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Wildlife Contamination Assessment of Nansei Shoto Islands (2005-2007)

WWF Japan

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SUMMARY

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Nansei Shoto Islands including Okinawa, the characteristic coral reef area in Japan, is known to have many rare species of biota and thus highly reputed as having biologically and ecologically pristine endemic coastal waters. However, the chemical pollution and its adverse effects by the industrial and human activities in this area due to bounding economic growth rates in the developing countries in Eastern Asia is of great concern. Additionally, domestic usage of chemicals for agricultural and health purposes in Nansei Shoto Islands has also been apprehended as one of the reasons for the environmental contamination and resulting ecological risk in coastal waters.

Based on the above background, the present project "Wildlife Contamination Assessment of Nansei Shoto Islands" was conducted during the period from 2005 to 2007 aiming at elucidating the status of contamination by toxic chemicals in coastal waters for providing basic information to the stakeholders, decision-makers and specialists engaged in a variety of endeavors on environmental conservation. Further, the purpose of this project is also to support and promote the conservation activities in Nansei Shoto Islands by arousing interest amidst the local population. The present report is a summary of this project.

The research objectives principally focused on the following three subjects: 1) status of contamination in sea turtles and cetaceans, 2) status of contamination in fish, and 3) exposure test on coral larvae to toxic contaminants. The toxic contaminants in this study mainly targeted the chemicals of academic and social concern involving POPs (Persistent Organic Pollutants such as PCBs, DDTs etc), new POPs like brominated flame retardants, organotin compounds and their replacements, trace elements, agrichemicals and antifouling agent. In particular, POPs and new POPs are of high interest contaminants of this study, since these are posing worldwide concern in view of their persistence, long-range transport, bioaccumulation and highly toxic characteristics.

The most important finding of this study is the presence of considerable levels of Chlordane compounds and DDTs, which were largely used in Okinawa Island in the past, in fish. Further, PBDEs (polybrominated diphenylethers), the new POPs were detected in relatively higher levels in fish from Okinawa Island. High concentrations of PCBs (polychlorinated biphenyls) were also found in mullets from Manko-Hijya River of Okinawa Island. The pollution pattern found in Okinawa Island well reflects the history and status quo of the peculiar usage of chemicals in this area. While the present study could not affirm direct evidence such as diseases related to toxic effects of contaminants, from the present results we can say that additional research on endocrine disruption in some fish species is required. Additionally, concentrations of DDE (stable metabolite of DDT), PCBs and Hg in fish exceeded the NOAEL (no observed-adverse-effect level) levels for the predatory fish-eating birds, indicating the necessity of monitoring surveys and risk assessment in avian systems of Okinawa Island. As another significant finding of this study, relatively high levels of Hg were detected in Tilapia (*Oreochromis spp.*) and orange mud crab (*Scylla olivacea*) from Ishigaki Island. The reason for this is still unclear, but the transportation from surrounding developing countries may be implicated.

While monitoring sea turtles and cetaceans, detection of high levels of PBDEs in hawksbill turtle and Hg in Fraser's dolphin (*Lagenodelphis hosei*) was interesting. The conclusions to find the sources of these contaminants might be disguised by the possible exposure originating from developing countries, since these animals are migrating to southern waters where many developing countries are located.

Additionally, the exposure tests conducted on the larvae of reef-building corals to TBT (tributyltin) revealed adverse effects, even at levels close to the usual concentrations noticed in the ambient aquatic environments, implying concern on increasing coastal pollution and ecological risk caused by increasing number of foreign vessels visiting these islands. Further, the exposure test to DCMU [3-(3,4-Dichlorophenyl)-1,1-dimethylurea], which is used as a replacement of TBT as an antifouling agent and as a herbicide in the farming of sugar cane and pineapple plants, showed the same bearing of adverse effects, indicating the need for additional studies on the effects on the growth of reef-building corals, because Okinawa Island are using large quantities of DCMU.

With regard to POPs pollution in Nansei Shoto Islands, attention should also be paid to the usage patterns of same chemicals in the surrounding countries. The Stockholm Convention (POPs Treaty) signed on 23rd May, 2001 on the control/reduction of production, usage and unintentional formation of twelve POPs has become legally binding all over the world from 17 May 2004. As the production, usage and transportation of most of the target POPs were prohibited in almost all the developed nations and many developing countries, the extent of their environmental contamination are reducing almost linearly year by year. However, some chemicals which are designated as “New POPs” because of the similar physicochemical properties with POPs and their anticipated increase of global pollution and ecological risk are proposed for categorization into the group of legacy POPs, by a committee comprising representatives from several countries that has discussed about the control measures of them. For example, the levels of the brominated flame retardants that are present in electrical and plastic products are found to be increasing in the environment, wildlife and human and thus their trend and behavior of contamination are of great concern both academically and socially. Although the data set on the environmental monitoring of these new contaminants are still scarce in developing countries when compared to the developed nations, recent studies by our group in Ehime University witnessed apparently higher concentrations of PBDEs in fish samples from the East China Sea than from Japanese offshore waters, indicating the rapid expansion of PBDEs contamination originating from the recycling and dumping sites of e-wastes in China. Actually, recent reports have indicates poor management of e-wastes and concomitant environmental and human health problems in some Asian countries with high economic growth rates. Such a situation may lead to increasing contamination and toxic effect by these New POPs in Nansei Shoto Islands in the near future, prompting us to pay attention on the pollution not only from the domestic sources but also from the outside origins situated in the surrounding countries.

Collectively, we expect that this project report will be useful for designing an environmental monitoring system of toxic contaminants in East Asia and assisting the human network for the environmental conservation activities in future.

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(Names in no specific order and without honorific titles. Organizations are of the project period.)

CONTENTS

Survey on Hazardous Chemicals in Aquatic Organisms Inhabiting Nansei Shoto Islands
: Report on the Contamination Status of Cetaceans and Sea Turtles

Shinsuke Tanabe, Shin Takahashi, Govindan Malarvannan, Tokutaka Ikemoto,
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Survey on Hazardous Chemicals in Aquatic Organisms Inhabiting Nansei Shoto Islands: Report on the Contamination Status of Cetaceans and Sea Turtles

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Introduction

Nansei Shoto Islands located in the southern end of Japanese archipelago have one of world's leading coral reefs that are home to many rare fauna and flora. It is well known as a region for animal conservation because of its high bio-diversity. On the other hand, as Nansei Shoto Islands are in close proximity to rapidly industrializing countries such as China and other Southeast Asian countries, environmental pollution and adverse effects on ecosystems of the East China Sea (ECS) by hazardous chemicals is of serious concern. In this regard, our group has conducted monitoring surveys for persistent organic pollutants (POPs) in the Asia-Pacific region using skipjack tuna (*Katsuwonus pelamis*) and mussel as bioindicators (Ueno et al. 2003; 2004a; Monirith et al. 2003). Our results indicated that pollution by POPs such as DDTs is serious in the ECS and coastal areas of China than in other regions of Asia. Contamination levels of brominated flame retardants (BFRs) like polybrominated diphenyl ethers (PBDEs), which are drawing special attention at present as a new persistent pollutant, were higher in skipjack tuna samples from the ECS than those from other regions (Ueno et al. 2004a). Furthermore, relatively high concentrations of organotin compounds were found in seawater from Naha Port when compared to other Japanese coastal areas (Takeuchi et al. 2004), and hence it can be anticipated that similar levels of organotin compounds might be occurring in other ports and harbors of Nansei Shoto Islands.

In this survey, we analyzed organohalogen compounds, organotins, and trace elements in sea turtles and cetaceans stranded along the coasts or by-caught around Nansei Shoto Islands to investigate their pollution status and accumulation profiles of contaminants.

Materials and Method

1) Samples

The muscle, liver, kidney and blubber samples of cetaceans were collected from dead specimens of bottlenose dolphin (*Tursiops truncatus*), pantropical spotted dolphin (*Stenella attenuata*), sperm whale (*Physeter macrocephalus*) and Fraser's dolphin (*Lagenodelphis hosei*) stranded or drifted ashore along the coasts of Okinawa Island and Ishigaki and Iriomote Islands during 2003 to 2007. The liver samples of sea turtles were collected from green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), loggerhead turtle (*Caretta caretta*) and leatherback turtle (*Dermochelys coriacea*) stranded along the coasts or by-caught around Ishigaki Island and Kochi prefecture, Kapan during 1998 to 2006. Information on sampling locations, sample ID, biometry of cetaceans and sea turtles analyzed in the present study are listed in Table 1. All the samples were stored at -20 °C until analysis.

2) Analytical Method

Analysis of target compounds was performed following the previously reported procedures (Ueno et al. 2004a; 2006; Iwamura et al. 2000; Nam et al. 2005). Organochlorines (PCBs, DDTs, CHLs, HCHs, and HCB), PBDEs, and organotins (butyltins, phenyltins, and octyltins) were quantified using GC-ECD, GC-MS, and GC-MS, respectively. Trace elements (V, Cr, Mn, Co, Cu, Zn, Ga, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi) were measured using ICP-MS, CV-AAS, and HG-AAS. Chemical structures of the target compounds analyzed in this study are shown in Figures 1 and 2.

Results and discussion

1) Cetaceans

Organohalogen Compounds

Organohalogen compounds were analyzed in the blubber samples of bottlenose dolphin from Okinawa Island (Onna village), sperm whale from Iriomote Island and Fraser's dolphin from Ishigaki Island. Organohalogen compounds were detected in the all the cetacean blubber samples analyzed in this study (Table 2). Among the compounds detected, DDTs were dominant, followed by PCBs > CHLs ≈ HCHs ≈ PBDEs ≈ HCB. Concentrations of organohalogen compounds in cetacean samples analyzed in this study were apparently higher than those reported in skipjack tuna and sea turtles from Nansei Shoto Islands and other adjacent areas in the ECS (Ueno et al. 2003, 2004a). This indicates significant biomagnification of these compounds in higher tropic marine mammals. On the other hand, concentrations of organohalogen compounds (PCBs, DDTs and PBDEs) in cetaceans from Nansei Shoto Islands were lower than those from Japanese coastal waters and stranded along the coasts of Hong Kong and were comparable to offshore species (Table 3). Among the species investigated in this study, the highest concentrations of PCBs, DDTs, CHLs and PBDEs were found in Fraser's dolphin, followed by bottlenose dolphin > sperm whale. These organohalogen compounds have high lipophilicity and have been reported to biomagnify significantly through food chain. Such a significant accumulation of these compounds in Fraser's dolphin can be caused by eating fish of higher trophic level than the other cetaceans investigated. On the other hand, a juvenile bottlenose dolphin had these compounds at relatively low concentrations although this species is a fish eating species. Composition of organohalogen compounds detected in the cetaceans from Nansei Shoto Islands found to be similar to those reported in other cetacean species from Japanese coastal waters (Ueno et al. 2003, 2004a; Kajiwara et al. 2006): *p,p'*-DDT among DDTs, β-HCH among HCHs, *trans*-nonachlor among CHLs, and BDE-47 or BDE-154 among PBDEs were detected as predominant compounds/isomers (Table 2). Similar to the present study, relatively high proportions of BDE-154 has been detected in coastal cetaceans from Japan where Octa-BDE technical mixture had been used as major PBDE based BFR, while predominant accumulations of BDE-47, which would have been originated from Penta-BDE technical mixture, has been found in cetaceans from North American and European countries so far (Kajiwara et al. 2006).

Organotin Compounds

Organotin compounds were analyzed in the liver samples of pantropical spotted dolphin from Okinawa Island and Fraser's dolphin from Ishigaki Island. Butyltin, phenyltin and octyltin compounds were detected in the cetacean liver samples analyzed in this study (Table 4). The concentration levels of these compounds in cetaceans were higher than those in sea turtles from Nansei Shoto Islands as discussed in a later part of this report. This indicates the bioaccumulative nature of organotin compounds in cetaceans. Increasing trend in the concentrations of butyltin compounds with age until sexual maturity was reported in cetaceans (Kim et al. 1996). In addition, biomagnifications of phenyltin compounds was also observed in the marine ecosystem (Hu et al. 2006). On the other hand, concentrations of octyltin compounds, which have been extensively used as PVC stabilizers and catalysts for plastic resins, were very low or even less than the detection limits (Table 4), suggesting their relatively low bioaccumulative nature and/or less release into the marine environment in comparison with butyltins and phenyltins. Concentrations of organotin compounds in pantropical spotted dolphin from Okinawa Island were higher than those in other cetaceans from offshore waters but lower than those in a coastal species, finless porpoise, from Japanese coastal waters (Table 5). On the other hand, Fraser's dolphin from Ishigaki Island showed the lowest level of organotin residues among the cetaceans surveyed around Japan. As this species is an offshore animal, less exposure to organotin compounds can be speculated in comparison with other cetaceans. The TBT and DBT concentrations in the cetaceans investigated in this study were lower than the threshold levels reported for hapatotoxicity in rat (Ueno et al. 1994) and inhibition levels for lymphocyte proliferation by these compounds (estimated from liver: blood concentration ratio, Nakata et al. 2002).

