0.322	
Hg	0.957 ± 0.369	0.952 ± 0.213	

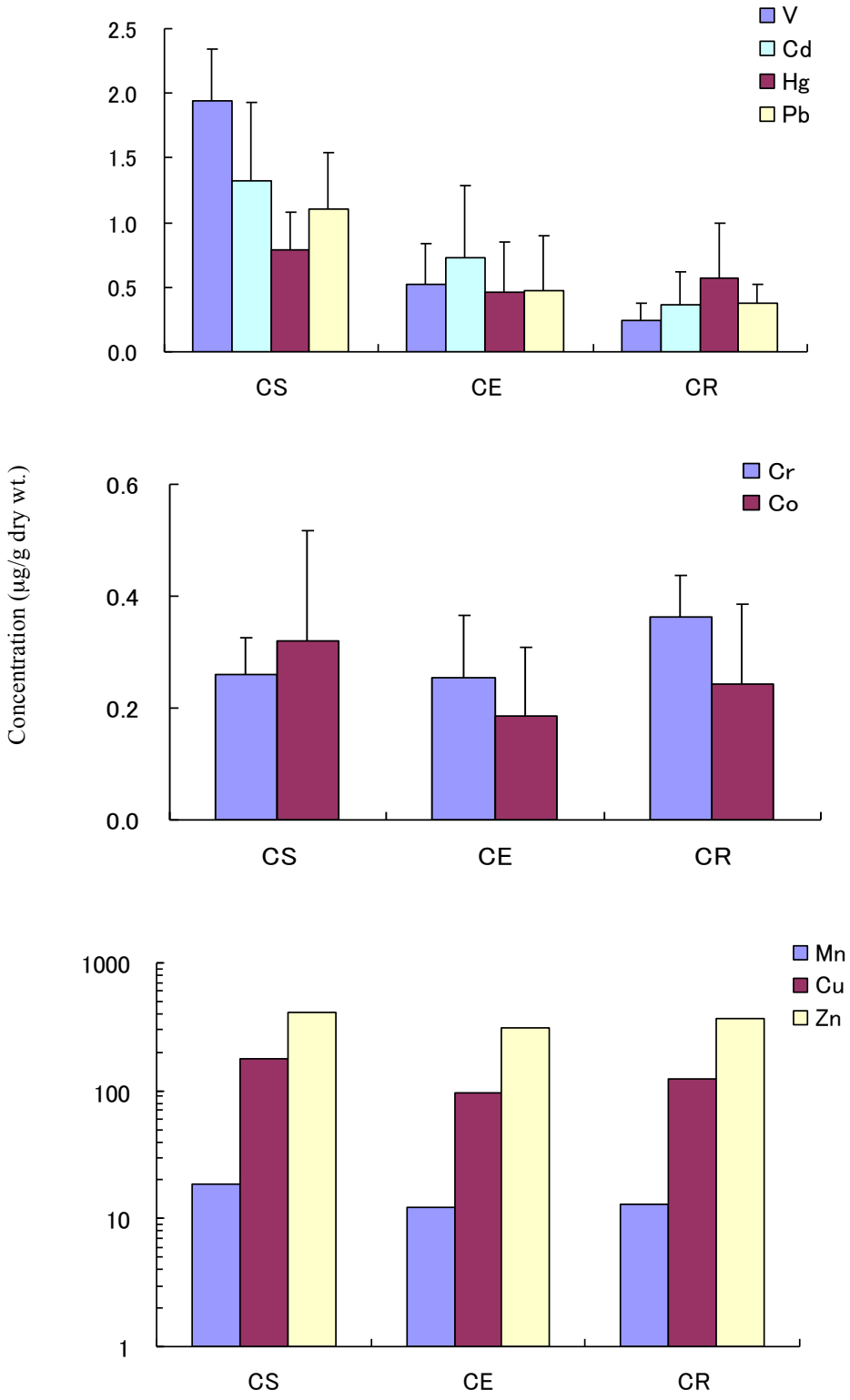
Note: (*) significant diffirence (α <0.05)

Table 6.4. Metal levels (μg/g dry wt.) in liver of Japanese eel *A. japonica* among migratory patterns

Elements	Mean concentrations ± SD		
	Sea eels (n=8)	Estuarine eels (n=5)	River eels (n=5)
V	1.948 ± 1.261 *	0.519 ± 0.318	0.246 ± 0.127
Cr	0.261 ± 0.065	0.255 ± 0.111	0.363 ± 0.074
Co	0.320 ± 0.198	0.187 ± 0.121	0.242 ±0.143
Mn	18.396 ± 4.702	12.368 ± 2.563	13.056 ± 2.079
Cu	175.46 ± 61.10	96.82 ± 33.30	124.98 ± 59.24
Zn	415.85 ± 60.90	312.78 ± 42.44	371.56 ± 93.43
Cd	1.322 ± 1.160	0.724 ± 0.567	0.359 ± 0.256
Pb	1.105 ± 0.436 *	0.477 ± 0.426	0.382 ± 0.135
Hg	0.943 ± 0.147	0.835 ± 0.221	0.715 ± 0.235

Note: (*) significant diffirence (α <0.05)

Figure 6.3. Difference of metal levels (μg/g dry wt.) in liver of Japanese eel *A. japonica* among migratory patterns



Note: CS – sea eel; CE – Estuarine eel; CR – River eel.

6.3.3. Relationship between trace metals and physical characteristics

In sites A and B, the Cd concentrations positively correlated with the Sr:Ca ratio (r = 0.67) (Figure 6.4 a), and a positive relationships was found between GSI and Cu levels (r = 0.78) (Figure 6.3 b). However, no significant relationships were found between the other metal levels and TL, BW or age.

