Vol. 13: 137–147, 2011
doi: 10.3354/ab00358

AQUATIC BIOLOGY
Aquat Biol

Published online August 23

Life history-related organotin body burden in the catadromous eels *Anguilla marmorata* and *A. bicolor pacifica* in Vietnam

Takaomi Arai[1,2,*], Naoko Chino[1], Dung Quang Le[1,3], Hiroya Harino[4]

[1]International Coastal Research Center, Ocean Research Institute, The University of Tokyo, 2-106-1 Akahama, Otsuchi, Iwate 028-1102, Japan

[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[3]Institute of Marine Environment and Resources, 246 Danang, Haiphong, Vietnam

[4]Department of Human Sciences, Kobe College, 4-1 Okadayama, Nishinomiya, Hyogo 662-8505, Japan

ABSTRACT: In order to understand the ecological risks caused by organotin compounds (OTs) in diadromous fish migrating between sea and freshwater, tributyltin (TBT) and triphenyltin (TPT) compounds, and their breakdown products, were determined in the catadromous eels *Anguilla marmorata* and *A. bicolor pacifica*, collected in Vietnam waters. Ontogenic changes in otolith strontium (Sr) and calcium (Ca) concentrations were examined along life history transects in order to determine habitat use in the eel. There were generally no significant correlations between TBT and TPT accumulation and various biological characteristics such as total length (TL) and body weight (BW). In *A. bicolor pacifica*, TBT and the total butyltin (BT) concentrations of yellow-stage eels (immature eels) were significantly higher than those in silver-stage eels (mature eels). This suggests that yellow-stage eels have a higher risk of contamination by TBT than silver-stage individuals. Positive linear relationships were found between Sr:Ca ratios, total BTs and total phenyltins. These results suggest that the ecological risk of OTs in these eels increases with increasing sea residence period. Thus, migratory history and maturation stage are the most important factor for OT accumulation in catadromous eels.

KEY WORDS: Tributyltin · Triphenyltin · Catadromous eel · Ecological risk · Habitat use · Migration

INTRODUCTION

Organotins (OTs) are used in a variety of consumer and industrial products such as marine antifouling paints, agricultural pesticides, preservatives, and plastic stabilizers. In particular, butyltins (BTs) and phenyltins (PTs) have in the past been extensively used in boat paints because of their excellent and long-lasting antifouling properties. Tributyltin (TBT) and triphenyltin (TPT) are responsible for many deleterious effects on non-target aquatic life (Fent & Meier 1994, Grzyb et al. 2003, Ohji et al. 2006). Despite recent regulation of their use in antifouling paints, high concentrations of TBT and TPT are still detected in the aquatic ecosystem (Arai & Harino 2009). OTs accumulate in marine, freshwater, diadromous and even in deep sea fishes (Arai 2009).

TBT accumulation in diadromous fish such as salmon and freshwater eel was significantly different between their life histories in a species (Ohji et al. 2006, 2010). TBT concentrations in sea-run brown trout *Salmo trutta* were significantly higher than those in freshwater-residents, and the proportion of TBT in the total BT in anadromous brown trout was significantly higher than that in the freshwater-resident type (Ohji et al. 2010). These results suggest that anadromous *S. trutta* had a higher ecological risk of TBT exposure during their life history than freshwater-resident *S. trutta*, even though both belong to the same species. Therefore, differences in TBT and TPT accumulation be-

*Email: arai@science.upm.edu.my

tween migratory types of other diadromous fish might be similar to that in *S. trutta*. However, there have been few studies to date on the relationship between OT accumulations and different migratory histories in diadromous species.

The catadromous eels *Anguilla marmorata* and *A. bicolor pacifica* are widely distributed in Asian countries including Vietnam. These eels are commercially important for both local consumption and exportation in Vietnam. They perform a spectacular migration between their freshwater and estuarine habitats, and their offshore spawning area. Larvae (leptocephali) drift from the spawning area toward coastal waters, then metamorphose into juveniles (glass eels) and begin their inshore migration. The glass eels start to become pigmented elvers when they enter estuaries, and in general, large numbers of elvers typically migrate up freshwater streams and rivers or enter lakes. After the yellow eel (immature) growth phase, they metamorphose into the silver eel (mature) stage, characterized by mature gonads and enlarged eyes, and move back downstream to the ocean to begin the journey to the spawning area.

Recently, the migratory history of several species of anguillid eels has been studied using microchemical analytical techniques to determine the ratios of strontium to calcium (Sr:Ca) in their otoliths. The Sr:Ca ratio in the otoliths of fishes such as anguillid eels differs depending on the amount of time they spend in freshwater versus seawater (Arai et al. 2004, 2006, Chino & Arai 2010a,b). Thus, the Sr:Ca ratios of otoliths may enable us to determine whether or not individual eels actually move between different habitats with differing salinity regimes. Chino & Arai (2010b) used these Sr:Ca ratios to classify the migratory histories of anguillid eels into 3 migratory types: (1) 'marine residents' (spent most of their life in the sea and did not enter freshwater), (2) 'estuarine residents' (inhabited estuaries or switched between different habitats), and (3) 'freshwater residents' (entered and remained in freshwater river habitats after arrival in the estuary). Therefore, there may be different ecological risks for pollutants including OTs among the 3 migratory types. However, there is little information available on OT accumulation in different life histories of anguillid eels. Such information is important to understand aquatic contamination levels and bioaccumulation using the eels as a biological indicator.

The objective of the present study was to examine differences in the accumulation patterns of BTs including TBT and its derivatives dibutyltin (DBT) and monobutyltin (MBT), and PTs including TPT and its derivatives diphenyltin (DPT) and monophenyltin (MPT), in the muscles of the different migratory types of *Anguilla marmorata* and *A. bicolor pacifica* in Vietnam. The environmental histories of these eels were reconstructed by means of the ontogenic changes in otolith Sr:Ca ratios along the life history transect used previously for determining the details of eel migration. The results of the present study may provide valuable clues for understanding the ecological risk of OTs and its variations according to migration in catadromous fish.

MATERIALS AND METHODS

Fish. The wild eel samples were collected by angling and electrofishing from rivers in 3 provinces in the central part of Vietnam, Quang Tri (QT), Quang Ngai (QN), and Phu Yen (PY; Fig. 1). Eels were collected from a river in QT (n = 10) in September 2007 and from rivers in QN (n = 10) and PY (n = 31) during February and March 2008 (Table 1). A total of 51 specimens were used in the present study. Total length (TL) and body weight (BW) were measured, gonad-somatic index (GSI) was calculated (Table 1), the skin color was noted to help in the categorization of each specimen as either a yellow (immature) or silver (mature) stage, and sex was determined by examining the gonads. In the present study, we only used female eels due to the limited number of male samples. Species identification was conducted based on morphological characteristics, and we were able to distinguish between *Anguilla marmorata* and *A. bicolor pacifica*. All QT and QN eels were *A. marmorata*, whereas PY specimens included 10 *A. marmorata* and 21 *A. bicolor pacifica*. Gonad weight (GW, to 0.01 g) and BW (to 0.1 g) were measured to determine the GSI, which was calculated as GSI = (GW/BW) × 100. Based on GSI and skin color, 3 *A. marmorata* (all from PY) were categorized as silver stage and the remainder as yellow stage. In *A. bicolor pacifica*, 10 specimens were categorized as yellow stage and the remainder as silver stage.