Trace elements

Trace elements were analyzed in the muscle sample of bottlenose dolphin from Okinawa Island and the muscle, liver and kidney of pantropical spotted dolphin from Okinawa Island and Fraser's dolphin from Ishigaki Island. Among the trace elements analyzed, concentrations of Zn were the highest in the almost all the samples, and relatively high concentrations of Hg and Cd were observed in the liver and kidney, respectively (Table 6). It has been well known that toothed whales accumulate Hg to very high levels (O'Shea 1999), and Hg in the tissues was detoxified by association with Se at the equimole ratio (Koeman et al, 1973; Martoja and Berry 1980). Similar to such previous studies, equivalent mole ratios of Hg and Se were found in pantropical spotted dolphin and Fraser's dolphin in this study, indicating that Hg would be retained less toxic form. In addition, concentrations of Cd in the kidney of pantropical spotted dolphin and Fraser's dolphin were lower than the threshold levels (100 µg/g wet wt) for renal dysfunction in human (Beyer 2000). Concentrations of most trace elements including toxic metals such as Hg and Cd were lower in bottlenose dolphin than in pantropical spotted dolphin because the bottlenose dolphin analyzed in this study was a neonate specimen as shown by its body length. On the other hand, concentrations of In and Bi, which are in use at electric and electronic industries recently, were low in the cetaceans. Comparing with the data on the toothed whales reported so far, Fraser's dolphin analyzed in this study showed high concentrations of Se, Sr, Ag, Cd, Ba and Hg. Particularly, significant accumulation of Hg in this species reflects its higher trophic level and fish eating habit as also noted in the accumulation of organohalogen compounds. Concentrations of other elements in the cetaceans investigated in this study were comparable to or lower than those in toothed whales reported so far (Table 6)

2) Sea Turtles

Organohalogen compounds

Concentrations of organohalogen compounds analyzed in three different turtle species (Green turtle, Hawksbill turtle and Loggerhead turtle) in this study are shown in Table 7. Among the compounds detected, the concentrations of PCBs, DDTs and CHLs were higher than HCHs, HCB and PBDEs. This pattern is similar to cetaceans and skipjack tuna from Japanese costal waters (Ueno et al. 2003; Kajiwara et al. 2006). Interestingly, PCB and CHL levels in hawksbill turtle, which is omnivorous and mainly feed on sea sponge, showed higher levels than in loggerhead turtle, which feed on higher trophic species, cephalopods and crustaceans. This might be the result of hawksbill turtle which are mostly distributed in coastal wastes along sub tropical Asian countries where recent industrial developments has been taken place and also chemical pollution levels increase. The coastal species of hawksbill turtle may be strongly affected by the influence of local pollution in coastal areas as compared to the other species which are distributed relatively in offshore waters.

Comparing with other two species the concentrations in green turtle were relatively low, and decreasing trend in the concentrations were noted with increasing carapace length. This would be due to low tropic level feeding habits (herbivorous). During juvenile stage green turtle mainly feeds on zooplankton and then change the diet to herbivorous food when it becomes an adult. As the turtles grew, the intake of organohalogens would decrease by the change in diet with age from carnivorous to herbivorous and hence the initial concentrations gets diluted. Such a decrease in DDTs and PCBs concentrations in green turtle depending on the body length was already reported in an earlier study on this species (Mckenzie et al. 1999). On the other hand, in comparison of pollution level between the locations using the data on loggerhead turtle, no significant difference were observed in the concentrations of organohalogen compounds between Ishigaki Islands and Kochi prefecture expect for PBDEs which shows higher concentrations in the turtles from Ishigaki Islands than Kochi. In the case of global monitoring for PBDEs using skipjack tuna as a bioindicator, high levels on PBDE concentrations were found in the East China Sea and South China Sea than off Kochi (Ueno et al. 2004a). This trend was similar to our findings in the case of loggerhead turtle. In hawksbill turtle large variation in the concentrations of PBDEs were observed even in the samples from same location. Sea turtles occasionally eat plastic bags and pellets which were discarded in the sea and this may cause exposure to these compounds in some specimens. Spatial variability in the concentrations of PBDEs in hawsbill turtle may reflect various potential sources and exposure routes for PBDEs in the coastal environment. To our knowledge, this is the first report on contamination by BFRs in sea turtles and hence the results could not be discussed in detail. Further study and monitoring on sea turtles are required to elucidate their contamination status and species specific accumulation of BFRs. Concentrations of organohologen compounds in sea turtles from Nansei Island and off Kochi were relatively low as compared to those from other locations in the world (Table 8), but comparable to those in fish from off shore waters around Japan (Ueno et al. 2003).

Organotin compounds

Concentrations of organotin compounds (OTs) in sea turtles from Nansei Shoto Islands and Kochi prefecture were generally low and the levels of octyltin and phenyltin compounds were below their respective detection limits (Table 9). Among the butyltin compounds, DBT was mostly detected in the sea turtles and TBT was detected only in one specimen of loggerhead turtle from Ishigaki Island. This may suggest the high metabolic capacities to degrade TBT to DBT and other tin compounds in sea turtles. Relatively high concentrations of DBT in hawksbill turtle may be due to exposure of butyltins in coastal areas of Asian countries similar to the exposure to some organohalogen compounds as noted above. Butyltin concentrations in sea turtles observed in this study were one order lower than those in loggerhead turtle collected at Kochi prefecture in 1990, indicating decreasing trend of butyltins in the environment (Iwata et al. 1997) after the restriction on TBT in 1989/1990 in Japan. In addition butyltin levels were lower in sea turtle from Ishigaki Island and Kochi prefecture than those in cetaceans (Table 5) and fish from Japanese coastal waters (Ueno et al. 2004b). This indicates less contamination and impact by organotin compounds on sea turtles living in these regions.

Trace elements

Among the trace elements, high concentrations of Zn, Cu, Cd were detected in all the sea turtle species analyzed in this study (Table 10). Particularly green turtle showed higher concentrations of Cu whereas loggerhead turtle and leatherback turtle showed high concentrations of Cd. These trends found in the two species agreed with those reported in our previous studies on sea turtles (Sakai et al. 2000a; Anan et al. 2001). Significant accumulations of Cd in loggerhead and leatherback turtles would be due to their crustacean and cephalopod feeding habits. The elements, Ga, In and Bi, were very low which are in use at electric and electronic industries. Spatial difference was found in the concentrations of some trace elements in loggerhead turtle: the higher concentrations of Mn, Rb, Mo, Co, Pb and Sb were found in specimens from Kochi, and Sr, Ba and Ga were higher in the same species from Ishigaki Islands. The alkaline earth metals, Sr, Ba and Ga, seem to have the possibility of similar environmental sources and behavior as Ca. The accumulation pattern found in loggerhead turtle may reflect that the sea turtle in Ishigaki Islands mainly feed in the coral reef environment, which are rich in Ca along with other alkaline earth metals. Distribution of the trace elements in the marine environment is variable and element species specific. Species specific difference and spatial variations in the element concentrations of sea turtles might be reflecting the difference in feeding behavior of the sea turtles in the marine environment. These tendency and concentration levels of trace elements detected in the sea turtles in this study are comparable to the reports so far published on sea turtles from the same region (Table 11).

Conclusion

Concentrations of organohalogen and organotin compounds in cetaceans and sea turtles from Nansei Shoto Islands seems to be lower than those reported in same or related species from Japanese coastal waters and East China Sea. This result suggests that environmental impact by anthropogenic contaminants in the Nansei Shoto Islands ecosystem is not serious at present. However, information on the contamination status of BFRs in sea turtles has been scarcely reported. Relatively high concentrations of PBDEs found in hawksbill turtle suggests that further monitoring is required to elucidate the potential sources and exposure to PBDEs in the migrating routes of sea turtles and to predict the trend of contamination in future. This study found that Fraser's dolphin, fish eating species, accumulated Hg at high concentrations and loggerhead turtle from Ishigaki Island, which inhabits the coral reef environment, retained alkaline earth metals at higher concentrations than those from coast of Kochi prefecture. Therefore, it is seem that detail analysis on the accumulation pattern of the anthropogenic compounds and trace elements in wild animals can be applied to elucidate their biological information such as migration behavior, distribution and feeding habits. Further monitoring on the chemical pollution of wild animals using the interdisciplinary approach are necessary to clarify their unknown biological/ecological facts and to assess the risk of exposure to chemicals in detail.

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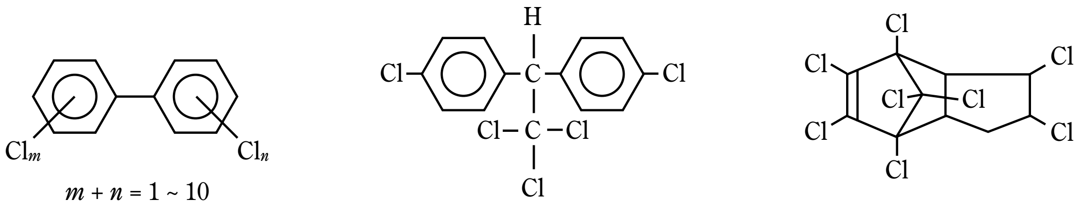
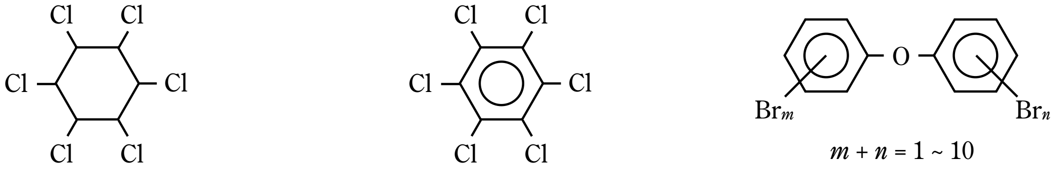
Fig. 1. Chemical structures and usage of organohalogen compounds analyzed in this study.



PCBs
Transformer/Condenser
(Insulation oil etc.)

DDTs
(*p*, *p*'-DDT, *p*, *p*'-DDE, *p*, *p*'-DDD)
Pesticide

CHLs
(*cis-/trans-* chlordane,
cis-/trans- nonachlor,
oxychlordane)
Termiticide

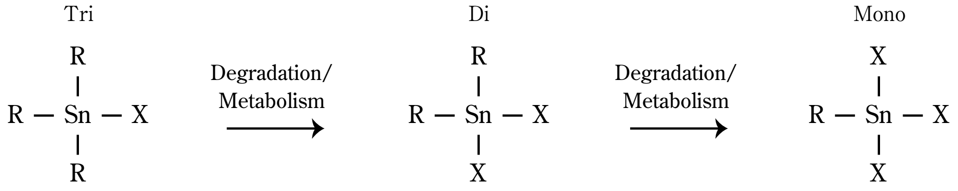


HCHs
(α, β, γ-HCH)
Pesticide

HCB
Fungicide
Synthetic intermediate

PBDEs
Flame retardant

Fig. 2. Chemical structures and usage of organotin compounds analyzed in this study.



$$R_3SnX \xrightarrow{\text{Degradation/Metabolism}} R_2SnX_2 \xrightarrow{\text{Degradation/Metabolism}} RSnX_3$$

TBT (Tributyltin)
TPT (Triphenyltin)
Antifouling
Biocide

DBT (Dibutyltin)
DOT (Diphenyltin)
PVC stabilizer
Polymer catalyst etc.

MBT (Monobutyltin)
MOT (Monophenyltin)
PVC stabilizer
Polymer catalyst etc.

R = Butyl, Phenyl, and Octyl group etc.
X = Cl and OH group etc.

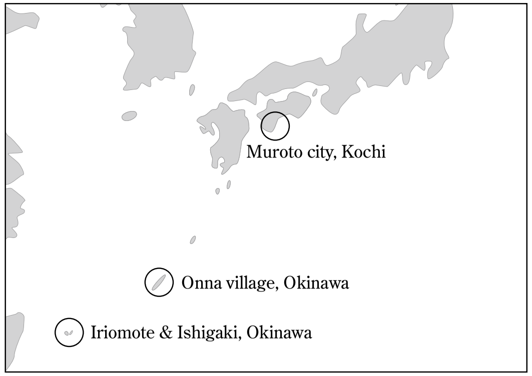
Table 1. Biological characterize of samples analyzed in this study

Species	Sample ID	Sampling locations	Year	Sex	Body length (cm)	Body weight (kg)	Tissue
Bottlenose Dolphin *(Tursiops truncatus)*	1286	Onna village, Okinawa	2003	Female	124	28.0	Muscle, Blubber
Pantropical Spotted Dolphin (*Stenella attenuata*)	1497	Onna village, Okinawa	2003	Male	198	53.8	Muscle, Liver, Kidney
Sperm Whale *(Physeter macrocephalus)*	1504	Iriomote Island, Okinawa	2003	Unknown	800	-	Blubber
Fraser's Dolphin (*Lagenodelphis hosei*)		Ishigaki Island, Okinawa	2007	Male	250	-	Blubber, Liver
Green Turtle *(Chelonia mydas)*	0304	Ishigaki Island, Okinawa	2003	Male	54.3*	-	Liver
	0324	Ishigaki Island, Okinawa	2003	Female	62.0*	-	Liver
	0332	Ishigaki Island, Okinawa	2003	Male	44.2*	-	Liver
	0424	Ishigaki Island, Okinawa	2004	Male	60.3*	-	Liver
	0428	Ishigaki Island, Okinawa	2004	Female	73.7*	-	Liver
	0522	Ishigaki Island, Okinawa	2005	Female	58.3*	-	Liver
Hawksbill Turtle *(Eretmochelys imbricata)*	0208	Ishigaki Island, Okinawa	2002	Female	37.9*	-	Liver
	0426	Ishigaki Island, Okinawa	2004	Female	31.9*	-	Liver
	0432	Ishigaki Island, Okinawa	2004	Male	31.3*	-	Liver
Loggerhead Turtle *(Caretta caretta)*	9802	Ishigaki Island, Okinawa	1998	Female	68.2*	-	Liver
	0508	Ishigaki Island, Okinawa	2005	Female	77.0*	-	Liver
	138	Muroto city, Kochi	2006	Male	70.7*	-	Liver
	139	Muroto city, Kochi	2006	Male	74.6*	61	Liver
	149	Muroto city, Kochi	2006	Female	67.8*	49	Liver
	186	Muroto city, Kochi	2006	Female	69.6*	48	Liver
Leatherback Turtle *(Dermochelys coriacea)*	221	Muroto city, Kochi	2006	Male	142.0*	260	Liver

*Carapace length

All samples were collected from dead specimens of stranding or bycatch

Sampling sites of Cetaceans and Sea Turtles



Bottlenose Dolphin

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Pantropical Spotted Dolphin

©WWF-Canon / William W. ROSSITER

Fraser's Dolphin

©Dolphin&Whale Save Project

Green Turtle

©WWF-Canon / Jürgen FREUND

Hawksbill Turtle

©WWF-Canon / Carlos DREWS

Loggerhead Turtle

©WWF-Canon / Michel GUNTHER

Leatherback Turtle

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Table 2. Concentrations of organohalogen compounds in the tissues of cetaceans from Okinawa, Iriomote and Ishigaki Islands (ng/g lipid wt.)