Cu and Zn levels of sites C had positive correlations with TL (r= 55 and r = 60, respectively), BW (r= 55 and r = 58, respectively) and age (r= 0.63 and r = 57, respectively) (Figure 6.5 a, b, c, d, e, f). Weak positive correlations between Hg and age (r=0.40), and between Cd and Sr:Ca ratio were found (r=0.4). Moreover, V and Pb showed positive relationships with Sr:Ca ratios (r = 0.81 and r = 0.68, respectively), whereas there was negative relationship between Cr levels and Sr:Ca ratios (Figure 6.6 a, b, c). No correlations were found between the studied metals and GSI.

Alternatively, the correlations between trace metals and biological characteristics of each migratory type in site C were distinctive. In order to examine the relationship between metal accumulation and growth stage of Japanese eels, the estuarine resident eels of site C and the eels of site B (estuarine eels) were pooled. Interestingly, the levels of trace metals such as Mn, Cu and Zn showed positive correlations with TL, BW and GSI. Moreover, level of V showed positive correlations with Sr:Ca ratios, TL and BW, whereas Hg levels negatively correlated with Sr:Ca ratios (Table 6.5).

Figure 6.4. Relationship between trace metals and physical characteristics from sites A and B in the Katsuura River.

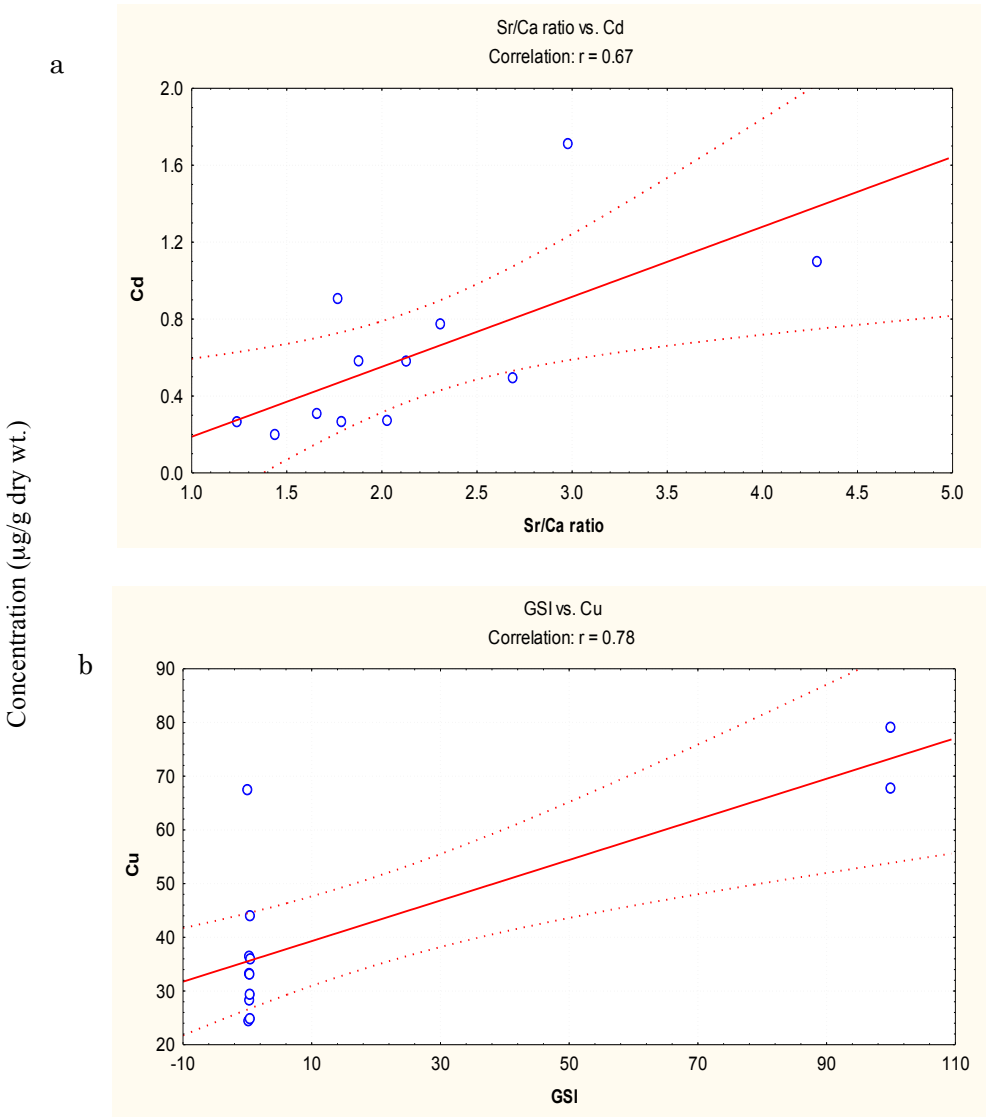
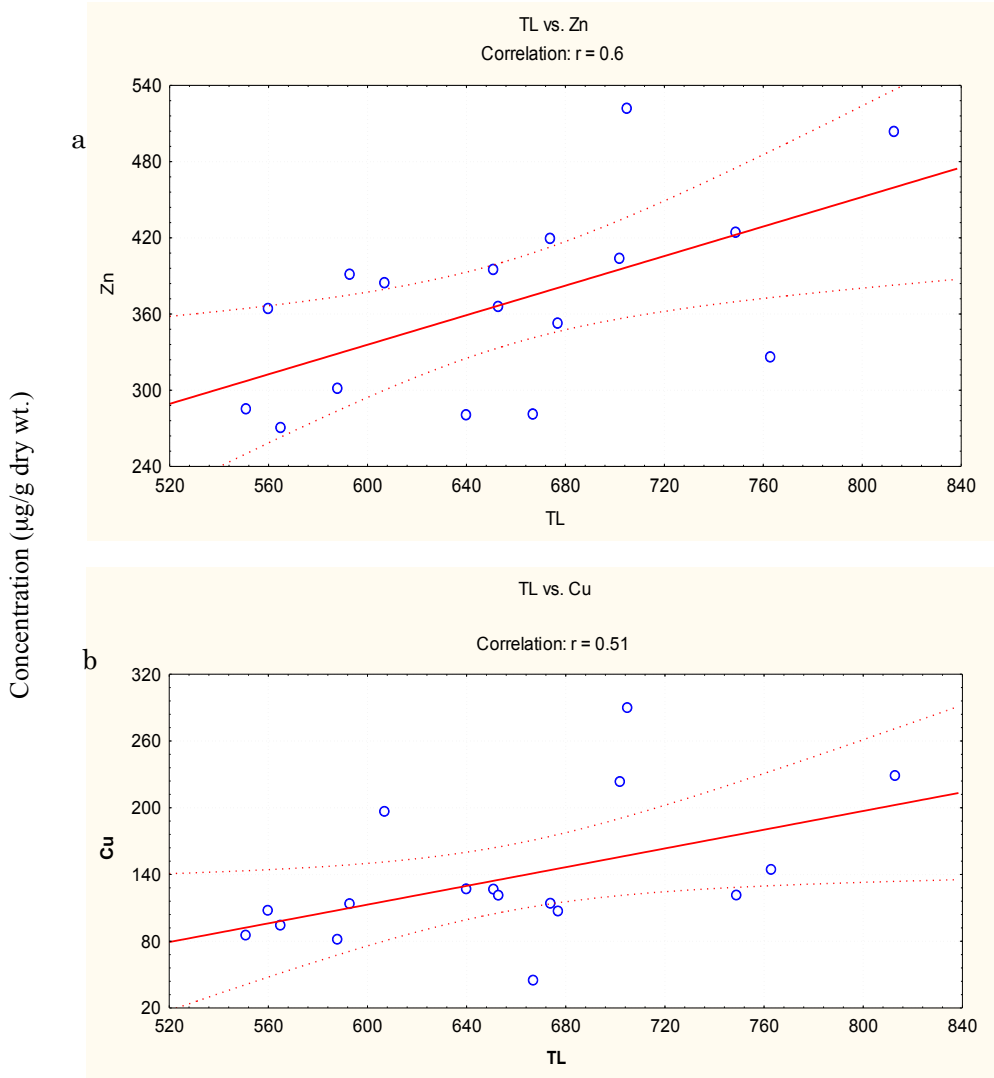
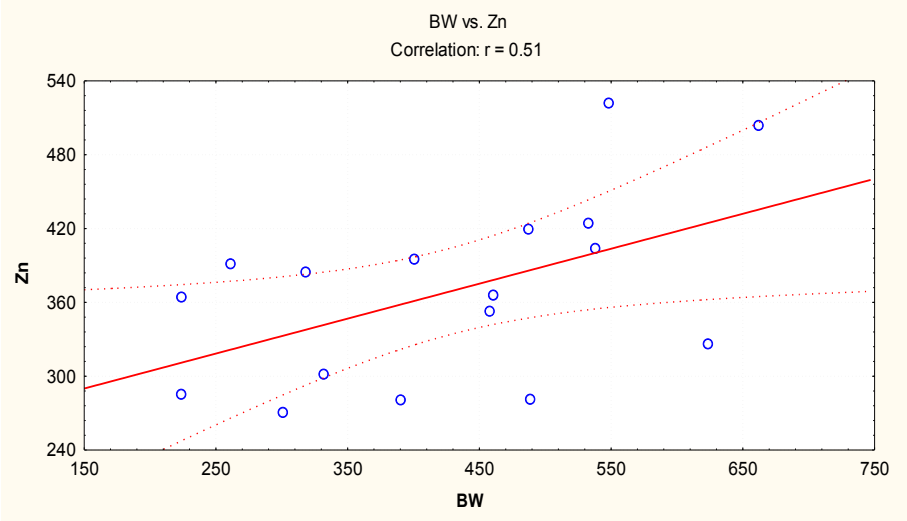