Muscle tissues were dissected out, weighed, put in clean polyethylene bags, and stored at –20°C in Vietnam. All samples were then transported to Japan in cool boxes with dry ice for further chemical analyses.

Chemical analysis of organotins in specimens. The method used to determine the concentrations of OTs in biological samples was based on that of Ohji et al. (2006, 2010) with some modifications.

One gram of homogenated liver of each eel was placed in a centrifuge tube and 100 µl of mixed acetone solution including 1 µg ml^{-1} of each tributyltin monochloride (TBTCl)-d_{27}, dibutyltin dichloride (DBTCl)-d_{18}, monobutyltin trichloride (MBTCl)-d_9, triphenyltin monochloride (TPTCl)-d_{15}, diphenyltin dichloride (DPTCl)-d_{10}, and monophenyltin trichloride (MPTCl)-d_5 was added to the centrifuge tube as a surrogate stan-

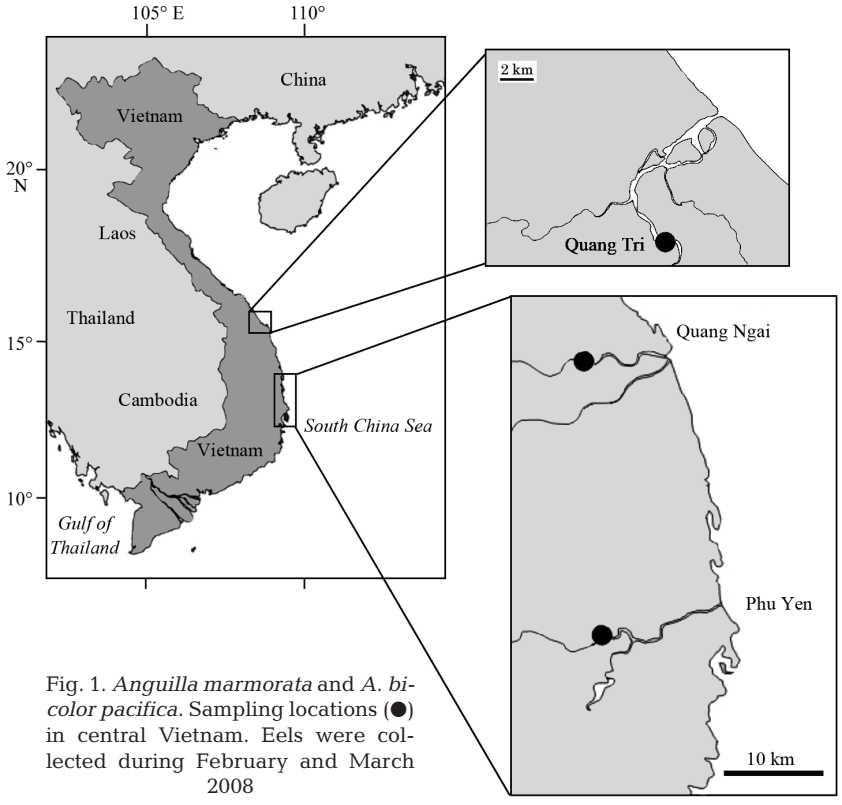

Fig. 1. *Anguilla marmorata* and *A. bicolor pacifica*. Sampling locations (●) in central Vietnam. Eels were collected during February and March 2008

dard. The mixture was extracted with 25 ml of 1 M HCl-methanol:ethyl acetate (1:1 ratio) by shaking for 10 min. After centrifugation at 4000 × *g* for 10 min, the residue was extracted and centrifuged again in the same way. The combined supernatants and 70 ml of saturated NaCl solution were transferred to a separating funnel. The analytes were extracted twice using 30 ml of ethyl acetate:hexane (3:2) solution. Next, 50 ml of hexane was mixed with the combined organic layers and the mixture was allowed to stand for 20 min. After removal of the aqueous layer, the organic layer was dried with anhydrous Na_2SO_4 and was concentrated up to trace level by a rotary evaporator, and further concentrated by means of a nitrogen purge. The analytes were diluted with 5 ml of acetic acid–sodium acetate buffer (pH 5.0) and ethylated using 1 ml of 5% $NaBEt_4$. The lipids were saponificated with 10 ml of 1 M KOH–ethanol solution by shaking for 1 h. Next, 50 ml of distilled water and 40 ml of hexane were added to the solution, and ethylated OTs in the mixed sample solution were extracted to an organic layer by shaking for 10 min. The ethylated OT residue in the aqueous layer was extracted again by shaking for 10 min with 40 ml of hexane. The combined organic layers were dried with anhydrous Na_2SO_4. After being concentrated to 1 ml by a rotary evaporator and nitrogen gas, the solution was cleaned using a florisil Sep-Pak column (Waters Associates). The analytes were eluted with 5% diethyl ether/hexane, and TeBT-d_{36} and TePT-d_{20} were added as internal standards. The final solution was then concentrated to 0.5 ml.

A Hewlett-Packard 6890 series gas chromatograph equipped with a mass spectrometer (5973 N) was used for analysis of OTs with selected ion monitoring. The separation was carried out in a capillary column coated with 5% phenyl methyl silicone (30 m length × 0.25 mm internal diameter, 0.25 µm film thickness; J&W Scientific). The column temperature was held at 60°C for the first 2 min, then increased to 130°C at 20°C min^{-1}, to 210°C at 10°C min^{-1}, to 260°C at 5°C min^{-1}, and to 300°C at 10°C min^{-1}. Finally, the column temperature was maintained at 300°C for 2 min. The interface temperature, ion source temperature and ion energy were 280°C, 230°C and 70 eV, respectively. Selected ion monitoring was performed under this program. Splitless injection (1 µl) of the sample was employed.

The concentrations of OTs in this study are expressed as Sn^{4+} on a wet weight basis for the biological samples.

Table 1. *Anguilla marmorata* and *A. bicolor pacifica*. Specimens used for organotin and otolith microchemistry analyses. GSI: gonad-somatic index

Sampling location	Growth stage	n	Total length (mm)		Body weight (g)		GSI	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
A. marmorata								
Quang Tri	Yellow	10	616.25 ± 210.45	342–1029	839.98 ± 1069.88	90.4–3392.4	0.21 ± 0.40	0.006–1.261
Quang Ngai	Yellow	10	399.15 ± 40.55	355–485	144 ± 49.99	100–260	0.13 ± 0.38	0.002–1.226
Phu Yen	Silver	3	878 ± 87.11	784–956	2094.67 ± 738.59	1259–2660	2.31 ± 1.39	1.502–3.920
	Yellow	7	805.29 ± 95.75	616–902	1421.71 ± 499.05	650–2025	0.46 ± 0.30	0.012–0.847
A. bicolor pacifica								
Phu Yen	Silver	11	751.32 ± 141.13	516.5–941	1094.95 ± 525.76	265–1906.4	2.24 ± 0.79	1.423–4.058
	Yellow	10	696.35 ± 78.55	614–889	754.25 ± 287.54	478–1498.5	0.63 ± 0.24	0.213–0.871

In order to examine the quality of the data obtained by the analytical procedure, the soft tissues of fish were spiked with 1 µg of BTs and PTs. The recoveries of the BTs and PTs were in the range of 85–103 and 87–105%, respectively, and their relative standard deviations (RSD) were in the range of 3.2–9.2 and 5.1–13%, respectively. The detection limits (signal-to-noise ratio of 3) of each OT were 0.001 ng g^{-1} wet wt, and the quantification limits (signal-to-noise ratio of 10) were 0.003 ng g^{-1} wet wt for the biological samples.