Species	Locations	Year	Sex	Tissue	Body Length (cm)	Lipid (%)	PCBs	DDT Compounds			DDTs	HCH Compounds			HCHs	CHL Compounds					CHLs	HCB
								p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT		α-HCH	β-HCH	γ-HCH		Oxy	t-CA	c-CA	t-nona	c-nona		
Bottlenose Dolphin	Okinawa Island	2003	Male	Blubber	124	48	**6600**	10000	1800	2500	**15000**	36	290	25	**350**	460	27	400	2300	630	**3800**	**240**
Sperm Whale	Iriomote Island	2003	Unknown	Blubber	800	7.5	**4500**	5100	710	680	**6500**	<0.1	44	<0.1	**44**	74	43	140	580	170	**1000**	**88**
Fraser's Dolphin	Ishigaki Island	2007	Male	Blubber	250	77.0	**12000**	24000	700	1000	**26000**	3.0	110	2.9	110	230	48	240	3400	520	4500	110
				Liver		4.8	**9500**	13000	540	87	**14000**	28	310	<0.1	340	110	<0.1	150	1200	210	1700	190
				Muscle		1.3	**16000**	20000	960	240	**21000**	74	270	<0.1	340	230	29	260	2200	490	3200	160

Species	Locations	Year	Sex	Tissue	Body Length (cm)	Lipid (%)	PBDE congeners									PBDEs
							BDE3	BDE15	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	
Bottlenose Dolphin	Okinawa Island	2003	Male	Blubber	124	48	<0.2	<0.2	10	200	19	35	6.0	22	<0.2	**290**
Sperm Whale	Iriomote Island	2003	Unknown	Blubber	800	7.5	<0.2	<0.2	2.4	40	5.3	8.3	6.4	21	<0.2	**83**
Fraser's Dolphin	Ishigaki Island	2007	Male	Blubber	250	77.0	<0.2	<0.2	3.5	100	15	45	67	160	4.3	**390**
				Liver		4.8	<0.2	<0.2	2.2	54	7.0	23	24	64	1.3	**180**
				Muscle		1.3	<0.2	<0.2	4.6	110	15	50	60	150	3.7	**390**

Table 3. Concentrations of organohalogen compounds in the tissues of cetaceans from Japanese and other Asian coasts (ng/g lipid wt.)

Species	locations	Year	Gonosomatic index	Sex	No. of samples	DDTs	PCBs	PBDEs	References
Sperm Whale	Iriomote Island	2003	-	-	1	6.5	4.5	83	This study
Bottlenose Dolphin	Okinawa Island	2003	Immature	Male	1	15	6.6	290	This study
Fraser's Dolphin	Ishigaki Island	2007	-	Male	1	26	12	390	This study
Killer Whale	Rausu, Hokkaido	2004	Mature	Male	1	220	57	270	Kajiwara et al. (2006)
Melon-headed Whale	Kagoshima	2001	Mature	Male	5	27 (18-33)	24 (15-30)	320 (300-340)	Kajiwara et al. (2006)
Finless Porpoise	Seto-Inland sea	1998-2001	Mature	Male	6	64 (7.5-270)	220 (44-820)	730 (410-1300)	Kajiwara et al. (2006)
Stejneger's beaked Whale	Japan Sea	1996-2001	Mature	Male	12	72 (10-130)	31 (9.7-45)	530 (390-650)	Kajiwara et al. (2006)
Dall's Porpoise	Off-Sanriku	2000	Mature	Male	5			57 (29-100)	Kajiwara et al. (2006)
Pacific humpback Dolphin	Hong Kong	1994-1997	-	Male	7	130 (67-200)	67 (43-160)		Minh et al. (1999)
Pacific humpback Dolphin	Hong Kong	1997-2001	Mature	Male	5			2200 (280-6000)	Ramu et al. (2005)
Finless Porpoise	Hong Kong	1993-1997	-	Male	3	110 (43-210)	27 (6.8-64)		Minh et al. (1999)
Finless Porpoise	Hong Kong	2000-2001	Mature	Male	4			710 (200-980)	Ramu et al. (2005)
Spinner Dolphin	Mindanao, Philippines	1996	Mature	Male	2	7.4 (6.6-8.2)	3.8 (3.6-3.9)		Minh et al. (2000)
Fraser's Dolphin	Mindanao, Philippines	1996	Mature	Male	3	11 (8.3-14)	6.3 (5.8-13)		Minh et al. (2000)

Table 4. Concentrations of organotin compounds in the tissue of cetaceans from Okinawa and Ishigaki Islands (ng/g wet wt.)

Species	Locations	Year	Sex	Body Length (cm)	BT Compounds			BTs	PT Compounds		PTs	OcT Compounds			OcTs
					MBT	DBT	TBT		DPT	TPT		MOcT	DOcT	TOcT	
Pantropical Spotted Dolphin	Okinawa Island	2003	Male	198	44	610	270	**950**	2.4	13	**15**	<0.4	<0.6	<0.6	**<0.6**
Fraser's Dolphin	Ishigaki Island	2007	Male	250	12	47	37	**96**	12	7.0	**19**	2.3	5.6	<0.6	**7.9**

Table 5. Concentrations of butyltin compounds in the liver of cetaceans from Japanese coasts (ng/g wet wt.)

Species	Locations	Year	Body Length (cm)	No. of samples	MBT	DBT	TBT	BTs	References
Pantropical Spotted Dolphin	Okinawa Island	2003	198	1	44	610	270	920	This study
Fraser's Dolphin	Ishigaki Island	2007	250	1	12	47	37	96	This study
Stejneger's beaked Whale	Niigata	1993	429	1	67	280	52	400	Tanabe et al. (1998)
Ginkgo-toothed beaked Whale	Yamagata	1993	479	1	120	130	76	330	Tanabe et al. (1998)
Baird's beaked Whale	Ayukawa	1988	930-1060	3	46.0 (17-95)	140 (80-180)	23 (9-30)	210 (110-310)	Tanabe et al. (1998)
Bottlenose Dolphin	Taiji	1986	254-287	4	480 (310-560)	1900 (1600-2100)	470 (390-540)	2800 (2600-3000)	Tanabe et al. (1998)
Rough-toothed Dolphin	Taiji	1986	169	1	450	2200	670	3300	Tanabe et al. (1998)
Dwarf sperm Whale	Toyohashi	1993	265	1	200	470	55	730	Tanabe et al. (1998)
Pygmy sperm Whale	Tottori	1997	270	1	75	120	31	230	Tanabe et al. (1998)
Killer Whale	Taiji	1986	598-636	3	710 (480-1100)	1600 (1500-1900)	180 (150-220)	2500 (2200-2700)	Tanabe et al. (1998)
Dall's Porpoise	Sanriku Coast	1995	163-195	3	97 (50-120)	430 (180-600)	230 (110-310)	760 (340-1000)	Tanabe et al. (1998)
Finless Porpoise	Seto-Inland Sea	1985	162	1	3000	6100	1100	10000	Tanabe et al. (1998)
	Ise Bay	1994	139	1	680	1800	810	3300	Tanabe et al. (1998)
	Chiba, Pacific Coast	1981	151	1	130	790	200	1100	Tanabe et al. (1998)

Table 6. Concentrations of trace metals in the tissue of cetaceans from Okinawa and Ishigaki Islands and the Brazilian coast (µg/g wet wt.)

Species	Locations	Year	Tissue	No. of samples	V	Cr	Mn	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	In	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi	References
Bottlenose Dolphin	Okinawa Island	2003	Muscle	1	0.020	0.63	0.657	0.011	4.88	111	0.57	4.87	0.203	0.040	0.002	0.002	0.002	0.016	0.01	0.09	0.008	0.43	0.079	<0.001	0.002	This study
Pantropical Spotted Dolphin	Okinawa Island	2003	Muscle	1	0.017	0.27	0.433	0.005	4.37	43.1	2.5	6.93	0.103	0.032	<0.001	0.102	0.001	0.192	<0.01	0.13	0.011	7.8	<0.001	0.004	<0.001	This study
			Liver	1	0.11	0.19	10.5	0.019	15.7	115	47	6.76	0.240	1.44	1.4	22.2	0.014	1.86	0.02	0.08	0.015	77	0.011	0.170	0.017	This study
			Kidney	1	0.021	0.15	1.66	0.011	15.4	116	29	8.01	0.531	0.219	0.19	97.7	<0.001	0.284	<0.01	0.10	0.011	24	0.014	0.034	0.009	This study
Fraser's Dolphin	Ishigaki Island	2007	Muscle	1	0.48	0.56	5.45	0.11	6.45	110	65	3.86	56.9	0.203	0.61	10.4	0.003	0.062	<0.01	0.08	0.71	170	0.005	0.201	0.005	This study
			Liver	1	0.37	0.28	11.80	0.072	45.2	128	660	4.16	21.9	2.90	8.90	64.5	0.003	0.175	<0.01	0.06	0.32	1,600	0.022	0.211	0.014	This study
			Kidney	1	0.062	0.24	2.59	0.073	19.0	93.7	30	4.21	3.07	0.163	0.30	178	0.003	0.078	<0.01	0.08	0.17	39	0.020	0.049	0.005	This study
Estuarine Dolphin	Brazilian coast	1997-1999	Liver	20	0.13	0.93	9.84	0.027	31.6	192	38	4.09	0.384	2.55	1.9	0.654			<0.01	0.08	0.009	77	0.003	0.070		Kunito et al. (2004)
Franciscana Dolphin	Brazilian coast	1997-1999	Liver	23	0.089	0.49	14.8	0.040	44.5	152	9.1	5.34	0.523	2.28	2.4	0.404			<0.01	0.08	0.008	3.5	0.002	0.026		Kunito et al. (2004)
Atlantic spotted Dolphin	Brazilian coast	1997-1999	Liver	2	0.24	0.64	14.9	0.023	40.6	361	79	4.69	0.317	2.74	1.5	30.9			0.01	0.11	0.152	140	0.004	2.58		Kunito et al. (2004)
Common Dolphin	Brazilian coast	1997-1999	Liver	1	0.30	0.42	12.4	0.043	27.7	158	30	4.33	0.128	2.95	0.82	2.55			<0.01	0.06	0.014	23	0.003	0.062		Kunito et al. (2004)
Striped Dolphin	Brazilian coast	1997-1999	Liver	1	0.061	0.23	12.3	0.041	33.4	287	190	4.31	0.299	2.34	3.2	7.83			<0.01	0.08	0.005	290	0.015	0.074		Kunito et al. (2004)

Table 7. Concentrations of organohalogen compounds in the liver of sea turtles from Ishigaki Island and coast of Kochi prefecture (ng/g lipid wt.)