Figure 6.5. Relationship between Cu and Zn with total length (TL), body weight (BW) and age from site C

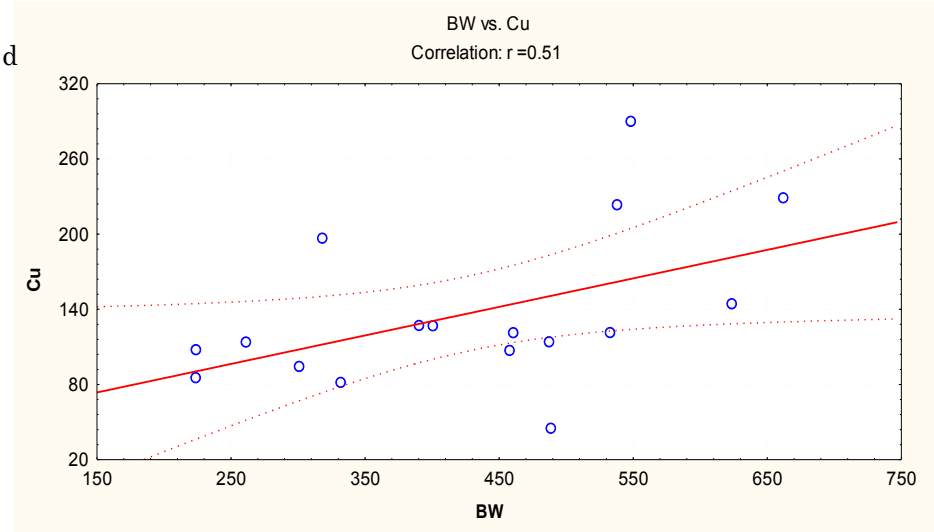


Concentration (µg/g dry wt.)