Otolith preparation and otolith X-ray microprobe analysis. Sagittal otoliths were extracted from each eel, embedded in epoxy resin (Epofix; Struers), and mounted on glass slides. 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 (Discoplan-TS; Struers), and polished further with OP-S suspension on an automated polishing wheel (RotoPol-35; Struers,) equipped with a semi-automatic specimen mover (PdM-Force-20; Struers). Finally, they were cleaned using distilled water and ethanol, and dried at 50°C in an oven prior to examination.

For electron microprobe analyses, all otoliths were platinum-palladium coated by a high vacuum evaporator. 'Life-history transect' analysis of the Sr and Ca concentrations in all specimens was performed by measuring along a line down 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 Chino & Arai (2010a,b). Wollastonite ($CaSiO_3$) and tausonite ($SrTiO_3$) were used as standards, and the accelerating voltage and beam current were 15 kV and 1.2×10^{-8} A, respectively. The electron beam was focused on a point 10 µm in diameter, with measurements spaced at 10 µm intervals.

Chino & Arai (2010a,b) determined the migratory patterns of *Anguilla marmorata* and *A. bicolor pacifica* using otolith Sr:Ca ratios outside the 'high Sr core', which corresponded to the period of ocean life during the leptocephalus and early glass eel stages (Arai et al. 1997). In accordance with the criteria of Chino & Arai (2010a,b), we omitted the high Sr core (mean: 150 µm radius from the otolith core), and only values outside the high Sr core were used to obtain a mean otolith Sr:Ca ratio for each specimen. We then grouped these specimens into the 3 general categories, 'marine residents' (Sr:Ca $\geq 6.0 \times 10^{-3}$), 'estuarine residents' ($2.0 \times 10^{-3} \leq$ Sr:Ca $< 6.0 \times 10^{-3}$) and 'freshwater eels' (Sr:Ca $< 2.0 \times 10^{-3}$), using their mean otolith Sr:Ca ratios to enable statistical comparisons between eels with different habitat use histories.

Following 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 Chino & Arai (2010a,b).

Statistics. All data are presented as mean ± SD. Differences between data were analyzed using the Mann-Whitney *U*-test, and considered significant at $p < 0.005$. Differences among data were examined by an analysis of variance (ANOVA), and afterwards Scheffe's multiple range tests for the combination of 2 data. Significance of the correlation coefficient and regression slope were tested by Fisher's *Z*-transformation and an analysis of covariance (ANCOVA) (Sokal & Rohlf 1995).

RESULTS

Environmental habitat use by eels

The Sr:Ca ratios in the transects along the radius of each otolith showed common features in all specimens. The high Sr:Ca ratios in the central core region, inside the elver mark, corresponded to the leptocephalus and early glass eel stages during their oceanic life (Arai et al. 1997).

The life history transects of Sr:Ca ratios outside the elver mark in the otolith showed 3 distinctive patterns: (1) constantly living in freshwater (freshwater resident); (2) constantly living in brackish water with no freshwater life (estuarine resident); and (3) constantly living in sea water with no freshwater life (marine resident) (Fig. 2). *Anguilla marmorata* and *A. bicolor pacifica* had a wide range of otolith Sr:Ca ratios, from 0.92×10^{-3} to 6.14×10^{-3} and from 2.52×10^{-3} to 6.32×10^{-3}, respectively (Fig. 3). The wide range of otolith Sr:Ca ratios indicated that the habitat use of both species were variable after their recruitment to coastal waters as glass eels. For *A. marmorata*, there were 3 freshwater residents and 7 estuarine residents in QT, 5 freshwater residents, 4 estuarine residents and 1 marine resident in QN, and 9 estuarine residents and 1 freshwater resident in PY. For *A. bicolor pacifica*, there were 19 estuarine residents and 2 marine residents.

Relationship between organotin accumulation and each biological characteristic

No relationships were observed between total BT levels and TL (ANCOVA, $p > 0.5$), or total PT levels and TL (ANCOVA, $p > 0.5$) in either species. There were also no relationships between TBT levels and TL, or between TPT levels and TL (ANCOVA, $p > 0.5$) in either species.

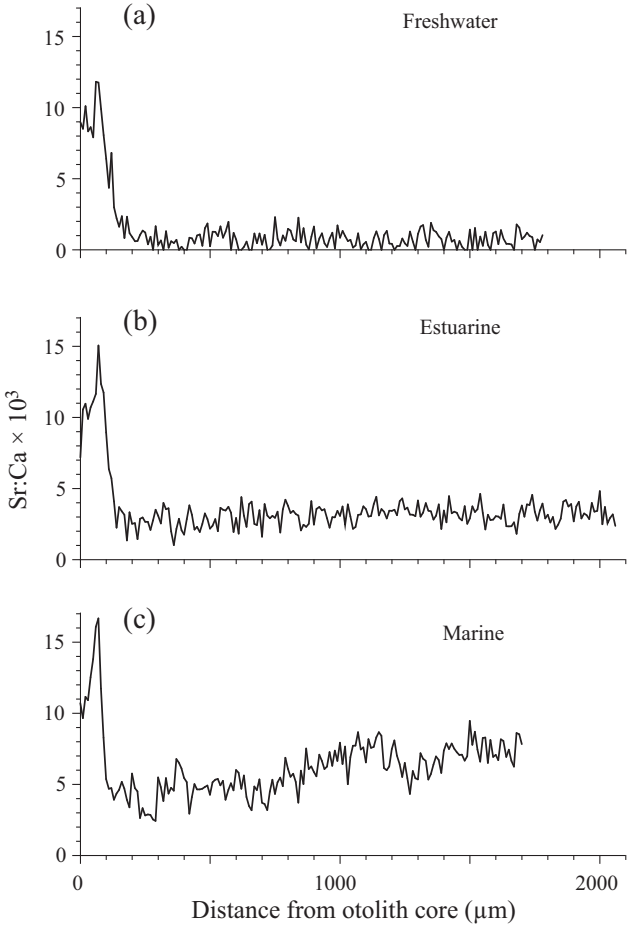

Fig. 2. *Anguilla marmorata* and *A. bicolor pacifica*. Typical changes in otolith Sr:Ca ratio along line transects from the core to the edge in the frontal plane of sagittal otoliths of tropical anguillid eels collected in central Vietnam. Specimens were classified based on the Sr:Ca mean ratio outside the elver mark (ca. 150 µm from the core): (a) freshwater resident, (b) estuarine resident, (c) marine resident

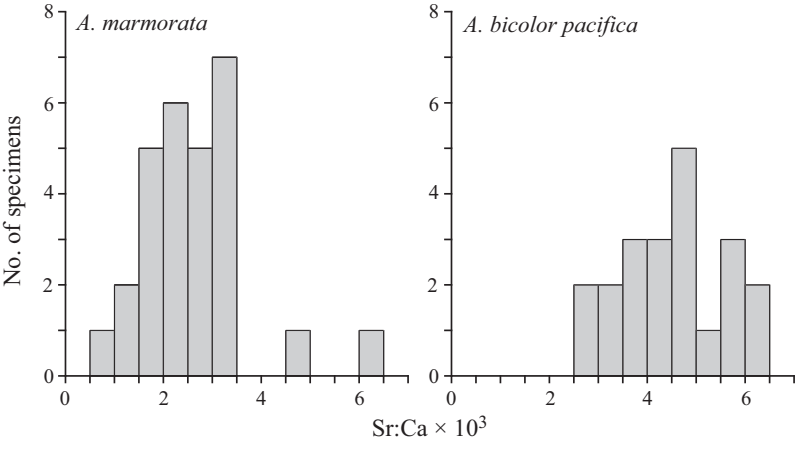

Fig. 3. *Anguilla marmorata* and *A. bicolor pacifica*. Frequency distribution of mean Sr:Ca ratios outside the elver mark (≥150 µm from the core) of sagittal otoliths

There was no relationship between the total BT levels and BW (ANCOVA, $p > 0.5$), or between the total PT levels and BW in either species (ANCOVA, $p > 0.5$). No relationships were observed between TBT and TPT levels and BW in either species (ANCOVA, $p > 0.5$).