Species	Sample ID	Locations	Year	Sex	Straight carapace length (cm)	Lipid (%)	PCBs	DDT Compounds			DDTs	HCH Compounds			HCHs	CHL Compounds					CHLs	HCB
								p,p'-DDE	p,p'-DDD	p,p'-DDT		α-HCH	β-HCH	γ-HCH		Oxy	t-CA	c-CA	t-nona	c-nona		
Green Turtle	0304	Ishigaki Island	2003	Male	54.3	7.7	**2.0**	0.11	<0.02	<0.02	**0.11**	0.25	0.33	<0.02	**0.59**	0.17	<0.01	<0.01	0.089	<0.01	**0.26**	**0.36**
	0332	Ishigaki Island	2003	Male	44.2	8.4	**11**	3.2	<0.02	<0.02	**3.2**	<0.01	0.97	<0.02	**1.0**	0.29	<0.01	<0.01	1.4	0.12	**1.8**	**0.60**
	0424	Ishigaki Island	2004	Male	60.3	15	**0.47**	0.15	<0.02	<0.02	**0.15**	<0.01	0.30	<0.02	**0.30**	<0.02	<0.01	<0.01	0.10	<0.01	**0.10**	**0.20**
	0428	Ishigaki Island	2004	Female	73.7	13	**0.31**	<0.02	<0.02	<0.02	**<0.02**	<0.01	2.0	<0.02	**2.0**	<0.02	<0.01	<0.01	0.20	<0.01	**0.20**	**0.36**
	0522	Ishigaki Island	2005	Female	58.3	15	**0.65**	0.69	<0.02	<0.02	**0.69**	<0.01	1.2	<0.02	**1.2**	10	<0.01	<0.01	0.61	0.050	**11**	**0.43**
Hawksbill Turtle	0208	Ishigaki Island	2002	Female	37.9	5.6	**240**	10	<0.02	<0.02	**10**	<0.01	5.7	<0.02	**5.7**	13	<0.01	<0.01	11	<0.01	**25**	**0.24**
	0426	Ishigaki Island	2004	Female	31.9	3.6	**170**	20	0.71	<0.02	**20**	<0.01	3.7	<0.02	**3.7**	6.5	<0.01	<0.01	11	0.42	**18**	**0.14**
	0432	Ishigaki Island	2004	Male	31.3	8.2	**30**	110	<0.02	<0.02	**110**	<0.01	0.33	<0.02	**0.33**	12	<0.01	<0.01	4.3	<0.01	**16**	**0.67**
Loggerhead Turtle	9802	Ishigaki Island	1998	Female	68.2	6.5	**20**	30	2.1	<0.02	**30**	<0.01	2.3	<0.02	**2.3**	1.9	<0.01	<0.01	6.7	0.61	**9.3**	**2.0**
	0508	Ishigaki Island	2005	Female	77.0	19	**20**	20	1.5	<0.02	**20**	<0.01	2.7	<0.02	**2.7**	1.4	<0.01	<0.01	5.6	0.61	**7.6**	**0.61**
	138	Kochi	2006	Male	70.7	8.5	**25**	20	0.27	0.76	**20**	0.14	1.8	<0.02	**1.9**	1.1	<0.01	<0.01	3.9	0.62	**5.9**	**0.68**
	139	Kochi	2006	Male	74.6	7.9	**15**	30	0.11	0.26	**30**	0.20	2.4	<0.02	**2.6**	0.47	<0.01	<0.01	2.9	0.18	**3.7**	**0.72**

Species	Sample ID	Locations	Year	Sex	Straight carapace length (cm)	Lipid (%)	PBDE Congeners														PBDEs
							BDE3	BDE15	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE196	BDE197	BDE206	BDE207	BDE209	
Green Turtle	0304	Ishigaki Island	2003	Male	54.3	7.7	<0.05	<0.07	<0.06	0.074	0.1	<0.05	0.21	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**0.38**
	0332	Ishigaki Island	2003	Male	44.2	8.4	<0.05	<0.07	<0.06	2.1	1.3	2.9	0.2	0.17	0.27	0.09	0.25	<0.07	<0.07	<1.5	**7.3**
	0424	Ishigaki Island	2004	Male	60.3	15	<0.05	<0.07	<0.06	0.056	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**0.06**
	0428	Ishigaki Island	2004	Female	73.7	13	<0.05	<0.07	<0.06	0.12	<0.05	<0.05	0.08	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**0.20**
	0522	Ishigaki Island	2005	Female	58.3	15	<0.05	<0.07	<0.06	0.12	<0.05	<0.05	0.16	<0.05	0.14	0.07	<0.05	<0.07	<0.07	<1.5	**0.49**
Hawksbill Turtle	0208	Ishigaki Island	2002	Female	37.9	5.6	<0.05	<0.07	<0.06	0.09	<0.05	0.11	4.2	<0.05	1.2	0.710	0.54	<0.07	<0.07	<1.5	**6.9**
	0426	Ishigaki Island	2004	Female	31.9	3.6	<0.05	<0.07	<0.06	<0.03	0.14	2.7	47	3	39	23	21	<0.07	0.45	<1.5	**140**
	0432	Ishigaki Island	2004	Male	31.3	8.2	<0.05	<0.07	<0.06	<0.03	0.06	0.2	0.81	0.09	0.25	<0.05	<0.05	<0.07	<0.07	<1.5	**1.4**
Loggerhead Turtle	9802	Ishigaki Island	1998	Female	68.2	6.5	<0.05	<0.07	<0.06	0.05	<0.05	0.13	0.29	0.33	0.33	0.21	0.13	<0.07	<0.07	<1.5	**1.5**
	0508	Ishigaki Island	2005	Female	77.0	19	<0.05	<0.07	0.1	0.08	0.08	0.18	0.53	0.43	1.1	0.96	0.76	<0.07	0.32	<1.5	**4.5**
	138	Kochi	2006	Male	70.7	8.5	<0.05	<0.07	<0.06	0.07	<0.05	0.11	0.12	0.15	0.19	<0.05	0.065	<0.07	<0.07	<1.5	**0.7**
	139	Kochi	2006	Male	74.6	7.9	<0.05	<0.07	<0.06	0.08	0.06	0.12	0.11	0.13	0.22	<0.05	0.1	<0.07	<0.07	<1.5	**0.8**

Table 8. Concentrations of organochlorine compounds in the liver of sea turtles from various locations in the world (ng/g wet wt.)

Species	Locations	Year	Life stage	Body weight (kg)	Curved carapace length (cm)	Straight carapace length (cm)	No. of samples	SPCB	SDDT	SCHLs	HCB	References
Green Turtle	Florida, USA	Unknown (1983- before)		4~12			4	<5.0-70	<1.0			McKim & Johnston (1983)
	California - peninsula, Mexico	Unknown (2003- before)		14~45			7	<3.0-44.7	<3.0-7.8	<3.0-10.4	nd-18.6	Gardner et al. (2003)
	Mediterranean sea - Cyprus	1995-96			30-57		9	<1.1-47	<1.0-21	<0.4-3.7		Mckenzie et al. (1999)
	Ishigaki Island, Japan	2003-2005		about 20-50		54-74	5	0.04-0.92	0.008-0.27	0.015-1.7	0.028-0.065	This study
Loggerhead Turtle	Florida- USA	Unknown (1983- before)		22~61			8	<5.0-133	<0.1-51			McKim & Johnston (1983)
	North Carolina- USA	Unknown (1995- before)	juvenile				17	8.3-514	<2.0-458			Rybitski et al. (1995)
	California - peninsula, Mexico	Unknown (2003- before)					1	58.1	10.4	45.3	3.5	Gardner et al. (2003)
	Mediterranean sea	1993		11~23			4	69-205				Corsolini et al. (2000)
	Mediterranean sea - Cyprus	1994-95			23-67		4	50-102	49-77	1.8-5.0		Mckenzie et al. (1999)
	UK	1995			21		1	159	152	nm		Mckenzie et al. (1999)
	Ishigaki Island, Japan	1998-2005		about 50-60		68-77	2	1.3-3.8	2.0-3.8	0.6-1.4	0.12-0.13	This study
	Kochi prefacture, Japan	2006				71-75	2	1.2-2.1	1.7-2.4	0.29-0.50	0.057-0.058	This study
Hawksbill Turtle	Ishigaki Island, Japan	2002-2004				31-38	3	2.5-13	0.56-9.0	0.65-1.4	0.005-0.055	This study
Olive Ridley Turtle	California - peninsula, Mexico	Unknown (2003- before)					1	41	<3.0	<3.0	<3.0	Gardner et al. (2003)
Kemph's Ridley Turtle	Long Island, USA	1980-89	juvenile				?	272-655				Lake et al. (1994)
Leatherback Turtle	UK	1993-95			141-151		2	3.1-3.7	9.1-14	2.3-2.3		Mckenzie et al. (1999)

Table 9. Concentrations of organotin compounds in the liver of sea turtles from Ishigaki Island and coast of Kochi prefecture (ng/g wet wt.)

Species	Sample ID	Locations	Year	Sex	Straight carapace length (cm)	MBT	DBT	TBT	MOT	DOT	TOT	DPT	TPT
Green Turtle	0304	Ishigaki Island, Japan	2003	Male	54.3	4.7	<0.2	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
	0324	Ishigaki Island, Japan	2003	Male	62.0	<4.0	13.0	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
	0332	Ishigaki Island, Japan	2003	Female	44.2	<4.0	<0.2	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
	0424	Ishigaki Island, Japan	2004	Male	60.3	<4.0	<0.2	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
	0428	Ishigaki Island, Japan	2004	Female	73.7	<4.0	0.4	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
	0522	Ishigaki Island, Japan	2005	Female	58.3	<4.0	<0.2	<0.3	<5.0	<0.3	<0.5	<0.5	<0.5
Hawksbill Turtle	0208	Ishigaki Island, Japan	2002	Female	37.9	6.8	16	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5
	0426	Ishigaki Island, Japan	2004	Female	31.9	<4.0	65	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5
	0432	Ishigaki Island, Japan	2004	Male	31.3	<4.0	51	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5
Loggerhead Turtle	9802	Ishigaki Island, Japan	1998	Female	68.2	<4.0	2.6	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5
	0508	Ishigaki Island, Japan	2005	Female	77.0	8.2	18	0.8	<0.3	<0.3	<0.5	<0.5	<0.5
	138	Kochi, Japan	2006	Male	70.7	<4.0	18	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5
	139	Kochi, Japan	2006	Male	74.6	<4.0	1.8	<0.3	<0.3	<0.3	<0.5	<0.5	<0.5

Table 10. Concentrations of trace metals in the liver of sea turtles from Ishigaki Island and coast of Kochi prefecture (µg/g dry wt.)

Species	Sample ID	Locations	Year	Sex	Straight carapace length (cm)	V	Cr	Mn	Co	Cu	Zn	Ga	Se	Rb	Sr	Mo	Ag	Cd	In	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi
Green Turtle	0304	Ishigaki Island, Japan	2003	Male	54.3	0.44	0.58	3.92	0.11	165	155	0.005	4.0	4.83	8.27	0.830	13	17.1	<0.001	0.046	0.33	<0.01	0.060	3.1	0.002	0.331	0.005
	0324	Ishigaki Island, Japan	2003	Male	62.0	0.28	0.34	3.75	0.70	70.1	84.2	0.018	1.7	1.67	7.59	0.567	6.7	6.89	<0.001	0.009	0.24	<0.01	0.23	0.42	0.001	0.175	0.004
	0332	Ishigaki Island, Japan	2003	Male	44.2	0.15	0.41	6.42	1.0	113	137	0.009	19	2.96	8.81	0.426	0.62	9.89	<0.001	0.043	0.03	<0.01	0.14	0.11	0.002	1.75	<0.001
	0424	Ishigaki Island, Japan	2004	Male	60.3	0.50	0.39	5.20	0.55	30.0	109	0.012	1.6	4.20	2.15	0.845	2.5	13.1	0.001	0.008	0.46	<0.01	0.087	0.26	0.002	0.185	0.004
	0428	Ishigaki Island, Japan	2004	Female	73.7	0.35	0.36	2.28	0.048	90.2	115	0.007	1.6	2.28	19.0	0.786	2.2	8.71	<0.001	0.018	0.47	<0.01	0.061	0.96	0.002	0.050	0.005
	0522	Ishigaki Island, Japan	2005	Female	58.3	0.78	0.37	4.00	0.62	54.8	92.5	0.014	2.2	3.00	6.98	0.819	5.3	9.89	<0.001	0.010	0.37	<0.01	0.14	0.77	0.001	0.268	0.004
Hawksbill Turtle	0208	Ishigaki Island, Japan	2002	Female	37.9	0.65	0.27	4.89	1.3	58.5	178	0.095	10	3.56	225	0.763	1.6	5.61	<0.001	0.088	0.02	<0.01	1.7	0.49	0.005	0.340	0.002
	0426	Ishigaki Island, Japan	2004	Female	31.9	0.083	0.21	10.3	0.50	32.4	152	0.003	16	6.72	1.80	0.288	0.053	2.48	0.001	0.228	< 0.01	<0.01	0.036	0.08	0.002	0.137	0.002
	0432	Ishigaki Island, Japan	2004	Male	31.3	0.21	0.27	7.37	0.82	21.2	109	0.015	20	4.53	7.25	0.528	0.45	4.14	0.001	0.226	0.02	0.01	0.30	0.48	0.002	0.141	0.002
Loggerhead Turtle	9802	Ishigaki Island, Japan	1998	Female	68.2	4.0	0.23	2.63	0.15	19.1	114	0.034	25	2.64	17.0	0.504	0.10	29.8	<0.001	0.084	0.05	<0.01	0.62	0.76	0.003	0.512	<0.001
	0508	Ishigaki Island, Japan	2005	Female	77.0	2.6	0.18	2.32	0.13	46.6	65.6	0.041	15	2.02	15.7	0.467	0.40	25.3	<0.001	0.044	0.04	<0.01	0.77	0.60	0.005	0.212	<0.001
	138	Kochi, Japan	2006	Male	70.7	6.5	0.30	8.68	0.33	28.0	112	0.010	25	12.4	1.41	0.820	0.28	41.3	<0.001	0.040	0.11	0.01	0.16	1.2	0.021	0.782	<0.001
	139	Kochi, Japan	2006	Male	74.6	5.7	0.40	6.77	0.69	16.7	95.4	0.010	20	10.1	2.73	1.49	0.043	32.2	<0.001	0.022	0.16	0.01	0.15	1.0	0.008	0.493	<0.001
	149	Kochi, Japan	2006	Female	67.8	4.7	0.27	7.60	0.31	19.7	141	0.006	25	8.27	1.76	0.834	0.18	47.0	0.001	0.025	0.10	<0.01	0.084	1.0	0.014	0.732	0.001
	186	Kochi, Japan	2006	Female	69.6	2.3	0.33	7.55	0.33	23.3	104	0.010	23	8.12	3.26	0.826	0.13	26.5	<0.001	0.036	0.07	<0.01	0.18	0.61	0.013	0.667	<0.001
Leatherback Turtle	221	Kochi, Japan	2006	Male	142.0	3.1	0.25	7.53	0.57	83.5	188	0.032	47	7.79	4.15	2.03	0.86	65.0	<0.001	0.021	0.07	<0.01	0.61	2.5	0.011	1.52	0.001

Table 11. Concentrations of trace metals in the liver of sea turtles from various locations of the world (µg/g dry wt.)