c

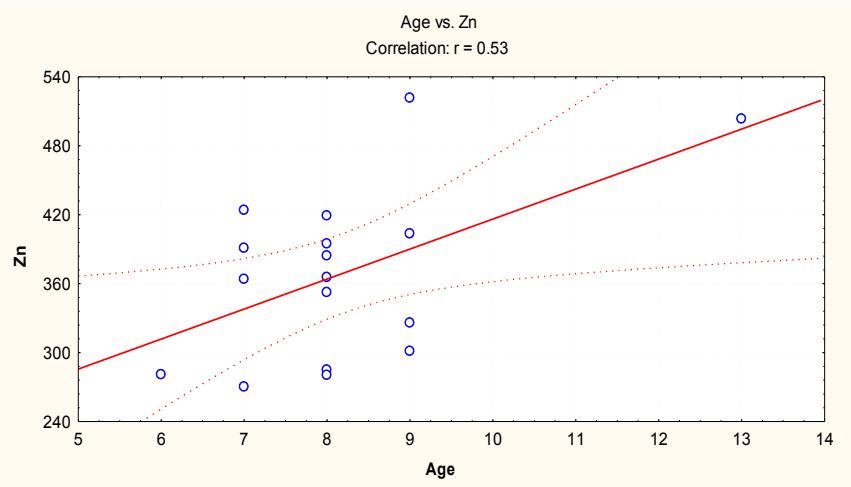


d



Concentration (µg/g dry wt.)