In *Anguilla bicolor pacifica,* a negative relationship was found between total BT levels and GSI, and between TBT levels and GSI (ANCOVA, $p < 0.05$) (Fig. 4). However, positive relationships were observed between total PT levels and GSI, and between TPT levels and GSI (ANCOVA, $p < 0.05$) (Fig. 4). We did not evaluate the relationship between each OT level and GSI in *A. marmorata* due to the limited number of higher GSI samples.

Relationship between organotin accumulation and maturation stage

In *Anguilla bicolor pacifica*, the TBT concentration in yellow stage eels was 51.52 ± 54.20 ng g^{-1} wet wt, and the value was significantly higher than that in silver stage eels (13.90 ± 8.38 ng g^{-1} wet wt) (Mann-Whitney *U*-test, $p < 0.05$) (Table 2). However, the TPT concentration in yellow stage eels (2.22 ± 1.78 ng g^{-1} wet wt) was significantly lower than that in silver stage eels (16.73 ± 15.18 ng g^{-1} wet wt) (Mann-Whitney *U*-test, $p < 0.005$) (Table 2). In *A. marmorata* from PY, no significant differences were observed between each TBT and TPT concentration and maturation stages (Mann-Whitney *U*-test, $p > 0.5$).

With respect to TBT and TPT concentrations, significant differences were found between yellow stage *A. bicolor pacifica* and *A. marmorata* (Mann-Whitney *U*-test, $p < 0.01–0.001$). Similarly significant differences between those species were also observed for both the TBT and TPT concentrations in silver stage eels (Mann-Whitney *U*-test, $p < 0.05–0.001$).

Accumulation of organotin in eels with different habitat use

In PY, no significant differences were found in the mean otolith Sr:Ca ratios between *A. bicolor pacifica* (4.50×10^{-3} ± 1.08×10^{-3}) and *A. marmorata* (3.33×10^{-3} ± 0.69×10^{-3}) (Mann-Whitney *U*-test, $p > 0.05$). Environmental habitat uses were similar for these 2 species. Sr:Ca ratios were compared with ∑BT (= TBT + DBT + MBT) and ∑PT (= TPT + DPT + MPT) concentrations for all samples. Positive linear relationships were

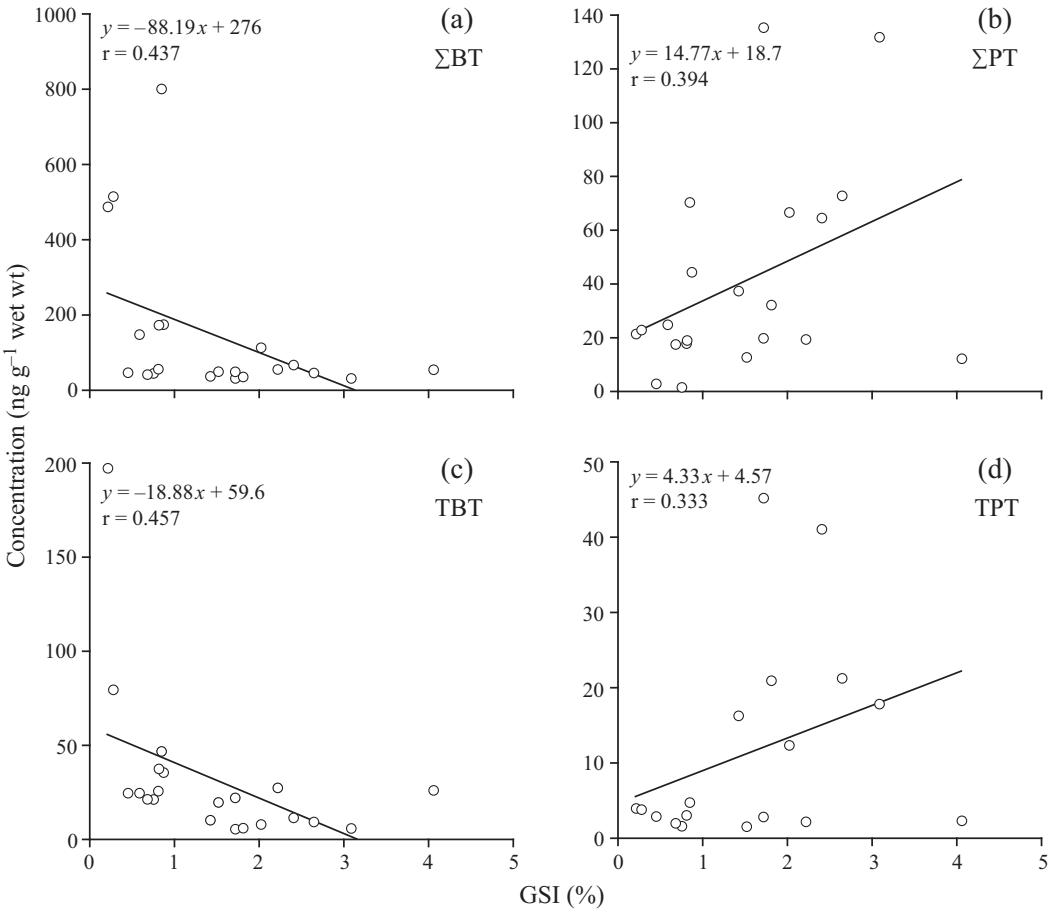

Fig. 4. *Anguilla bicolor pacifica.* Relationships between organotin concentrations in eel livers and gonad-somatic index (GSI) values: (a) total butyltin, (b) total phenyltin, (c) tributyltin, (d) triphenyltin

found both between the Sr:Ca ratio and the ∑BT concentration (ANCOVA, $p < 0.05$), and between the Sr:Ca ratio and the ∑PT concentration (ANCOVA, $p < 0.005$) (Fig. 5). These results suggest that OT accumulation differs depending on their environmental habitat uses.