Species	No. of samples	Locations	V	Cr	Mn	Co	Cu	Zn	Ga	Se	Rb	Sr	Mo	Ag	Cd	In	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi	References
Green Turtle	6	Ishigaki Island, Japan	0.41	0.41	4.26	0.51	87.3	116	0.011	5.0	3.16	8.80	0.712	5.1	10.9	0.001	0.022	0.32	< 0.01	0.12	0.94	0.002	0.461	0.004	This study
	26	Yaeyama, Japan	0.90	2.2	4.7	0.30	139	87.0		5.2	7.10	1.40	0.60	3.2	18.0			0.22		0.70	0.40	0.002	0.500		Anan et al. (2001)
	2	Yaeyama, Japan			6.3	<0.1	37	194							27						0.60		0.2		Sakai et al. (2000a)
	50	Yaeyama, Japan			6.2	<0.1	167	101							19						1.0		<0.1		Sakai et al. (2000b)
	6	Mediterranean sea													5.9						0.60		<0.01		Godley et al. (1999)
	13	Hawaii	1.3	<0.7	5.3		304	112		2.2					17										Aguirre et al. (1994)
Hawksbill Turtle	3	Ishigaki Island, Japan	0.31	0.25	7.52	0.89	37.4	146	0.038	16	4.94	78.1	0.526	0.69	4.08	0.001	0.181	0.014	0.007	0.686	0.35	0.003	0.207	0.002	This study
	17	Yaeyama, Japan	0.30	1.0	8.20	0.60	64.0	109		45	8.10	2.10	1.30	0.50	7.40			0.09		0.5	1.1	0.007	0.2		Anan et al. (2001)
	3	Australia						59-101		11					8.0-21										Gordon et al. (1998)
Loggerhead Turtle	2	Ishigaki Island, Japan	3.3	0.20	2.48	0.14	32.9	89.7	0.037	20	2.33	16.4	0.486	0.25	27.6	<0.001	0.064	0.05	< 0.01	0.69	0.68	0.004	0.362	<0.001	This study
	4	Kochi prefacture, Japan	4.8	0.33	7.65	0.41	21.9	113	0.009	23	9.71	2.29	0.992	0.16	36.8	0.001	0.031	0.11	0.01	0.14	0.95	0.014	0.668	0.001	This study
	7	Yaeyama, Japan			6.9	<0.1	60	93							31						5		0.3		Sakai et al. (1995: 2000a)
	8	Australia						76		7.4					54						0.05				Gordon et al. (1998)
	12	Mediterranean sea		11						16					7.6						1.7		1.2		Storelli et al. (1998)
	5	Mediterranean sea													8.6						2.4		<0.01		Godley et al. (1999)
	7	Atlantic coast of France					27	83							8.6										Caurant et al. (1999)
Olive Ridley Turtle	1	Australia						49							21										Gordon et al. (1998)
Leatherback Turtle	1	Kochi prefacture, Japan	3.1	0.25	7.53	0.57	83.8	188	0.032	38	7.79	4.15	2.03	0.86	65.0	<0.001	0.021	0.07	< 0.01	0.61	2.5	0.011	1.52	0.001	This study
	1	UK		<0.06			31	132		20					88						1.2		14		Godley et al. (1998)
	1	UK					0.15	2.6		1.4					0.2						0.4		0.1		Davenport and Wrench (1990)
	18	Atlantic coast of France					29	97							23										Caurant et al. (1999)

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Introduction

Nansei Shoto Islands located in the southern end of Japanese archipelago have one of world's leading coral reefs that are home to many rare fauna and flora. It is well known as a region for animal conservation because of its high bio-diversity. On the other hand, as Nansei Shoto Islands are in close proximity to rapidly industrializing countries such as China and other Southeast Asian countries, environmental pollution and adverse effects on ecosystems of the East China Sea (ECS) by hazardous chemicals is of serious concern. In this regard, our group has conducted monitoring surveys for persistent organic pollutants (POPs) in the Asia-Pacific region using skipjack tuna (*Katsuwonus pelamis*) and mussel as bioindicators (Ueno et al. 2003; 2004a; Monirith et al. 2003). Our results indicated, pollution by POPs such as DDTs is more serious in the ECS and coastal areas of China than in other regions of Asia. Contamination levels of brominated flame retardants like polybrominated diphenyl ethers (PBDEs), which are drawing special attention at present as a new persistent pollutant, were higher in skipjack tuna samples from the ECS than those from other regions (Ueno et al. 2004a). Furthermore, relatively high concentrations of organotin compounds were found in seawater from Naha Port when compared to other Japanese coastal areas (Takeuchi et al. 2004), and hence it can be anticipated that similar levels of organotin compounds might be occurring in other ports and harbors of Nansei Shoto Islands.

In this survey, we analyzed organohalogen compounds, organotins, and trace elements in fish and shellfish from the rivers, coastal areas and offshore locations of Okinawa Island and Ishigaki Island to investigate the pollution status of aquatic ecosystems of Nansei Shoto Islands. Additionally, histological observation and measurement of vitellogenin in blood samples of two fish species were also conducted to examine the relationship with target compounds.

Materials and Method

1) Samples

Skipjack tuna (*Katsuwonus pelamis*) samples were collected in September 2005 from two locations of off-Ishigaki Island (East China Sea side: N24°38.081', E123°58.720' and Pacific Ocean side: N24°09.546', E124°20.461'). Tilapia (*Oreochromis spp.*), mullet (*Mugilidae spp.*), tropical mussel (*Modiolus au-*

riculatus), kanoko-gai (*Clithon sowerby*), common spider conch (*Lambis lambis*), mud crab (*Scylla spp.*), orange mud crab (*Scylla olivacea*) and sentinel crab (*Macrophthalmus convexus*) were also collected from Okinawa Island and Ishigaki Island between August 2005 and July 2006. Information on sampling locations, sample numbers, biometry of fish and shellfish analyzed in the present study are listed in Table 1. All the samples were stored at -20 °C until analysis.

2) Analytical Method

Analysis of target compounds was performed following the previously reported procedures (Ueno et al. 2004a; 2006; Iwamura et al. 2000; Nam et al. 2005). Organochlorines (PCBs, DDTs, CHLs, HCHs, and HCB), PBDEs, and organotins (butyltins, phenyltins, and octyltins) were quantified using GC-ECD, GC-MS, and GC-MS, respectively. Trace elements (V, Cr, Mn, Co, Cu, Zn, Ga, Se, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi) were measured using ICP-MS, CV-AAS, and HG-AAS. Chemical structures of the target compounds analyzed in this study are shown in Figures 1 and 2. In addition, vitellogenin (VTG) in the blood of fish (tilapia and mullet) were determined using Mancini method. After dissection, external observation of gonad, sex determination, mesurement of gonadal weight (GW), and histological observation were preformed. Gonad somatic index (GSI = gonadal weight/body weight) was calculated.

Statistical analysis was performed using *t*-test for parametric data and *U*-test for non-parametric data to determine differences among various locations or species. Correlations between concentrations of each compound and VTG, GW and GSI were checked using Spearman's rank correlation method.

Results and discussion

Organohalogen Compounds

1) Skipjack tuna

Organohalogen compounds were detected in all the skipjack tuna samples analyzed in this study (Tables 2 and 3). PCBs were dominant, followed by DDTs > CHLs ≥ PBDEs > HCB > HCHs. Concentrations of organohalogen compounds in muscles of skipjack tunas collected off Ishigaki Island were relatively lower than those from off-Japan, off-Taiwan, and other adjacent areas in the ECS reported previously (Ueno et al., 2003, 2004a). In addition, significantly higher levels of PCBs, CHLs, HCB, and HCHs were detected in skipjack tuna samples from the Pacific side than the ECS side of Ishigaki Island ($p<0.05$, T-test), indicating that these may be two different populations or these two sampled groups of fishes might have different migratory routes.

2) Tilapia and mullet

Organohalogen compounds were detected in all the fish samples collected in Okinawa Island and Ishigaki Island (Tables 4 and 5). Generally, CHLs and DDTs were predominant, followed by PCBs > PBDEs > HCHs > HCB. No sex difference of organohalogen levels was observed in both tilapia and mullet ($p>0.05$, U-test). Concentrations of organohalogen compounds in muscles of tilapia and mullet were relatively higher than in skipjack tuna analyzed in this study, and the levels varied among sampling sites. Especially, elevated levels of CHLs, DDTs, PCBs, and PBDEs were detected in tilapia and mullet samples from Okinawa Island, suggesting the possible existence of region-specific pollution sources of these contaminants. Eutrophication mainly by human sewage has been noticed in Manko-Noha River. In recent years, organic pollution indices tend to recover, but it is likely that organochlorines such as CHLs, which was used heavily as a termiticide in Okinawa, are still present in this river system. Into Hija River, which has the largest watershed area in Okinawa Island, domestic and agricultural sewages are discharged, and also the American army base is located along this river. Especially, elevated levels of DDTs and PBDEs and higher proportion of γ-HCH among HCH isomers were found in tilapia and mullet samples from Hija River. These accumulation patterns were notably different from those in Japanese coastal and offshore fish species reported previously (The Ministry of the Environment, 2005, 2006; Ueno et al., 2003, 2004a). The usage of γ-HCH, commercially known as lindane, was very less compared with BHC (technical HCH) in Japan. Considering these observations, the above results imply that lindane and DDTs might have been used in the American army base in the past. In addition, it has been reported that contamination levels of PBDEs were pronounced in aquatic ecosystem in and around North America, where pentaBDE products have been heavily consumed as flame retardants (Dodder et al., 2002; Rayne et al., 2003), and hence it can be suspected that PBDE sources are present in the American army base. PBDE levels detected in tilapia and mullet samples from Hija River were comparable to those in fish species from North America and higher than those from Japan (Akutsu et al., 2001; Ueno et al., 2004a). Furthermore, elevated levels of PBDEs were also found in tilapia and mullet samples from Noha and Shikaza Rivers, indicating extensive pollution of PBDEs in Okinawa Island. Concentrations of DDTs, CHLs, and HCHs in tilapia and mullet samples from Okinawa Island were also higher than those in Japanese coastal and offshore fish species reported previously (The Ministry of the Environment, 2005, 2006; Ueno et al., 2003). Especially, CHLs in Okinawa fish samples showed the highest levels when compared to all the previous data available in Japanese fish species, indicating the heavy use of technical CHL in Okinawa Island in the past. In fact, it was reported that 59 tons of technical CHL were sold in Okinawa during 1979, and CHLs accumulated at very high levels in fish species of Okinawa region (Ohshiro, 1981).

Different accumulation profiles of CHLs and PCBs were observed between tilapia and mullet. In general, ratios of *cis*-chlordane to oxychlordane were higher in mullet than in tilapia, and higher residue levels of PCBs were found in samples from Manko-Noha River System. Mullets are bottom dwelling species, whereas tilapia feeds on the water column, and hence the different profiles of organochlorines between these fish species may be attributed to exposure from their ambient sediment. Under reductive condition of the sediment, it is likely that *cis*-chlordane is less degraded to oxychlordane. Additionally, paints containing PCBs were used for Naha Bridge, which was built on Manko-Noha River. Therefore, it can be suspected that large amounts of PCBs are present in the sediment of this river system. Concentrations of PCBs in mullet samples from Manko-Noha River were comparable to those in fish species collected from Tokyo Bay and Osaka Bay, close to metropolitan areas (The Ministry of the Environment, 2005, 2006).

3) Invertebrate species

Organohalogen compounds were detected in all the invertebrate species analyzed in this study (Tables 6 and 7). Organohalogen concentrations in the invertebrates, except mud crab from Manko-Noha River, were comparable to those in tilapia and mullet from Ishigaki Island and lower than those in fish species from Okinawa Island. In addition, no difference of organohalogen levels in shelled organisms was found between the sampling areas (Okinawa Island - Ishigaki Island). This may be attributed to restricted habitat of these species, whereas fishes such as tilapia and mullet reflect organohalogen pollution from estuary and brackish-water regions, where land-based contaminants are predominant. Considering these observations, it can be suspected that organohalogen compounds are accumulating even now in the estuary and brackish-water regions of Okinawa Island, especially Manko-Noha River System. In fact, similar or even higher levels of organohalogen compounds were found in mud crabs from Manko-Noha River, compared with the concentrations of these contaminants in tilapia and mullet from the same river system.