e



f

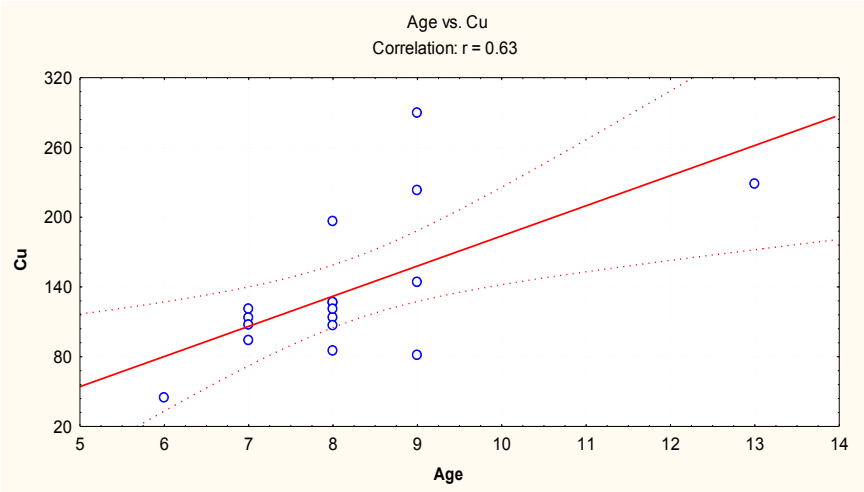


Figure 6.6. Relationship between Cr, V and Pb with Sr:Ca ratios from site C

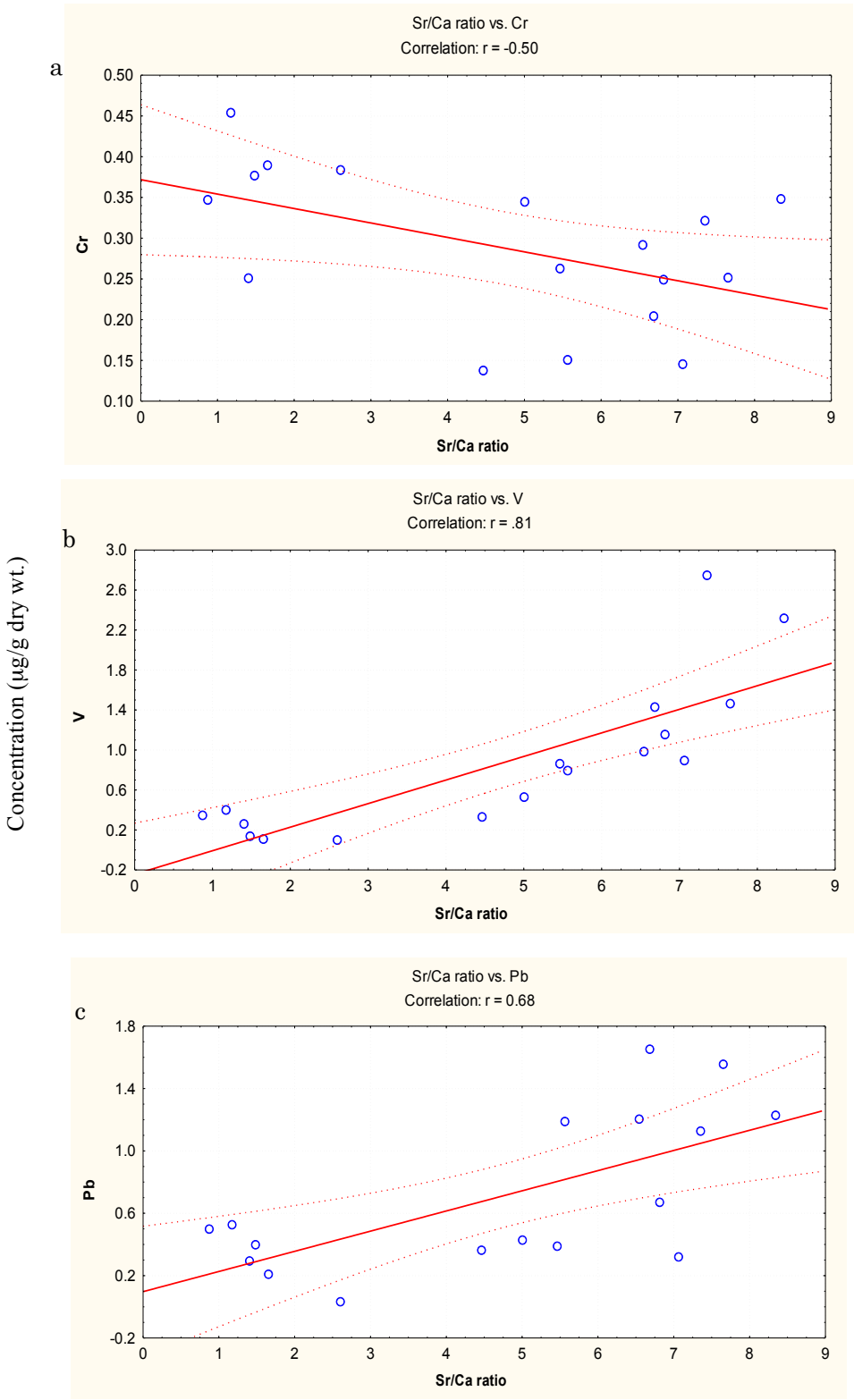


Table 6.5. Relationship between trace metal levels in pooled data of estuarine eels (sites B and C) and physical characteristics

Elements	TL	BW	Age	GSI	Sr/Ca ratio
V	0.64	0.65	-0.38	0.46	0.81
Cr	-0.46	-0.48	0.34	-0.40	-0.44
Co	-0.49	-0.49	0.33	0.05	-0.60
Mn	0.87	0.86	-0.28	0.70	0.52
Cu	0.64	0.60	0.14	0.66	0.42
Zn	0.84	0.81	-0.25	0.79	0.42
Cd	-0.33	-0.46	0.52	0.37	0.22
Pb	0.39	0.44	-0.47	0.18	0.49
Hg	-0.53	-0.47	0.34	-0.45	-0.85

Note: Red numbers are significant relationships between metal concentration and physical characteristics

6.4. Discussion

6.4.1. Metal accumulation and migratory patterns

Recently, fish has been widely used as potential bioindicators to assess environmental quality and risk for human health, because fish uptake trace metals from polluted environment and bioaccumulate in their tissues. Furthermore, they are important protein sources for human consumption (Farkas et al., 2000; Burger et al., 2002; Burger and Gochfeld, 2005; Agusa et al 2005, 2007; Has-Schon et al., 2008). The level of metal uptake in fish varied depending on the status of metal pollution in the environment where they reside (Linder et al., 1996; Vincente-Martorell et al., 2009). However, not all fish species are useful bioindicator for all trace metals, and the species which regulate the levels of any metal in their body or limited distribution, should be rejected for such studies. Temperate anguillid eels have been used as bioindicator for trace metals in aquatic system for decades, especially in Europe and United States because of their wide distribution (Linde et al., 1996; Edward et al. 1999; Langston et al. 2002; Usero et al. 2003; Maes et al., 2007). Many studies have been revealed that some temperate eels, *A. anguilla* and *A. japonica*, can spend their entire lives in brackish- and sea-water environment by examining the Sr:Ca ratios in their otolith (Tsukamoto and Arai, 2001; Tzeng et al., 2002; Arai et al., 2003). In the present study, the Sr:Ca ratio in each otolith of Japanese eel was examined before the comparison of metal accumulations in eels' liver was executed. The study showed that the difference of metal accumulation in the eels related to migratory types. Almost all metals in the silver sea eels were slightly higher than in other migratory types; in particular, V and Pb in the silver sea eels were at higher levels than those in silver river eels. This might relate to the reserve of various anthropogenic sources including trace metals in coastal areas where the rivers bring industrial and domestic waste from human activities. Although the silver eels were collected in Kii Channel, the silver eels might grow in some coast of Seto Inland Sea where is well known to be a trace metal pollution area (Hoshika et al., 1991). This can explain the higher levels of trace metals, such as Co,

Mn, Cu, Zn, and Cd, in silver sea eels than those in silver estuarine eels or silver river eels from site C. In case of Pb, Pb is a nonessential and poisonous, which damages nerve connections and causes blood and brain disorders. In addition, lead compounds are not decomposed and are accumulated locally in biological organisms (Merian, 1991). Due to this, the eel has been widely recognized as a bioindicator of pollution; the metal concentrations in the liver of a resident eel can reflect the status of the local environment (Bird et al., 2008; Batty et al., 1996; Barak and Mason, 1990). Therefore, the elevated Pb concentration in the liver of silver sea eels might relate to the Pb sources that derived from local activities along the coast in the study area such as marine ports and lead-acid battery production. In contrast to Pb, the elevation of V in silver sea eels and silver estuarine eels compared to silver river eels may relate to V uptake at different habitat use (Bell et al., 1981). When soluble vanadium in rivers reaches the sea, much of it is precipitated, and the marine mud becomes enriched with vanadium rather than freshwater sediment (National Academy of Science, 1974). Anguillid eels are known to inhabit mud or weed beds and they feed on benthic invertebrates and other aquatic fauna within their habitats (Tesch, 1977). Vanadium, however, is not a common pollutant except in the case of pentavalent vanadium (Roshchin, 1967) and its compounds can be slowly excreted from the European eel *Anguilla anguilla* under experimental conditions (Bell et al, 1981), although it has been shown to be nutritionally essential for rats and chicks (Merian, 1991).