Differences in organotin accumulation among sites

Total BT and total PT concentrations ranged from 12.77 to 801.1 ng g^{-1} wet wt and from <0.001 to 135.4 ng g^{-1} wet wt, respectively (Table 2). Those of TBT and TPT ranged from 1.53 to 197.4 ng g^{-1} wet wt

Table 2. *Anguilla marmorata* and *A. bicolor pacifica*. Mean ± SD and ranges of organotin concentrations (ng g^{-1} wet wt) in eel livers. M-/D-/TBT: mono-/di-/tributyltin; M-/D-/TPT: mono-/di-/triphenyltin; ND: not detected

Site	Stage	MBT	DBT	TBT	Total BT	MPT	DPT	TPT	Total PT
A. marmorata									
Quang Tri	Yellow	20.28 ± 12.26 10.22–33.43	5.94 ± 2.75 2.58–12.94	2.74 ± 1.58 1.69–5.57	28.96 ± 13.25 16.71–59.92	9.85 ± 8.51 ND–26.44	0.22 ± 0.53 ND–1.57	0.33 ± 1.15 ND–3.99	10.41 ± 8.42 ND–26.44
Quang Ngai	Yellow	26.55 ± 13.70 16.45–59.22	22.46 ± 29.29 5.58–100.2	26.78 ± 15.26 4.07–58.52	75.79 ± 47.62 36.53–199.7	8.58 ± 3.47 3.32–15.61	0.64 ± 1.02 ND–3.17	0.25 ± 0.54 ND–1.41	9.47 ± 3.79 3.32–15.61
Phu Yen	Yellow	11.81 ± 4.20 7.89–19.95	5.30 ± 1.94 3.36–9.06	2.52 ± 0.73 1.53–3.66	19.63 ± 5.95 12.77–31.60	7.58 ± 3.38 4.70–13.51	ND	ND	7.58 ± 3.38 4.70–13.51
	Silver	16.01 ± 12.58 7.88–30.50	6.54 ± 0.18 6.34–6.68	3.23 ± 0.98 2.44–4.32	25.78 ± 13.61 16.67–41.42	4.88 ± 5.50 ND–10.84	ND	ND	4.88 ± 5.50 ND–10.84
A. bicolor pacifica									
Phu Yen	Yellow	30.41 ± 21.39 11.15–78.85	167.5 ± 218.0 8.49–675.4	51.52 ± 54.20 21.31–197.4	249.4 ± 261.5 42.34–801.1	19.33 ± 16.36 ND–52.21	2.76 ± 4.03 ND–13.42	2.22 ± 1.78 ND–4.76	24.32 ± 20.06 1.62–70.39
	Silver	24.79 ± 8.83 16.67–48.38	13.81 ± 22.08 1.65–79.70	13.90 ± 8.38 5.62–27.52	52.50 ± 23.13 32.18–113.6	7.17 ± 8.93 ND–23.51	31.14 ± 40.81 ND–90.20	16.73 ± 15.18 1.56–45.23	55.04 ± 44.57 12.25–135.4

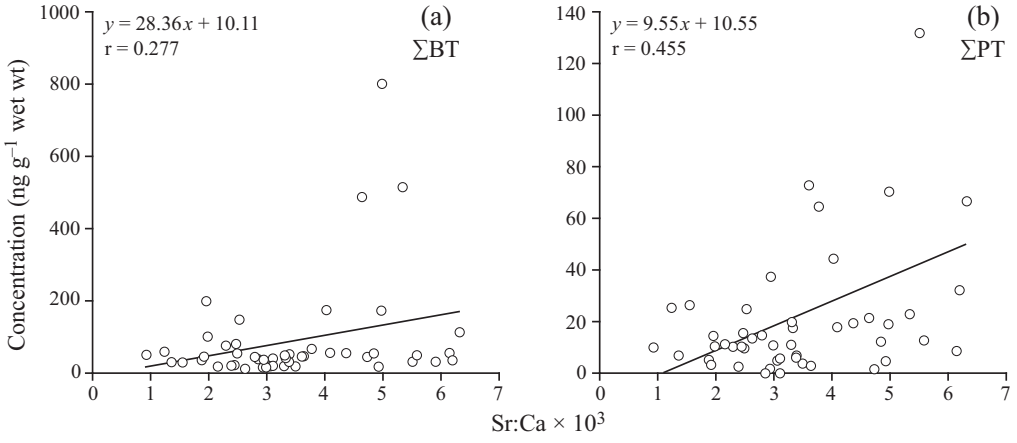

Fig. 5. *Anguilla marmorata* and *A. bicolor pacifica*. Relationship between organotin concentrations in eel livers and otolith Sr:Ca ratios: (a) total butyltin and (b) total phenyltin

and from <0.001 to 45.23 ng g^{-1} wet wt, respectively. OT accumulation was affected by both environmental habitat uses (migratory types) and maturation stage. Therefore, OT levels were compared within the same migratory types and maturation stage to examine the regional differences in OT levels.

In yellow stage freshwater residents, the total BT and PT concentrations (mean ± SD) were 87.08 ± 67.85 and 8.72 ± 4.49 ng g^{-1} wet wt in QN, and 24.25 ± 25.16 and 11.75 ± 13.24 ng g^{-1}wet wt in QT, respectively (Table 3); there were no significant differences in either the total BT and the total PT concentrations between those regions (Mann-Whitney *U*-test, p > 0.05) (Fig. 6a). There was a significant difference in TBT concentration between QN and QT (Mann-Whitney *U*-test, p < 0.05–0.005), although no significant differences were found for other OTs between those sites (Mann-Whitney *U*-test, p > 0.05–0.5).

In yellow stage estuarine residents, the total BT and total PT concentrations (mean ± SD) were 66.48 ± 14.58 and 10.61 ± 3.64 ng g^{-1} wet wt in QN, 23.18 ± 8.21 and 6.19 ± 5.24 ng g^{-1} wet wt in QT, 19.85 ± 6.49 and 4.61 ± 4.83 ng g^{-1} wet wt in PY (all for *Anguilla marmorata*), and 249.4 ± 261.5 and 24.32 ± 20.06 ng g^{-1} wet wt in PY *A. bicolor pacifica*, respectively (Table 4). There were significant differences in total BT concentrations between QN and QT, between QN and PY and between PY *A. marmorata* and PY *A. bicolor pacifica* (ANOVA, p < 0.05–0.005), while no significant differences were found in other combinations (ANOVA, p > 0.5–0.05) (Fig. 6b). In total PTs, significant differences were found between QT *A. marmorata* and PY *A. bicolor pacifica* and between PY *A. marmorata* and PY of *A. bicolor pacifica* (ANOVA, p < 0.05), while no significant differences were found in other combinations (ANOVA, p > 0.5–0.05). In TBT, significant differences were found between QN and QT, between QN and PY, between QT and PY *A. bicolor pacifica* and between PY *A. marmorata* and PY *A. bicolor pacifica* (ANOVA, p < 0.05), while no significant differences were found in other combinations (ANOVA, p > 0.5–0.05). There were no significant differences in TPT among sites (ANOVA, p > 0.1–0.05).

In silver stage estuarine residents, the total BT and the total PT concentrations (mean ± SD) were 25.78 ± 13.61 and 4.88 ± 5.50 ng g^{-1} wet wt in PY *Anguilla marmorata* and 47.53 ± 11.82 and 56.28 ± 48.98 ng g^{-1} wet wt in PY *A. bicolor pacifica*, respectively (Table 5). There was a significant difference in the total PT concentrations between those species (Mann-Whitney *U*-test, p < 0.05), but no significant difference in the total BT concentrations between those regions (Mann-Whitney *U*-test, p > 0.05) (Fig. 6c). There was a significant difference in TBT concentration between those species (Mann-Whitney *U*-test, p < 0.005), although no significant differences in other combinations (Mann-Whitney *U*-test, p > 0.5) (Fig. 6c).

OT levels differed among sites within the same migratory types and maturation stage (Fig. 6).