Organotin Compounds

1) Skipjack tuna

DBT and TBT were detected in all the skipjack tuna samples analyzed in this study (Table 8). In addition, MBT, MOT, and DPT were detected in some samples. Concentrations of butyltins (BTs = MBT+DBT+TBT) in samples collected off Ishigaki Island were relatively lower than those from off-Japan and other adjacent areas in the ECS (Ueno et al., 2004b). In addition, significantly higher levels of BTs were detected in skipjack tuna samples from the ECS side than the Pacific side of Ishigaki Island ($p<0.01$), indicating the existence of two different populations of this fish species or different migratory routes between these two sampled groups, as also described in organohologen section. For organohalogen compounds, however, levels in samples from the Pacific side were significantly higher than from the ECS side, indicating different pollution sources and environmental behavior between organohalogens and BTs.

2) Tilapia and mullet

MBT, DBT, TBT, MOT, and DOT were detected in some tilapia samples collected in Okinawa Island and Ishigaki Island (Table 9). In addition, MBT, DBT, TBT, and TPT were detected in some mullet samples from both sampling sites (Table 9). MBT, DBT, MOT, and DOT were detected in more specimens of tilapia from Manko-Noha River. Considering the fact that mono- and di-organotin compounds have been used as PVC stabilizers and polymer catalysts, it can be suspected that domestic sewage into Manko-Noha River System may be the main pollution source. On the other hand, organotin levels detected in mullet samples were close to detection limit values and no regional difference was observed in this species. Concentrations of organotin compounds in tilapia and mullet were comparable to or lower than those in other Japanese fish species (The Ministry of the Environment, 2005; Yamabe et al., 2004, 2005).

3) Invertebrate species

MBT and MOT were detected in some shell species collected from Okinawa Island and Ishigaki Island, but the levels were close to detection limit values. In addition, organotin concentrations in all the crab samples were below detection limit values (Table 10).

Trace elements

1) Skipjack tuna

Results obtained on the trace element levels in skipjack tuna from off Ishigaki Island showed that concentrations of Zn in muscle and liver tissues were the highest among trace elements, followed by Rb, Cu, and Cs in muscle and Cd, Cu, and Se in liver (Table 11). Concentrations of Cu, Se, Sr, Mn, Cs, and Co in muscle and Zn, Se, Sr, and Tl in liver of skipjack tuna from the Pacific were significantly higher than those from the ECS ($p<0.05$, T-test). On the other hand, the opposite trend was observed for Rb concentrations in muscle ($p<0.05$, T-test). Although the reason for such regional differences of these elements is still unclear, our results may provide a representative baseline data on the distribution of trace elements in the study area.

2) Tilapia and mullet

Among the trace elements analyzed in the present study, Zn was the highest in the muscle of tilapia and mullet from Okinawa Island and Ishigaki Island, followed by Rb (Table 12). Moreover, concentrations of many trace elements (e.g., Mn, Cu, Rb, Ag, Bi, and Hg in tilapia and Mn, Co, and Rb in mullet) in fishes from Ishigaki Island were higher than those from Okinawa Island ($p<0.05$, U-test). We could not indicate the factors effecting such regional differences. Further investigation on the differences in the distribution and the contamination status of trace elements in the present study area is needed.

Species differences of trace element concentrations in muscles of skipjack tuna, tilapia, and mullet were observed: Rb was high in tilapia and mullet while Cu was high in skipjack tuna. Watanabe and Tanabe (2003) reported that concentrations of Rb in freshwater fish were higher than those in marine fish. Thus, the differences in trace element concentrations among different species in this study can be attributed to the species differences in the uptake, depending on salinities of brackish water, coastal and oceanic regions from where these species were collected. It could also be seen that low concentrations of Hg and high levels of Cd and Cu in muscles occur in skipjack tuna which might be due to the fact that they mainly feed on lower invertebrates. For other trace elements, the levels were similar among fish species. Concentrations of toxic elements such as Cd, Hg, and Pb in skipjack tuna, tilapia, and mullet were comparable to those in marine fish from Japan (Watanabe and Tanabe, 2003) and Southeast Asia (Agusa et al., 2007).

3) Invertebrate species

Strontium, which is chemically similar to Ca, was found to be the highest in soft tissues of shellfishes, followed by essential elements including Zn and Cu (Table 13). In the shellfish specimens of same species, concentrations of V, Ag, Ba, and Tl were high in Okinawa Island and the Rb concentrations were high in Ishigaki Island ($p<0.05$, U test). The highest concentration of Zn was found in muscles of crab, followed by Sr and Cu (Table 13). Concentrations of Mn, Co, Cu, Zn, Rb, Mo, Cd, Cs, Ba, and Hg in crabs from Ishigaki Island were higher than those from Okinawa Island, while the levels of Ag and Pb showed opposite trends ($p<0.05$, U test). The finding that concentrations of many trace elements were higher in shellfishes from Ishigaki Island than Okinawa Island is consistent with those found in fishes.

Similar results could be seen in shellfishes. Ag concentrations in crabs from Okinawa Island were higher than those in Ishigaki Island. Because Ag is recently used in fungicides, the burden is higher in animals from main island which is having a larger population. Mud crabs accumulated one order higher levels of Cu, Zn, Mn, Cd, and Ag than fishes. Concentrations of trace elements in soft tissue of shellfishes were higher than those in muscle of fishes and orange mud crabs. It is known that shellfishes concentrate many trace elements such as Cu, Zn, Ag, Pb, and V in their hepatopancreas (Rainbow, 1996). Watanabe et al. (2002) also found that concentrations of many trace elements in invertebrates were higher than those in fishes. Results obtained in this study are almost consistent with these previous studies.

Relationships among gonad abnormality, GSI, and VTG

Some abnormalities in gonads such as blister, discoloration, and agglutination were observed in specimens of tilapia from Ishigaki Island, and Hija and Manko-Noha River System of Okinawa Island (Table 14). However, no clear relation was found between abnormal gonads and concentrations of chemicals analyzed in this study and thus these aberrations might have been resulted from other factors.

Vitellogenins were detected only in female tilapias. There was no correlation between concentrations of VTG and chemicals measured in this study. On the other hand, concentrations of HCHs were negatively correlated with GSI in male tilapias ($p<0.05$, Speaman's Rank Correlation Test)(Fig.3). In vivo studies using medaka or guppy indicate that β-HCH act like an estrogenic substance, which induces VTG production or formation of ovotestis in male fishes (Wester et al., 1985; Wester and Canton, 1986). Although VTG production could not be detected in male tilapia in this study, it can be presumed that HCHs might affect gonadal development in fishes of this area.

Risk assessment for human and piscivorous birds

The Canadian Government has recently assessed organochlorine risks via food intake and calculated tolerable daily intake (TDI) as follows; DDTs = 20 μg/kg bw/day, PCBs (Sum of main 14 isomers) = 1μg/kg bw/day, HCHs (Sum of α-, β-, γ-isomers) = 0.3 μg/kg bw/day, CHLs = 0.05 μg/kg bw/day (Oostdam et al., 2005). In addition, allowable daily intake (ADI) for TBT proposed by the Ministry of Health and Welfare in Japan is 1.6 μg/kg bw/day (Sugita, 1992). Tolerable average residue level (TARL) in fish used for human consumption can be estimated using the following formula.

$$\mathrm{TARL}\ (\mu g/g) = \mathrm{TDI}\ (\mu g/kg\ bw/day) * \text{body weight (kg)} \ / \ \text{daily intake of fish (g/day)}$$

Assuming that body weight and daily intake of an adult are 60 kg and 110g (average maximum intake suggested by a national nutrition survey conducted by the Ministry of Health and Welfare), respectively, TARLs of organochlorines and TBT for human are found as follows: DDTs = 11 μg/g, PCBs = 0.55 μg/g, HCHs = 0.16 μg/g, CHLs = 0.027 μg/g, and TBT = 0.87 μg/g (wet basis). In this study, the highest concentrations (wet basis) of DDTs, PCBs, HCHs, CHLs, and TBT detected in fish and invertebrate species were 0.105 µg/g (tilapia from Manko-Noha River), 0.093 µg/g (mullet from Manko-Noha River), 0.00061µg/g (orange mud crab from Ishigaki Island), 0.095µg/g (tilapia from Hija River), and 0.020 µg/g (skipjack tuna from off-Ishigaki), respectively, These levels were below TARLs, except for CHLs. Hg concentrations in the muscles of fish and invertebrate species analyzed in this study (maximum value of 0.35 µg/g wet wt. was found in orange mud crab) were also below the provisional regulation value (0.4 µg/g wet wt.) for food proposed by the Ministry of Health, Labour, and Welfare of Japan. TARLs mean that adverse effects by contaminants may not occur even though human continued consuming the food with TARL through the lifetime. Fish specimens analyzed in this study were not from the food market and fish culture. Usage of CHLs in Japan was prohibited in 1986, hence the CHL levels exceeding TARL in some fish do not mean significant risk for human health, but it must be better to reduce the contamination levels in the ecosystem of Okinawa Island in view of environmental conservation.

Meanwhile, Hinck et al. (2006a, 2006b) calculated no effect hazard concentration (NEHC) of organochlorines in fish for piscivorous birds by using no observed adverse effect level (NOAEL) reported in experimental animals, and the body weight and daily intake of fish-eating birds, using the following formula.

NEHC (µg/g) = NOAEL (mg/kg bw/day) * body weight (kg) / daily intake of prey fish (kg/day)

NEHCs of *p,p'*-DDE, PCB (Aroclor1260) and Hg were estimated to be 0.07, 1.4 and 0.05µg/g for osprey, and 0.003, 0.05 and 0.002µg/g for belted kingfisher, respectively (Hinck et al., 2006a, 2006b). NEHCs are several orders of magnitude lower than TARLs for human, because it is known that avian species are more sensitive to *p,p'*-DDE and Hg toxicities. Therefore, avian species are considered to be important bioindicators for risk assessments of toxic substances in the ecosystem (ospreys, common kingfisher, herons, and gulls inhabit Nansei Shoto Islands as main piscivorous birds). Despite of no available data on NOEL and NEHC of CHLs for avian species, CHLs were detected at concentrations ranging from 0.09 to 18.4 µg/g in avian feed (beetles) collected from the areas where chlordane poisonings of large number of wild birds was observed in New Jersey and Ohaio States, U.S.A. (Stansley et al., 2001).

When concentrations of organochlorines detected in fish and invertebrate species from Okinawa Island and Ishigaki Island were compared with NEHCs, Hg and *p,p'*-DDE levels in some mud crab and fish samples were higher than the NEHCs for osprey and kingfisher, and PCB levels in some fish were also higher than the NEHCs for kingfisher (Fig. 4). The maximum concentration of CHLs (0.095 µg/g) in fish was comparable to the lower levels found in beetles from the chlordane polluted fields. NEHCs mean that toxic effects by contaminants may not occur even though piscivorous species continued consuming the prey fish with NEHC through their lifetime. However, it is unclear whether toxic effects will occur if piscivorous birds feed on prey fish containing the pollutants at higher levels than the NEHC. The above results suggest that further studies on risks of toxic substances for piscivorous birds inhabiting Nansei Shoto Islands are indispensable.

Conclusion

Contamination by organohalogens, especially CHLs and DDTs, were prominent in fish and shellfish collected from Okinawa Island rather than from Ishigaki Island and offshore areas, indicating that even now relatively greater pollution sources of these contaminants are present in Okinawa Island. In addition, higher proportion of γ-HCH among HCH isomers was found in fish species from Hija River, along which the American army base is located. This implies the past use of lindane for army base activities, because very low quantity of this pesticide was used in Japan. PBDEs were relatively higher in fish and shellfish species from Okinawa Island compared with those from other Japanese locations reported previously. It is suspected that use of flame-retardant plastic and fabric products imported from US may be a main pollution source. Furthermore, elevated PCB levels were detected in mullet samples from Manko-Noha River, indicating the presence of region-specific pollution sources in Okinawa Island. Significantly negative correlation between HCH concentrations and gonad somatic indices (GSI) was observed in male tilapias, and hence additional testing and further studies on the consequences of endocrine disruption are needed in this species. In addition, Hg and *p,p'*-DDE levels in some fish and shellfish samples exceeded NEHCs for piscivorous birds. Considering these results, further ecotoxicological monitoring is necessary to assess the risk on wildlife in Nansei Shoto Islands in detail.

Contamination levels by organotin compounds were relatively low in fish and shellfish from Okinawa Island and Ishigaki Island. But, elevated concentrations of some trace elements including Hg were detected in tilapia and orange mud crab samples from Ishigaki Island. Further studies on the sources of trace elements are essential to elucidate why relatively higher concentrations of some trace elements including Hg were observed in the Ishigaki ecosystem. Recently, it was reported that about half of the Hg released into the atmosphere is from human-induced sources and Hg release is increasing in the Asian region, especially China, where the industrial activities are rapidly expanding (Pacyna et al., 2006). Although it is anticipated that human-induced sources of trace elements are less in Ishigaki Island, it is highly possible that pollutants released from countries such as China located along the ECS reach as far as this Island. Therefore, it is crucial to pay attention to pollution trend not only in these island areas (i.e. Okinawa Island and Ishigaki Island) but also in the neighboring countries, especially China, to preserve Nansei Shoto Island ecosystem. Cross-border network formation for environmental monitoring and ecosystem conservation in East Asian regions is an urgent issue.