In the sites of the Katsuura River, based on the otolith Sr:Ca ratio indexes of Tsukamoto and Arai (2001), the eels collected from sites A are river resident eels because the otolith Sr:Ca ratio below 2.5×10^{-3}. However the higher of Sr:Ca ratio values of eels from site B indicates that their habitat use, 1-2 km from river mouth, were influenced by the sea water in some degree. The concentrations of Zn and Cd in the eels from the site B were found to be higher levels than those in the eels from site A, and a positive correlation between Cd and the Sr:Ca ratio was observed. This means that the trend of Cd increases from the upper to lower reaches of the Katsuura River. Additionally, the increased Zn levels correlated with the increase in Cd levels of eels from sites A

and B, likely relating to the detoxicity mechanism in the liver, because the relationship between these metals were found in some studies (Noël-Lambot et al., 1977; Kargin and Cogun, 1999). Thus, the estuarine eels can face rather potential risk of Cd than river eels because Cd is very toxic metal and it acts as a strong endocrine disrupter for eels by stimulating the hypothalamus-pituitary-gonad axis. Furthermore Cd could also cause the exhaustion of female silver eels when they migrate to spawning area (Pierron et al., 2008). However the Cd levels in the eels from studied sites may not threaten because these levels are still lower than the threshold or adverse effect levels as reported by Pierron et al. (2008).

In the case of Hg, there were no significant differences among migratory types, indicating that eels were evenly exposed to mercury contamination in all parts of the study sites. The levels of Hg in Japanese eels in this study were higher than those recorded in range of 0.057 – 0.501µg/g dry wt. and 0.153 – 0.312 µg/g dry wt. in muscle and liver of European eels *A. anguilla* from Yare River and Thames River, respectively (Edward et al. 1999; Yamaguchi et al., 2003). Furthermore, the Hg levels in the eels of this study were also higher than those in tropical eels *A. bicolor pacifica* from the Ba River, but to be lower than those in *A. marmorata* (Chapter 3, Chapter 5). The high level of Hg accumulation might relate to the metal body burden due to the energy-storing phase of the eels. Anguillid eels were known as effective accumulators of Hg contaminants due to the fact that they are susceptible to biomagnification and bioaccumulation (Edwards et al., 1999). Therefore, anguillid eels often uptake much Hg level rather than the other species collected from the same habitat (Yamaguchi et al., 2003). While the most toxic form of Hg was methylmercury (MeHg), and nearly all (95–99%) Hg in fish flesh is MeHg (Chapter 3; Bloom, 1992; Grieb et al., 1990), the liver was the main target for inorganic mercury, and this form is easily excreted. Moreover, the levels of Hg in the eel of this study were lower than the adverse effect levels reported by Friedmann et al. (2002). Therefore, the eels might not be seriously affected by the Hg levels in the study sites.

6.4.2. Metal accumulation and growth stage

The trace metals, such as Mn, Cu, and Zn, showed high levels in eels' livers because livers were target organ for metal accumulation to execute their functions (Chapter 3). The higher levels of these trace metal uptakes in the silver eels than those in yellow eels might relate to anthropogenic sources, because these silver eels collected in Kii channel, but they could grow up from some coasts in Seto Inland Sea with regard to silver sea and estuarine eels. While Kii Channel connects with Osaka Bay where is one of the most industrialized areas in Japan, and its marine environment has been significantly affected by the anthropogenic impacts over the last four decades (Hoshika et al., 1991; Takeoka, 2002). Otherwise, Mn, Cu and Zn are considered as the essential metals and they play important functions in biological mechanism in organisms. The higher concentrations of these metals in silver eels compared to yellow eels, therefore, might even relate to biological metabolism. Once the maturing fishes start developing their gonads, the metabolic processes in the livers usually enhance due to large blood supply to proteins. However, the details of such mechanisms in fish are little known. Alternatively, the liver is known to play important roles in detoxification to protect against toxic metals, such as Cd, Pb and Hg (Langston et al., 2002). The increased levels of non-essential metals such as Cd and Pb may increase the requirements for Mn, Cu and Zn in the liver (Hidalgo et al., 1986; Weber, 1993; Brusle, 1990). The increased production of the detoxification enzymes against toxic metals in the liver may cause decreased fecundity of maturing fish due to the reduced capacity of the liver to manufacture yolk proteins (Lawrence and Hemingway, 2003; Adams et al. 1992a,b). However, the levels of toxic metals in the eels were lower than effect threshold levels as discussed above. Therefore, the trace metal levels in livers of maturing eels may not threaten to the physiological processes during gonadal maturation of eels.

6.4.3. Relationship between metal accumulations and physical characteristics

Not all trace metal levels showed the correlation with Sr:Ca ratios of otolith, except for V, Cd and Pb. The correlations of these metals coincide with the higher levels in the sea eels than those in

river eels. This suggests that the accumulation of these metals increase with increasing Sr:Ca ratios. Therefore, it is seemed that the sea eels are higher risk of these trace metals than the other migratory types of eels. In estuarine eels, no significant differences were observed in any metal levels compared to sea and river eels. Therefore the uptake of these metals in estuarine eels might be considered to be at an intermediate level between those of sea and river eels. While estuarine eels are thought to move between freshwater and marine habitats during their yellow phase, their trace metal exposure varies in relation to their habitat use. Although Hg levels was no significant difference among migratory types and sites, the negative relationship between Hg level and otolith Sr:Ca ratio exposed that the Hg sources originated from freshwater bodies. This suggests that the river and estuarine eels might be the higher potential risk of Hg than the sea eels.

Like *A. marmorata* and *A. bicolor pacifica* from the Ba River (Chapter 5), the relationships between metal levels and physical characteristic of *A. japonica* were found, especially the levels of essential metals such as Mn, Cu and Zn, strongly correlated with GSI and body size. This indicated that these metals play some important roles of biological mechanisms in eels' liver during maturation (Chapter 3).

In short, the present study suggests that otolith analysis is necessary together with pollution analyses when using anguillid eels as bioindicators.