Table 3. *Anguilla marmorata*. Mean ± SD and range of organotin concentrations (ng g^{-1} wet wt) in livers of yellow stage freshwater resident eels. ND: not detected

Site	MBT	DBT	TBT	Total BT	MPT	DPT	TPT	Total PT
Quang Tri	18.23 ± 20.93 ND–50.40	4.44 ± 5.48 ND–12.94	1.58 ± 1.44 ND–2.83	24.25 ± 25.16 ND–59.92	11.75 ± 13.24 ND–26.44	ND	ND	11.75 ± 13.24 ND–26.44
Quang Ngai	21.94 ± 10.69 16.45–41.02	32.57 ± 40.32 5.58–100.2	32.57 ± 18.55 12.04–58.52	87.08 ± 67.85 36.53–199.7	7.37 ± 3.39 3.32–10.49	0.85 ± 1.38 ND–3.17	0.50 ± 0.70 ND–1.41	8.72 ± 4.49 3.32–14.55

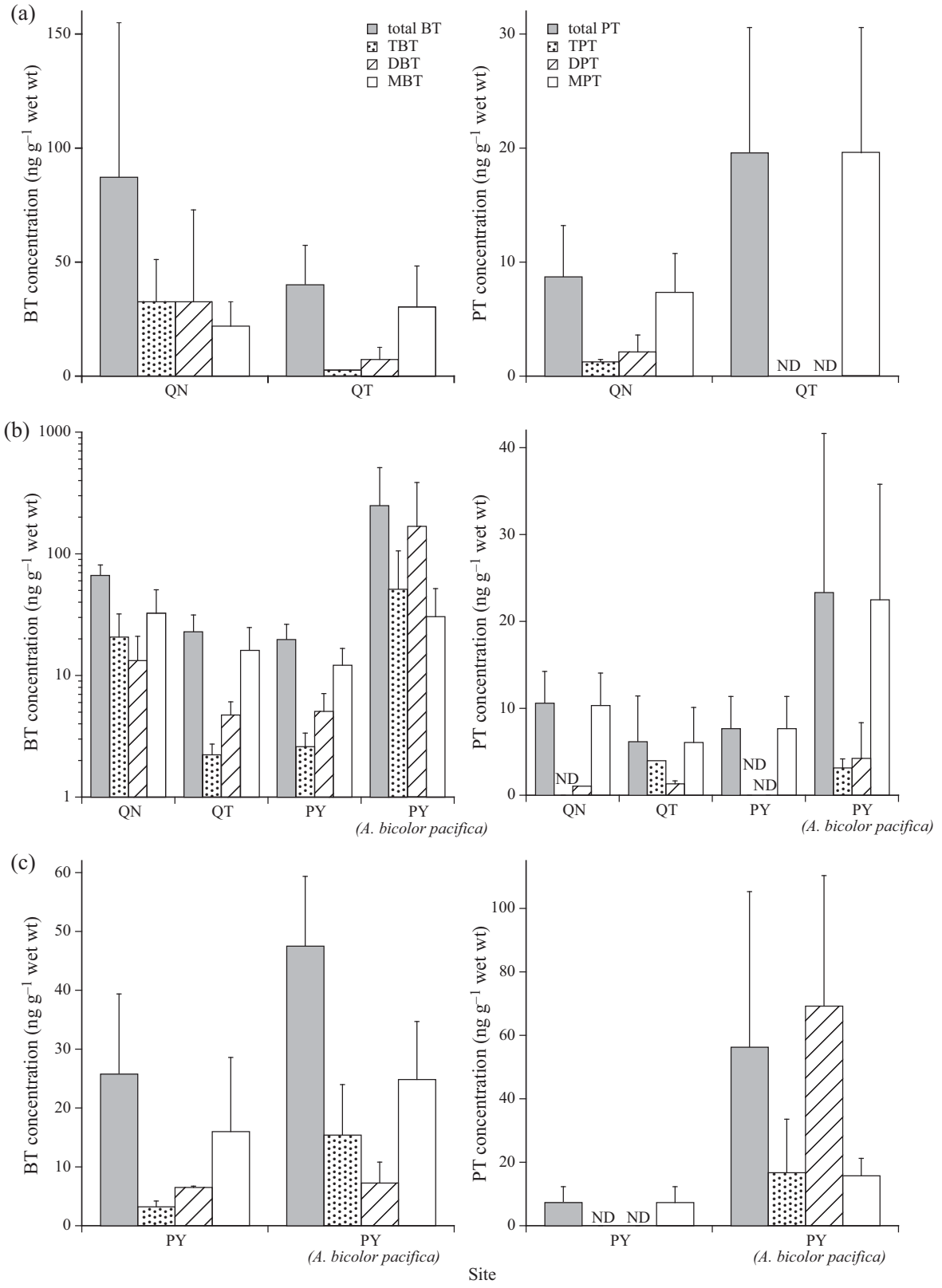

Fig. 6. *Anguilla marmorata* and *A. bicolor pacifica.* Mean (+SD) butyltin (BT, left) and phenyltin (PT, right) concentrations in eel livers within the same migratory types and maturation stage from each site (QT: Quang Tri; QN: Quang Ngai; PY: Phu Yen). (a) yellow stage freshwater residents, (b) yellow stage estuarine residents, (c) silver stage estuarine residents. T/D/MBT: tri-/di-/monoBT; T/D/MPT: tri-/di-/monoPT; ND: not detected (below detection limit)

Table 4. *Anguilla marmorata* and *A. bicolor pacifica*. Mean ± SD and range of organotin concentrations (ng g^{-1} wet wt) in livers of yellow stage estuarine resident eels. ND: not detected

Site	MBT	DBT	TBT	Total BT	MPT	DPT	TPT	Total PT
A. marmorata								
Quang Tri	16.20 ± 8.57 11.04–33.43	4.74 ± 1.33 2.90–6.78	2.24 ± 0.49 1.77–3.07	23.18 ± 8.21 16.71–39.40	6.10 ± 4.01 ND–10.37	0.44 ± 0.70 ND–1.57	0.67 ± 1.63 ND–3.99	6.19 ± 5.24 ND–11.26
Quang Ngai	32.46 ± 18.13 19.05–59.22	13.29 ± 7.72 6.52–21.70	20.74 ± 11.29 4.07–28.54	66.48 ± 14.58 52.62–81.28	10.35 ± 3.71 6.90–15.61	0.26 ± 0.52 ND–1.05	ND	10.61 ± 3.64 6.90–15.61
Phu Yen	12.17 ± 4.48 7.89–19.95	5.08 ± 2.03 3.36–9.06	2.61 ± 0.76 1.53–3.66	19.85 ± 6.49 12.77–31.60	7.68 ± 3.69 4.70–13.51	ND	ND	4.61 ± 4.83 4.70–13.51
A. bicolor pacifica								
Phu Yen	30.41 ± 21.39 11.15–78.85	167.5 ± 218.0 8.49–675.4	51.52 ± 54.20 21.31–197.4	249.4 ± 261.5 42.34–801.1	19.33 ± 16.36 ND–52.21	2.76 ± 4.03 ND–13.42	2.22 ± 1.78 ND–4.76	24.32 ± 20.06 1.62–70.39

Table 5. *Anguilla marmorata* and *A. bicolor pacifica*. Mean ± SD and range of organotin concentrations (ng g^{-1} wet wt) in livers of silver stage estuarine resident eels in Phu Yen. ND: not detected

Species	MBT	DBT	TBT	Total BT	MPT	DPT	TPT	Total PT
A. marmorata	16.01 ± 12.58 7.88–30.50	6.54 ± 0.18 6.34–6.68	3.23 ± 0.98 2.44–4.32	25.78 ± 13.61 16.67–41.42	4.88 ± 5.50 ND–10.84	ND	ND	4.88 ± 5.50 ND–10.84
A. bicolor pacifica	24.85 ± 9.85 16.67–48.38	7.27 ± 3.54 1.65–10.78	15.41 ± 8.57 5.62–27.52	47.53 ± 11.82 32.18–67.76	8.76 ± 9.16 ND–23.51	30.77 ± 44.33 ND–21.16	16.75 ± 16.84 1.56–45.23	56.28 ± 48.98 12.25–135.4

DISCUSSION

Based on our results, TBT and TPT accumulations in the tropical anguillid eels *Anguilla marmorata* and *A. bicolor pacifica* did not generally depend on TL and BW. Concentrations of TBT were also independent of TL and BW in the European eel *A. anguilla* and the Japanese eel *A. japonica* (Harino et al. 2002, Ohji et al. 2006). Likewise, TBT concentration and fish length did not correlate in Japanese sea perch *Lateolabrax japonicus*, white crocker *Pennehia argentatu* or yellowtail *Seriola quinqueradiata* (Harino et al. 2000). BT residues in fish are not greatly affected by size, but more likely reflect the recent history of TBT contamination in their environment.