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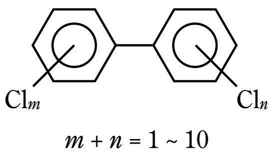
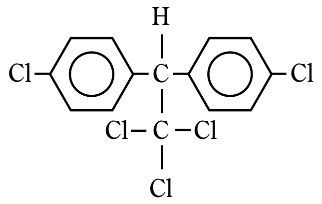
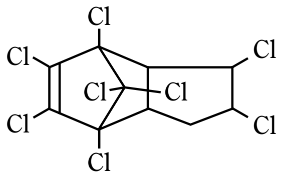
Fig. 1. Chemical structures and usage of organohalogen compounds analyzed in this study.



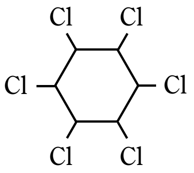
PCBs
Transformer/Condenser
(Insulation oil etc.)



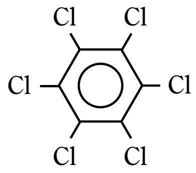
DDTs
(*p, p'*-DDT, *p, p'*-DDE, *p, p'*-DDD)
Pesticide



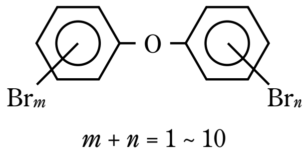
CHLs
(*cis-/trans-* chlordane,
cis-/trans- nonachlor,
oxychlordane)
Termiticide



HCHs
(α, β, γ-HCH)
Pesticide

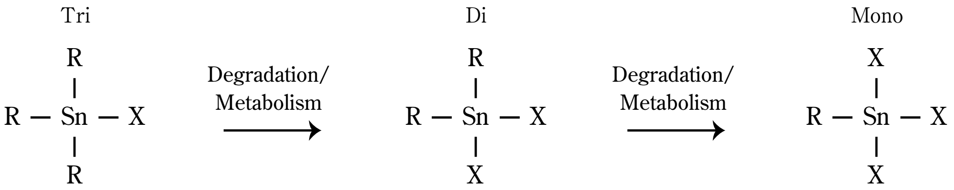


HCB
Fungicide
Synthetic intermediate



PBDEs
Flame retardant

Fig. 2. Chemical structures and usage of organotin compounds analyzed in this study.



$$\underset{\text{Tri}}{\begin{array}{c} R \\ | \\ R - Sn - X \\ | \\ R \end{array}} \xrightarrow[\text{Metabolism}]{\text{Degradation/}} \underset{\text{Di}}{\begin{array}{c} R \\ | \\ R - Sn - X \\ | \\ X \end{array}} \xrightarrow[\text{Metabolism}]{\text{Degradation/}} \underset{\text{Mono}}{\begin{array}{c} X \\ | \\ R - Sn - X \\ | \\ X \end{array}}$$

TBT (Tributyltin)
TPT (Triphenyltin)
Antifouling
Biocide

DBT (Dibutyltin)
DOT (Diphenyltin)
PVC stabilizer
Polymer catalyst etc.

MBT (Monobutyltin)
MOT (Monophenyltin)
PVC stabilizer
Polymer catalyst etc.

R = Butyl, Phenyl, and Octyl group etc.
X = Cl and OH group etc.

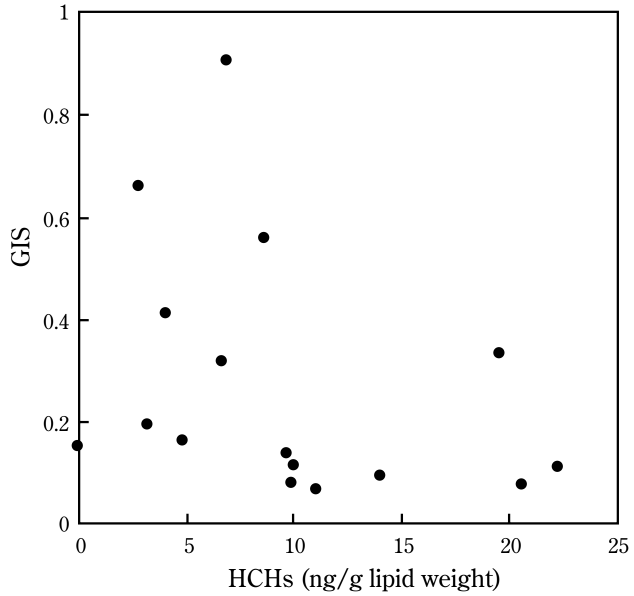


Fig. 3. Relationship between HCHs (sum of α, β, and γ isomers) concentrations and gonad-osomatic index (GSI) in males of tilapia (p<0.05, Spearman' s rank correlation).

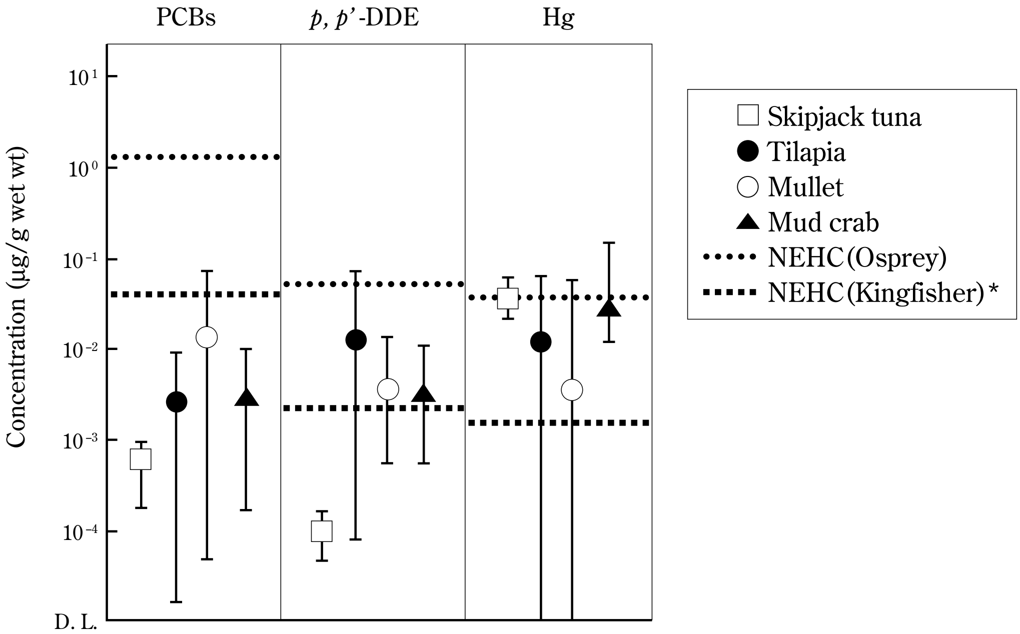


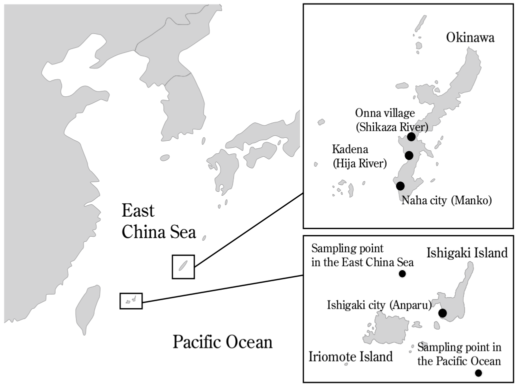
Fig. 4. Comparison between the concentrations of PCBs, *p,p'*-DDE and Hg in fish and shellfish from Okinawa Island and Ishigaki Island and the no effect hazard concentrations (NEHCs) for osprey and kingfisher

[*NEHCs for kingfisher was cited from the data for belted kingfisher presented by Hinck et al. (2006a, 2006b)]

Table 1. List of samples analyzed in this study

Species	Sampling site	Years of collec-tion	Number of samples	Body length (mm)	Body weight (g)	Analyzed tissue
Skipjack tuna (*Katsuwonus pelamis*)	Off Ishigaki Island (Pacific side)	2005	5	367±7 (360–375)	1146±67 (1038–1211)	Muscle/Liver
	Off Ishigaki Island (East China Sea side)	2005	5	366±8 (355–375)	1196±73 (1117–1313)	Muscle/Liver
Tilapia (*Oreochromis spp.*)	Okinawa-Manko (Noha River)	2006	8	240±17 (208–268)	476±92 (340–628)	Muscle
	Okinawa-Kadena (Hija River)	2006	8	232±31 (189–272)	452±148 (265–650)	Muscle
	Okinawa-Onna village (Shikaza River)		8	194±30 (163–228)	249±106 (142–379)	Muscle
	Ishigaki Island (Anparu mudflat)	2005	8	243±23 (205–282)	393±162 (304–830)	Muscle
Mullet (*Mugilidae spp.*)	Okinawa-Manko (Noha River)	2006	4 (1 pool)	196±37 (147–225)	163±78 (65–238)	Muscle
	Okinawa-Kadena (Hija River)	2006	6	273±12 (257–291)	359±81 (276–508)	Muscle
	Okinawa-Onna village (Shikaza River)		5 (1 pool)	122±17 (98–141)	39±15 (20–60)	Muscle
	Ishigaki Island (Anparu mudflat)	2005	5 (1 pool)	150±35 (120–200)	83±62 (35–180)	Muscle
Tropical mussel (*Modiolus auriculatus*)	Okinawa-Onna village	2006	311 (1 pool)	-	-	Soft tissue
	Ishigaki Island	2005	99 (1 pool)	-	-	Soft tissue
Kanoko-gai (*Clithon sowerby*)	Okinawa-Onna village	2005	392 (1 pool)	-	-	Soft tissue
	Ishigaki Island	2005	712 (1 pool)	-	-	Soft tissue
Common spider conch (*Lambis lambis*)	Okinawa-Onna village	2006	6 (1 pool)	-	-	Soft tissue
	Ishigaki Island	2005	11 (1 pool)	-	-	Soft tissue
Mud crab (*Scylla spp.*)	Okinawa-Manko (Noha River)	2006	6 (1 pool)	-	-	Muscle/Hepatopancreas
Orange mud crab (*Scylla olivacea*)	Ishigaki Island	2005	9 (2 pool)	-	-	Muscle/Hepatopancreas
Sentinel crab (*Macrophthalmos convexus*)	Ishigaki Island	2005	<100 (1 pool)	-	-	Whole body

Sampling Site of Fish and Shellfish



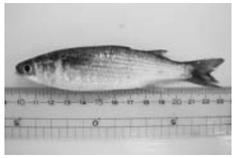
Skipjack tuna

©Ehime University CMES

Tilapia

©Ryukyu University/ Katsuko SAKAKI

Mullet



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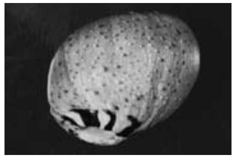
Tropical mussel

©Ehime University CMES

Kanoko-gai



©Jun NAWA

Common spider conch

©WWF Japan/ Shigeki YASUMURA

Orange mud crab

©Ehime University CMES

Sentinel crab

©WWF Japan/ Masahiro KOBAYASHI

Table 2. Concentrations (ng/g lipid wt.) of organochlorine compounds in muscles of skipjack tuna collected off Ishigaki Island

Sample ID	Sampling site	Lipid(%)	PCBs	DDT Compounds				HCH Compounds				CHL Compounds						HCB
				p,p'-DDE	*p,p'*-DDD	*p,p'*-DDT	DDTs	α-HCH	β-HCH	γ-HCH	HCHs	Oxy	*t*-CA	*c*-CA	*t*-nona	*c*-nona	CHLs	
2005 PC-ST01	Pacific Ocean	0.63	84	19	1.8	24	45	<0.3	1.1	<0.1	1.1	1.1	1.1	1.7	3.4	0.95	8.3	3.3
2005 PC-ST02	Pacific Ocean	0.94	41	9.2	1.8	7.1	18	<0.3	0.94	<0.1	0.94	1.7	1.0	1.6	1.9	1.2	7.4	1.7
2005 PC-ST03	Pacific Ocean	0.66	150	45	5.1	32	82	<0.3	0.71	<0.1	0.71	0.87	1.7	2.5	2.1	2.0	9.2	4.6
2005 PC-ST04	Pacific Ocean	0.73	95	7.5	1.5	9.3	18	<0.3	0.61	<0.1	0.61	8.1	1.5	2.3	4.9	1.5	18	3.9
2005 PC-ST05	Pacific Ocean	0.69	99	41	4.7	21	67	<0.3	1.0	<0.1	1.0	16	1.5	2.2	4.7	1.9	26	2.4
2005 ECS-ST01	East China Sea	1.6	28	8.8	8.9	5.8	24	<0.3	0.55	<0.1	0.55	0.38	0.56	0.75	1.5	0.30	3.5	1.0
2005 ECS-ST02	East China Sea	1.2	79	16	21	19	56	<0.3	0.61	<0.1	0.61	1.7	1.0	1.5	2.9	0.86	8.0	3.0
2005 ECS-ST03	East China Sea	1.5	35	9.4	9.4	8.2	27	<0.3	0.57	<0.1	0.57	0.11	0.45	0.57	1.4	0.38	2.9	1.6
2005 ECS-ST04	East China Sea	2.1	23	8.7	9.9	6.2	25	<0.3	0.43	<0.1	0.43	0.70	0.45	0.71	1.7	0.51	4.1	1.5
2005 ECS-ST05	East China Sea	1.4	51	14	9.8	9.6	33	<0.3	0.60	<0.1	0.60	0.23	0.70	0.92	0.94	0.38	3.2	1.4