Chapter 7

General discussion

7.1. Can tropical eel be used as biomonitor for environmental pollution in Vietnam?

During last decades, Vietnam has built up environmental monitoring programme for specific contaminants. The programme has been conducted along coastal area since 1992, however the contaminants were merely measured in abiotic compartments such as water and sediment and monitoring area was limited in marine water while freshwater or brackish water was disregard. In fact, it is clear that a lot of measurements of trace metals in water fall below the detection limit (Agtas et al., 2007; Belpaire et al., 2008), or very high levels in sediment but they originate from local geological traits instead of anthropogenic sources such as Mn or Pb (Tran et al., 2001; Duzgoren-Aydin and Aydin, 2009; Chapter 2). In contrast, concentrations of metals in eels are always measurable and attain higher values than in sediment such as Hg, Cd (Chapter 2 and 3). Additionally, combining the results in the chapter 2 and 4, the trace metal profile of sediment and the eel collected in the different rivers (Thach Han River in QT and Ba River in PY) indicated that the metal levels in eels' tissues can reveal the status of metal pollution pressure in the specific locations, especially liver. For instance, hepatic Cd, Pb and Zn residues in the eels from the Thach Han River (QT) were higher than those levels in the ones from the Ba River (PY), this related to the elevated levels of these metals derived from anthropogenic sources in sediment from Thach Han River as explained in Chapter 2. Furthermore, the metal body residues in specific organs such as liver in anguillid eels increase with increasing exposure concentrations in laboratory (Usero et al., 2003; Karayakar et al., 2010). Otherwise, metal accumulation in the eels even reflect the bioavailability of contaminants from environment by uptake pathways (Dallinger et al., 1987; Brusle 1990; Belpaire et al., 2008) or result the biomagnification of contaminants through food chain (Gray, 2002). Thus, anguilid eel was chosen as suitable bioindicator to examine the health of

environment in European. For instance, in some countries like The Netherlands and Belgium, a nationwise monitoring network is operational (Maes et al., 2008; Belpaire and Goemans 2007) or other countries like Sweden, Finland, Denmark, German, United Kingdom, France, Spain and Italia, eel biomonitoring studies have been undertaken on a local scale (Batty et al. 1995; Van der Oost et al., 1996; Langston et al., 2002; Yamaguchi et al., 2003; Usero et al., 2003; Oliveira et al., 2005; Belpaire and Goemans 2007; Bird et al., 2008). Anguillid eels were also used as biomonitors in other countries such as United States (Brusle 1991; Hodson et al. 1994; Ashley et al., 2007), Australia or New Zealand (Beumer et al., 1982; Redmayne et al., 2000). While most anguillid eels widely distribute in tropical region (Tesch, 2003) and there is limited study on using tropical eels as biomonitor to assess environmental quality in Vietnam as well as tropical countries. Even these species meet the characters of indicator species such as size, long life span, fat content, feeding and habitat ecology, wide distribution, one reproductive cycle (Belpaire and Goemans 2007; Belpaire et al. 2008). Thus, the finding in this study partly provides the helpful information of using eel species as bioindicator to assess water system quality in Vietnam.

7.2. Metal accumulation in the eel related to migratory patterns

Metal accumulations in eel varied depending on the migratory patterns. Due to the eels, *A. marmorata* and *A. bicolor pacifica*, were collected in the rivers or lower part of river (Chapter 3, 4, 5) and they dominates in the middle to upper reaches of the river or middle to lower reaches of river, respectively. Therefore, the migratory history of the eel in the study area might mainly reflect the freshwater- or brackish-water- resident. Knowledge of these tropical anguillid eels' life history is still limited. Shiao et al., 2003 reported that *A. marmorata* tended to reside in freshwater and seemed to avoid seawater during the yellow eel, while Chino and Arai, 2010 supposed that none of *A. bicolor pacifica* had a general life history as a freshwater resident. By contrast, *A. japonica* was revealed the flexible migration strategies and an ability to adapt to various habitats and salinities by determined the

mean of Sr:Ca ratios in the otolithes (Arai et al. 2003; 2006; Tzeng et al. 2003). Furthermore, eels are fairly sedentary, they live in small home range until migrating downstream at maturing silver stage (Laffaille et al., 2005). This is an advantage for evaluating metal accumulations in the same eel species at various habitats (fresh, brackish or sea water) in order to elucidate the ecological risk. Hence, *A. japonica* was used as bioindicator to assess the ecological risk of TBT in previous study (Ohji et al. 2006), they suggested that the levels of organotin compound (OT) accumulated in the eel depended on the migratory types and maturation stages. However, migratory type was a more important factor for OT accumulation than maturation stage. In this study, thus, the eel *A. japonica* was used to examine whether levels of trace metals in relation to ecological migratory types and maturity stages (Chapter 5). The study was in agreement with Ohji et al. (2006) to indicate that sea eels showed a higher risk of pollutants than other migratory types of eels (estuarine and river eels) at the same maturity stage. This might reflect the status of different water bodies in the location where the eels live. The metal contaminant levels in eel can, therefore, pinpoint local sources of pollution. However, unlike OT accumulations between maturity stages, the elevated levels of essential metals, Mn, Cu, and Zn, in mature eels compared to those in immature eels related to maturity development rather than influenced by metal pollution in the ambient environment (Chapter 5). The study suggested that immature eel might be better valuable bioindicator for trace metals such as Mn, Cu and Zn than maturing silver eel; however, when using the maturing silver eel to understand the ecological risk caused by pollutants in aquatic systems, life history analysis should be carried out for accurate interpretation of the results.