The variation in accumulation of TBT and TPT depends on maturation stage in *Anguilla bicolor pacifica*. The TBT concentrations were significantly higher in yellow stage than in silver stage eels. Furthermore, a negative correlation was found between GSI values and both total BT and TBT concentrations. Yellow stage eels have a higher risk of TBT contamination than silver stage. De Silva et al. (2002) reported biochemical features of ready-to-migrate silver and premigratory yellow stages of the shortfin eel *A. australis* of southeastern Australian waters; the percent of moisture, protein and ash content was significantly higher in yellow stage than silver stage eels. Eels are not thought to feed just before the spawning migration, and oocytes are not fully developed at that time (Lokman et al. 1998), so a certain proportion of their energy reserves will need to be channeled towards oocyte maturation. Therefore, biochemical changes, including the build-up of large energy reserves, take place during the transformation from yellow to silver stage in preparation for the long oceanic spawning migration (Lokman et al. 1998). Feeding activity in yellow stage eels in relation to accumulated protein might be higher than that in silver stage eels. Since TBT is a hydrophobic compound, it is likely to bind to the protein. Therefore, higher protein content in yellow stage than silver stage eels might lead to elevated concentration of TBT in the yellow stage. Further, TBT is more easily metabolized and eliminated as di- and mono-OTs than TPT. Thus, silver stage eels might have metabolized and eliminated more accumulated TBT during starvation for their spawning migration than yellow stage eels, which continuously take up TBT from the ambient environment. However, the accumulation pattern for TPT was different from that of TBT: concentrations of TPT in yellow stage eels were significantly lower than those in silver eels. The lower degradation capacity of TPT might result in greater accumulation in the silver stage than in the yellow stage due to the longer duration of the silver stage. Since studies on the relationship between TPT levels and biometric features have been rudimentary to date, further study on this topic is necessary.

In the present study, TBT and TPT were detected in all migratory types, i.e. freshwater, estuarine and

marine residents. Yellow stage eels are territorial and maintain local home-ranges in rivers, lakes, estuaries, and seas, residing in mud, weed beds or shady pools during the day (Naismith & Knights 1990). Eels feed on benthic invertebrates and other aquatic fauna occurring within their territories (Slayter 1981). Because of their benthic feeding habitat and close association with sediment, which is known to be a persistent sink of OTs (Langston & Pope 1995), eels have significant potential for bioaccumulation of OTs in all migratory types. The distribution of sediment-bound TBT is very similar to that in tissues in *Anguilla anguilla* collected along the Thames tideway (Langston et al. 2000, Harino et al. 2002), which confirms that sediment is an important vector for TBT uptake for these benthic fish.

The relationship between otolith Sr:Ca ratios and the total BT and PT concentrations clearly showed that OT concentrations increased significantly with increasing Sr:Ca ratio. Therefore, we consider that risk of OT exposure was higher in marine than in freshwater residents. Since TBT and TPT are used primarily in the coastal area, marine eels might be exposed to OTs throughout their whole life history. In contrast, since river eels remain in a freshwater environment throughout their life history, their exposure to OTs is likely much lower. The results of the present study suggest that not only marine fishes but also catadromous fishes are at risk of TBT exposure, and that their accumulation patterns differ according to their life histories. Since it is clear that there are differences in the risk of TBT and TPT among migratory patterns in anguillid eels, comparisons of the contamination level of OTs in anguillid eels of different regions should be conducted within the same migratory types.

Extremely high concentrations of BTs and PTs were detected in a few estuarine resident eels in the present study (Fig. 5). Yellow eels are known to have a restricted home range, although they sometimes move to different areas (Bozeman et al. 1985, Ford & Mercer 1986, Parker 1995, Oliveira 1997). The estuarine residents exhibited consistently intermediate Sr:Ca ratio from the high Sr core out to the otolith edge, suggesting that these eels had remained in a brackish water habitat for a long time. These results suggest that a constant Sr:Ca ratio corresponds to staying within a small home range. Therefore, even in the same category, there are various levels of risk for OTs in anguillid eels. The high OT concentrations found in some estuarine residents in the present study might have resulted from their spending long periods in a single small area which coincidentally was highly contaminated with OTs. Again, since TBT and TPT are used primarily in the coastal area, estuarine resident eels, like marine eels, might be exposed to OTs throughout their whole life history.

Significant differences were found between TBT and TPT concentrations among sites within the same migratory types and maturation stage. These results suggest that the usage of TBT and TPT were different among the regions. A few studies on TBT concentration in eels are available for comparison of the scale of impact. TBT and TPT concentrations ranged from 113 to 1050 and from 480 to 1500 ng g^{-1} dry wt, respectively, in eels *Anguilla anguilla* from marinas on Lake Grote Poel, the Netherlands (Stäb et al. 1996). The concentration of TBT in eels from the Thames Estuary and Weston Canal in the UK ranged from 180.0 to 1349 and from 292.6 to 319.8 ng g^{-1} wet wt, respectively (Harino et al. 2002). TPT concentrations in eels from the Thames Estuary and Weston Canal ranged from below the detection limit to 676.0 and from 998.4 to 1394.6 ng g^{-1} wet wt, respectively (Harino et al. 2002). TBT and TPT concentrations ranged from 1.1 to 1060.5 ng g^{-1} wet wt and from below the detection limit to 566.2 ng g^{-1} wet wt, respectively, in eels from Tokushima, Japan (Ohji et al. 2006). In the present study, TBT concentration ranged from 1.53 to 197.4 ng g^{-1} wet wt in eels from central Vietnam, generally lower than those previously reported, despite some overlap. TPT concentration in eels from the present study ranged from below the detection limit to 45.22 ng g^{-1} wet wt, and these values are much lower than those in previous reports. Though the sediment contamination status of OTs varied depending on countries, the concentration of OTs in Vietnam is within the range of levels found throughout the world (Arai & Harino 2009). The level of OTs in sediment from Vietnam was lower in comparison with those in non-regulated countries. Thus, OT concentrations in eels in Vietnam might be lower than those in eels from other countries.

The present study demonstrates that the diadromous eels *Anguilla marmorata* and *A. bicolor pacifica* have a different risk of OT accumulation based on intraspecific variation of the environmental habitat use (life history pattern), while the OT accumulation did not depend on body size. Therefore, comparisons of the contamination levels of OTs using eels from different regions should be conducted with specific reference to their migratory histories. Since marine and estuarine resident eels are considered to make a larger reproductive contribution than freshwater resident eels to the next generation in Japanese coastal waters (Chino & Arai 2009), OT contamination of coastal areas may represent a disproportionately high ecological risk to eel stocks. However, after freshwater resident yellow stage eels metamorphose into the silver stage, they move back downstream to the ocean to begin the migration to the same spawning area to which marine and estuarine resident eels also migrate. This implies that eels from marine, brackish and fresh waters can

mix during the spawning migration, participate in reproductive activity, and potentially contribute to the next generation together. TBT exposure detrimentally affects the reproductive activity of spermatozoa in the African catfish *Clarias gariepinus* (Rurangwa et al. 2002) and herring *Clupea harengus* (Grzyb et al. 2003), and significantly reduces fecundity and hatching success in the Japanese medaka *Oryzias latipes* (Walker et al. 1989). Additionally, embryonic exposure to TBT as well as TPT has been reported to delay hatching, decrease hatching success and increase mortality in the European minnow *Phoxinus phoxinus* (Fent 1992, Fent & Meier 1992, 1994). Therefore, TBT and TPT exposure might also affect the reproductive systems of marine and estuarine resident eels, resulting in a decline of the eel population. The higher accumulation of OTs found in both marine and estuarine resident eels may thus indirectly affect the reproductive success of freshwater resident eels, and lead to the decline of eel stocks.

Acknowledgements. This work was supported in part by Grant-in-Aid No. 20688008 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

LITERATURE CITED

Arai T (2009) Fish. In: Arai T, Harino H, Ohji M, Langston WJ (eds) Ecotoxicology of antifouling biocides. Springer Verlag, Berlin, p 291–305

Arai T, Harino H (2009) Contamination by organotin compounds in Asia. In: Arai T, Harino H, Ohji M, Langston WJ (eds) Ecotoxicology of antifouling biocides. Springer Verlag, Berlin, p 61–74

Arai T, Otake T, Tsukamoto K (1997) Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in the Japanese eel, *Anguilla japonica.* Mar Ecol Prog Ser 161:17–22

Arai T, Kotake A, Lokman PM, Miller MJ, Tsukamoto K (2004) Evidence of different habitat use by New Zealand freshwater eels *Anguilla australis* and *A. dieffenbachii*, as revealed by otolith microchemistry. Mar Ecol Prog Ser 266: 213–225

Arai T, Kotake A, McCarthy TK (2006) Habitat use by the European eel *Anguilla auguilla* in Irish waters. Estuar Coast Shelf Sci 67:569–578

Bozeman EK, Helfman GS, Richardson T (1985) Population size and home range of American eels in a Georgia tidal creek. Trans Am Fish Soc 114:821–825

Chino N, Arai T (2009) Relative contribution of migratory type on the reproduction of migrating silver eels, *Anguilla japonic*a, collected off Shikoku Island, Japan. Mar Biol 156:661–668

Chino N, Arai T (2010a) Migratory history of the giant mottled eel (*Anguilla marmorata*) in the Bonin Islands of Japan. Ecol Freshw Fish 19:19–25

Chino N, Arai T (2010b) Occurrence of marine resident tropical eel *Anguilla bicolor bicolor* in Indonesia. Mar Biol 157:1075–1081

De Silva SS, Gunasekera RM, Collins RO (2002) Some morphometric and biochemical features of ready-to-migrate silver and pre-migratory yellow stages of the shortfin eel of south-eastern Australian waters. J Fish Biol 61:915–928

Fent K (1992) Embryotoxic effects of tributyltin on the minnow *Phoxinus phoxinus.* Environ Pollut 76:187–194

Fent K, Meier W (1992) Tributyltin-induced effects on early life stages of minnows *Phoxinus phoxinus.* Arch Environ Contam Toxicol 22:428–438

Fent K, Meier W (1994) Effects of triphenyltin on fish early life stages. Arch Environ Contam Toxicol 27:224–231

Ford TE, Mercer E (1986) Density, size distribution and home range of American eels, *Anguilla rostrata*, in a Massachusetts salt marsh. Environ Biol Fishes 17:309–314

Grzyb K, Rychlowski M, Biegniewska A, Skorkowski EF (2003) Quantitative determination of creatine kinase release from herring (*Clupea harengus*) spermatozoa induced by tributyltin. Comp Biochem Physiol C 134:207–213

Harino H, Fukushima M, Kawai S (2000) Accumulation of butyltin and phenyltin compounds in various fish species. Arch Environ Contam Toxicol 39:13–19

Harino H, O'Hara SCM, Burt GR, Pope ND, Chesman BS, Langston WJ (2002) Butyltin and phenyltin compounds in eels (*Anguilla anguilla*). J Mar Biol Assoc UK 82:893–901

Langston WJ, Pope ND (1995) Determinations of TBT adsorption and desorption in estuarine sediments. Mar Pollut Bull 31:32–43

Langston WJ, Gibbs PL, Pascoe PE, Chesman BS, Burt GR, Pope ND, McEvoy J (2000) Tributyltin (TBT) impact in the Thames Estuary. Thames Estuary Water Quality Series 1. Environment Agency, London

Lokman PM, Vermeulen G, Lambert JGD, Young G (1998) Gonad histology and plasma steroid profiles in wild New Zealand freshwater eels (*Anguilla dieffenbachii and A. australis*) before and at the onset of the natural spawning migration. I. Females. Fish Physiol Biochem 19:325–338

Naismith IA, Knights B (1990) Modelling of unexploited and exploited populations of *Anguilla anguilla* (L.), in the Thames Estuary. J Fish Biol 37:975–986

Ohji M, Harino H, Arai T (2006) Differences in organotin accumulation among ecological migratory types of the Japanese eel *Anguilla japonica.* Estuar Coast Shelf Sci 69:270–290

Ohji M, Harino H, Arai T (2010) Bioaccumulation of organotin in relation to the life history of the brown trout *Salmo trutta.* Mar Ecol Prog Ser 399:285–293

Oliveira K (1997) Movement and growth rates of yellow phase American eels in the Annaquatucket River, Rhode Island. Trans Am Fish Soc 126:638–646

Parker SJ (1995) Homing ability and home-range of yellow phase American eels in a tidal dominated estuary. J Mar Biol Assoc UK 75:127–140

Rurangwa E, Biegniewska A, Slominska E, Skorkowski EF, Ollevier F (2002) Effect of tributyltin on adenylate content and enzyme activities of teleost sperm: a biochemical approach to study the mechanisms of toxicant reduced spermatozoa motility. Comp Biochem Physiol C 131:335–344

Slayter EM (1981) Unravelling the eel. Oceans 14(1):26–31

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. WH Freeman & Company, New York, NY

Stäb JA, Traas TP, Stroomberg G, van Kesteren J and others (1996) Determination of organotin compounds in the foodweb of a shallow freshwater lake in the Netherlands. Arch Environ Contam Toxicol 31:319–328

Walker WW, Heard CS, Lotz K, Lytle TF and others (1989) Tumorigenic, growth, reproductive and developmental effects in medaka exposed to bis(tri-n-butyltin) oxide. In: Proceedings of the Organotin Symposium, Oceans '89 Conference, Vol 2. Marine Technology Society and the Institute of Electrical and Electronics Engineers Council on Oceanic Engineering, Seattle, WA, p 516–524

Editorial responsibility: Helmut Segner, Bern, Switzerland

Submitted: March 11, 2011; Accepted: June 6, 2011
Proofs received from author(s): August 5, 2011