7.3. Risk assessment for eel populations and human consumption effect

Anguillid eels are one of top predators in aquatic system, they have a unique catadromous life cycle which can potentially lead to exposure of contaminants during all life stage (Brusle, 1990). The highest risks to eels would be expected to arise from long-term chronic exposure of pollutants

from water, sediments and diet in agricultural and/or industrialized catchments, estuaries and enclosed seas (Knight, 1997). During long biological energy reserving phase (yellow stage – few years to decades depended on species and hydrological condition), they uptake large quantities of persistent contaminants. Consequently, eels often potentially accumulate higher pollutants than other aquatic organisms in their habitat (Yamaguchi et al., 2003; Usero et al., 2003). In addition, result of bioaccumulation in eels even is relevant to maturity stage and body size, the silver maturing eels accumulate much higher persistent contaminant levels than yellow eels, especially Hg or Cd, even in less contaminated areas (Chapter 3 and 5). Therefore, the silver eels may pose higher ecological risk than yellow eels in polluted areas. The potential impacts of stored pollutants in eels can cause triggering toxic events when silver eels fast to migrate to spawning areas (Pierron et al. 2008; Robinet and Feunteun, 2002). Furthermore, many studies have shown numerous adverse effects of trace metals exposure on fish, including on growth, development, hematology, appetite, and behaviors (Verma and Tonk, 1982; Kirubagaran and Joy, 1995; Lawrence and Hemingway, 2003; Fiedmann et al, 2003; Raldua et al., 2007). In the present study, the metal accumulation in eels are at lower level when comparing with the threshold levels of other studies (Friedmann et al., 2002; Pierron et al., 2007; 2008), this may not relate to the decline of eel population in Vietnam. However, the elevated toxic metals in the eel from Quang Tri can be potential ecological risk for eel population in future. Hence, the use of eels in temporal and spatial biomonitoring and biomarker studies is important and necessary for risk assessment to minimize the adverse effects of anthropogenic pollutants to eel populations.

Like other countries, eels (fresh-cooked or smoked) are favorite dishes in Vietnam and they are important sources of protein and income for local people. However, the increasing environmental contamination can lead the effects on the eel quality and cause potential risk for human consumption. Although number of eel specimens accumulated Hg level in muscle, especially silver eels, exceed limits of the recommended level (USEPA, 2008) (Chapter 3), this raises concerns about human health effects by fish consumption in future. Because several health effects linked to

contaminated fish consumption was aware in the past such as Itai-Itai or Minamata disease (Tsuchiya et al. 1969). Dietary fish intake is its content of high trace metal levels such as Cd, Pb or As have been also associated to serious health effects on adult and children (Salonen et al., 2000; Castro-González and Méndez-Armenta, 2008). Therefore, critical environmental and body residue levels to protect eels and humans consumption must be formulated and interpreted with caution. Furthermore, using the daily intake dose estimation to assess human health risk by eel consumption can apply to investigate the other seafood along coast of Vietnam. Based upon contaminants in fish or seafood profile, the setting limits on food residues are able to build up and this is obviously a good precautionary approach to risk management in Vietnam.

Chapter 8

Conclusions and Recommendations

Conclusions

This study partly provides the helpful information of the eel species that is good indicator to assess water system quality in Vietnam and risk for human consumption. Additionally, some findings in this study are highlight as below:

- A strict link of metal accumulations between liver and gonad related to the Zn levels in these organs and the elevated Cd burden in gonad suggest that hepatic metals, both essential and nonessential, can be transferred to gonad during gonadal maturation.
- Anguillid eels are vulnerable to pollutants, they can take up large quantity of toxic metals such as Hg and Cd, even in less polluted areas.
- The metal levels in sediment show significant differences between rivers that coincided with the trace metal accumulation in eels.
- Migratory types of anguillid eels can consider as a further useful tool to aid the interpretation of metal pollution in fresh, brackish and coastal waters.
- Monitoring trace metals should be measured in eel liver instead of muscle tissue, as concentration of most metals are higher than in liver tissue. However metal measurements in muscle tissue are also easily detectable and present an added value towards human health risk assessment.
- In relation to potential risk to human consumers, critical residue limits in the eel have been recommended. The toxic metals in the study sites might not seriously affect yellow stage of eels. However small number of the maturing silver eel specimens contained the high levels of Hg in muscle or Cd in liver could pose a potential risk for eel population and human

consumption in the study site.

Recommendations

The anguillid eels are one of the most important commercial fish in Vietnam. However, the weakness of management in local authority has led the eels' habitat loosing and over-exploitation of the eel sources for recent years. The environmental quality has been decreased caused by urbanization and industrialization in the areas in recent, especially in Quang Tri province. These could be factors causing the decline of eel population in the region.

Although this study could not suggest the main reason caused the decline of eel population by trace metals, a number of organic persistent contaminants might have the possibility such as such as polychlorinated biphenyls (PCBs), organochlorine compounds (OCs), tributyltins (TBTs), because these contaminants may cause lipid storage and reproductive problems in anguillid eels when the eels migrate a long distance to spawning areas. Therefore, understanding of the concentrations of pollutants in eel species will give useful insights into the reasons of eel decline and explore new clues for elucidating their toxic effects on wildlife and human health. Otherwise, the elevated levels of mercury (mostly methylmercury) in maturing silver eels can be a potential risk to the eel population and human consumption.

Therefore, at regulatory authority levels, it is necessary to establish the sustainable management of anguillid eel sources in the region. It is especially interested in awareness raise for local fishermen in protecting eel habitats and sustainable exploitation of eel sources. Biomonitoring and food residues should be undertaken and applied on wider scale because of environmental problems in the past, present and future will be exposed. This will provide information to assists authorities make good decisions for environmental protection and management.

At the scientific level, there are still many gaps in the knowledge of tropical eel biology as well as adverse effects of pollutants on eel population in the region. Therefore, further study should be simultaneously conducted on the biology (especially at maturing or matured stage of eels),

migration history (lifecycle studies) of tropical anguillid eels and the effects of persistent organic pollutants (DDTs, HCHs, chlordane, PCBs and TBTs) on the eels. Further study should be continued on the interaction of Se and Hg levels in eels, especially in maturing silver eels. Maturing or matured silver stage of eels should be continued studying on bioaccumulation of toxic substances and how pollutants affect severely on fish health and reproductive capacity when the eels migrate to spawning areas. Advanced studies on eel physiological responds to the toxic contaminants should be carried out to elucidate insight the deleterious effects.

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