Table 3. Concentrations (ng/g lipid wt.) of PBDEs in muscles of skipjack tuna collected off Ishigaki Island

Sample ID	Sampling site	Lipid(%)	BDE 3	BDE 15	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	ΣPBDEs
2005 PC-ST01	Pacific Ocean	0.63	<0.1	<0.1	0.12	1.7	0.63	0.83	0.60	1.7	<0.1	5.6
2005 PC-ST02	Pacific Ocean	0.94	<0.1	<0.1	0.16	2.4	0.94	1.1	0.75	1.9	<0.1	7.3
2005 PC-ST03	Pacific Ocean	0.66	<0.1	<0.1	0.13	1.5	0.69	0.75	0.51	1.1	<0.1	4.7
2005 PC-ST04	Pacific Ocean	0.73	<0.1	<0.1	0.08	1.3	0.44	0.58	0.48	1.0	<0.1	3.9
2005 PC-ST05	Pacific Ocean	0.69	<0.1	<0.1	0.10	1.6	0.36	0.53	0.29	0.82	<0.1	3.7
2005 ECS-ST01	East China Sea	1.6	<0.1	<0.1	0.26	3.1	0.64	1.1	0.55	1.4	<0.1	7.1
2005 ECS-ST02	East China Sea	1.2	<0.1	<0.1	0.15	1.5	0.49	0.75	0.65	1.3	<0.1	4.8
2005 ECS-ST03	East China Sea	1.5	<0.1	<0.1	0.31	4.9	2.0	2.6	1.7	4.2	<0.1	16
2005 ECS-ST04	East China Sea	2.1	<0.1	<0.1	0.15	1.5	0.42	0.81	0.42	0.84	<0.1	4.1
2005 ECS-ST05	East China Sea	1.4	<0.1	<0.1	0.12	1.3	0.22	0.46	0.37	0.66	<0.1	3.1

Table 4. Concentrations (ng/g lipid wt.) of organochlorine compounds in muscles of tilapia and mullet collected from main Okinawa and Ishigaki Islands

Sample ID	Sampling site	Sex	Lipid (%)	PCBs	DDT Compounds				HCH Compounds				CHL Compounds						HCB
					p,p'-DDE	p,p'-DDD	p,p'-DDT	DDTs	α-HCH	β-HCH	γ-HCH	HCHs	Oxy	*t*-CA	*c*-CA	*t*-nona	*c*-nona	CHLs	
Tilapia																			
5	Ishigaki Island	female	0.19	**30**	50	7.0	3.1	**60**	< 3.5	2.7	< 3.8	**2.7**	5.4	<0.08	4.9	39	11	**60**	**0.84**
13	Ishigaki Island	female	0.18	**40**	150	29	6.0	**180**	< 3.5	5.2	< 3.8	**5.2**	10	<0.08	7.7	28	18	**64**	**0.43**
19	Ishigaki Island	female	0.18	**20**	40	8.1	4.9	**50**	< 3.5	4.6	< 3.8	**4.6**	2.0	<0.08	5.6	9.4	5.4	**22**	**0.95**
102	Ishigaki Island	female	0.25	**10**	40	10	4.6	**55**	< 3.5	2.6	< 3.8	**2.6**	4.8	<0.08	5.1	19	11	**40**	**0.43**
7	Ishigaki Island	male	0.20	**15**	90	36	14	**140**	< 3.5	3.2	< 3.8	**3.2**	17	<0.08	9.5	29	25	**80**	**0.54**
21	Ishigaki Island	male	0.18	**10**	70	20	8.0	**100**	< 3.5	4.9	< 3.8	**4.9**	13	<0.08	7.2	24	19	**63**	**< 0.08**
43	Ishigaki Island	male	0.11	**25**	125	40	10	**175**	3.6	16	< 3.8	**20**	19	<0.08	11	23	18	**72**	**0.54**
44	Ishigaki Island	male	0.16	**35**	85	3.6	2.3	**90**	< 3.5	4.1	< 3.8	**4.1**	1.0	<0.08	3.5	23	5.6	**34**	**1.7**
716	Okinawa-Kadena (Hija River)	female	0.52	**1200**	4700	330	70	**5100**	< 3.5	21	16	**37**	420	105	410	3500	490	**4800**	**6.3**
722	Okinawa-Kadena (Hija River)	female	1.9	**550**	4800	590	110	**5500**	< 3.5	6.4	5.3	**12**	450	220	735	3000	550	**5000**	**10**
734	Okinawa-Kadena (Hija River)	female	0.74	**620**	3100	530	100	**3700**	< 3.5	17	16	**33**	420	290	730	2000	570	**4000**	**1.4**
737	Okinawa-Kadena (Hija River)	female	0.32	**340**	1400	260	67	**1700**	< 3.5	19	20	**39**	310	90	250	1100	320	**2000**	**1.7**
725	Okinawa-Kadena (Hija River)	male	2.0	**350**	2100	350	45	**2500**	< 3.5	8.2	5.9	**14**	300	180	560	1600	350	**3000**	**7.9**
730	Okinawa-Kadena (Hija River)	male	2.9	**350**	2900	380	90	**3400**	< 3.5	6.1	5.0	**11**	310	200	560	1800	400	**3200**	**1.8**
801	Okinawa-Kadena (Hija River)	male	0.74	**820**	5700	710	180	**6600**	< 3.5	9.3	13	**22**	500	230	560	3000	880	**5100**	**0.85**
802	Okinawa-Kadena (Hija River)	male	2.5	**380**	1300	290	89	**1700**	< 3.5	12	8.7	**21**	330	210	580	1500	360	**3000**	**1.3**
601	Okinawa-Manko (Noha River)	female	0.19	**900**	530	56	17	**600**	< 3.5	< 2.4	< 3.8	**<3.8**	160	25	52	700	310	**1300**	**< 0.08**
612	Okinawa-Manko (Noha River)	female	0.90	**1300**	640	440	77	**1200**	3.7	9.9	< 3.8	**14**	240	480	840	1400	520	**3400**	**0.51**
628	Okinawa-Manko (Noha River)	female	1.0	**900**	670	280	84	**1000**	< 3.5	5.1	< 3.8	**5.1**	420	360	770	1900	710	**4200**	**< 0.08**
635	Okinawa-Manko (Noha River)	female	0.99	**650**	310	100	30	**450**	< 3.5	< 2.4	< 3.8	**<3.8**	330	250	570	1800	510	**3500**	**< 0.08**
604	Okinawa-Manko (Noha River)	male	0.42	**1100**	1300	570	110	**2000**	< 3.5	8.7	< 3.8	**8.7**	730	1400	2200	4200	1500	**10000**	**< 0.08**
605	Okinawa-Manko (Noha River)	male	0.24	**630**	760	260	61	**1100**	< 3.5	< 2.4	< 3.8	**<3.8**	630	820	1520	3400	1200	**7600**	**< 0.08**
634	Okinawa-Manko (Noha River)	male	0.56	**1000**	350	140	24	**510**	< 3.5	6.9	< 3.8	**6.9**	500	190	660	2400	820	**4600**	**1.3**
636	Okinawa-Manko (Noha River)	male	0.32	**2500**	1300	240	61	**1600**	< 3.5	2.8	< 3.8	**2.8**	390	110	330	3200	1200	**5200**	**< 0.08**
239	Okinawa-Onna village (Shikaza River)	female	1.2	**220**	630	120	43	**790**	0.89	6.6	0.22	**7.7**	110	57	160	650	180	**1200**	**< 0.08**
212	Okinawa-Onna village (Shikaza River)	female	0.33	**200**	440	57	28	**530**	0.55	13	< 3.8	**14**	120	39	110	510	180	**960**	**< 0.08**
213	Okinawa-Onna village (Shikaza River)	female	0.67	**200**	640	80	42	**760**	0.74	9.2	2.4	**12**	95	61	170	650	210	**1200**	**< 0.08**
215	Okinawa-Onna village (Shikaza River)	female	2.8	**100**	430	87	42	**560**	0.74	9	1.8	**12**	110	91	250	620	210	**1300**	**< 0.08**
208	Okinawa-Onna village (Shikaza River)	male	1.0	**88**	260	81	24	**370**	1.4	8.9	< 3.8	**10**	110	58	180	450	140	**940**	**< 0.08**
211	Okinawa-Onna village (Shikaza River)	male	0.21	**160**	1000	81	30	**1100**	0.84	5.3	0.59	**6.7**	63	27	78	470	180	**820**	**0.68**
218	Okinawa-Onna village (Shikaza River)	male	0.67	**140**	570	150	44	**760**	1.4	8.7	0.32	**10**	170	53	160	740	270	**1400**	**0.31**
220	Okinawa-Onna village (Shikaza River)	male	1.0	**150**	460	91	30	**580**	1.2	8.3	0.24	**9.7**	100	58	160	540	160	**1000**	**0.63**
Mullet																			
Pool	Ishigaki Island	female	0.16	**30**	320	90	30	**450**	2.2	2.3	0.99	**5.5**	35	45	140	150	80	**450**	**2.1**
702	Okinawa-Kadena (Hija River)	female	0.21	**830**	1000	100	40	**1150**	1.4	4.7	1.9	**8.0**	50	210	560	930	180	**1900**	**11**
703	Okinawa-Kadena (Hija River)	female	0.18	**2100**	1500	100	70	**1700**	2.2	14	2.8	**19**	40	440	1900	1900	500	**4800**	**9.1**
707	Okinawa-Kadena (Hija River)	female	0.20	**2500**	3000	150	80	**3200**	1.9	7.5	1.9	**11**	100	530	1900	2600	320	**5400**	**7.7**
704	Okinawa-Kadena (Hija River)	male	0.17	**380**	600	90	45	**700**	1.6	6.8	2.5	**11**	80	360	2200	1400	260	**4300**	**5.7**
705	Okinawa-Kadena (Hija River)	male	0.27	**600**	860	130	100	**1100**	1.3	6.0	2.5	**9.8**	60	270	630	860	220	**2100**	**8.1**
706	Okinawa-Kadena (Hija River)	male	0.24	**1800**	4300	340	150	**4800**	1.5	4.4	3.4	**9.3**	160	300	2000	3300	700	**6400**	**9.0**
Pool	Okinawa-Manko (Noha River)	female	0.70	**13300**	1200	300	170	**1700**	4.4	26	< 3.8	**30**	240	740	2000	3000	1000	**7000**	**4.0**
Pool	Okinawa-Onna village (Shikaza River)	female	0.65	**550**	2900	190	190	**3300**	< 3.5	10	< 3.8	**10**	360	1300	2600	2050	1030	**7500**	**4.0**

Table 5. Concentrations (ng/g lipid wt.) of PBDEs in muscles of tilapia and mullet collected from main Okinawa and Ishigaki Islands

Sample ID	Sampling site	Sex	Lipid (%)	BDE3	BDE15	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE196	BDE197	BDE206	BDE207	BDE209	ΣPBDEs
Tilapia																		
5	Ishigaki Island	female	0.19	<0.05	<0.07	<0.06	0.87	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**0.87**
13	Ishigaki Island	female	0.18	<0.05	<0.07	<0.06	1.9	0.66	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**2.6**
19	Ishigaki Island	female	0.18	<0.05	<0.07	<0.06	1.2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**1.2**
102	Ishigaki Island	female	0.25	<0.05	<0.07	<0.06	<0.03	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**<1.5**
7	Ishigaki Island	male	0.20	<0.05	<0.07	<0.06	1.1	<0.05	<0.05	<0.05	<0.05	2.6	<0.05	<0.05	<0.07	0.85	<1.5	**3.7**
21	Ishigaki Island	male	0.18	<0.05	<0.07	<0.06	1.5	0.70	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**2.2**
43	Ishigaki Island	male	0.11	<0.05	<0.07	<0.06	<0.03	1.2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**1.2**
44	Ishigaki Island	male	0.16	<0.05	<0.07	<0.06	1.7	0.68	<0.05	<0.05	1.0	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**3.4**
716	Okinawa-Kadena (Hija River)	female	0.52	<0.05	1.9	24	940	5.6	130	18	41	<0.05	<0.05	<0.05	0.43	0.45	<1.5	**1200**
722	Okinawa-Kadena (Hija River)	female	1.9	0.18	2.0	28	1300	24	190	38	48	<0.05	<0.05	0.21	<0.07	<0.07	<1.5	**1700**
734	Okinawa-Kadena (Hija River)	female	0.74	<0.05	2.0	26	1400	29	200	38	57	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**1800**
737	Okinawa-Kadena (Hija River)	female	0.32	<0.05	1.3	22	1100	29	140	26	38	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**1400**
725	Okinawa-Kadena (Hija River)	male	2.0	<0.05	1.8	19	710	10	99	17	26	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**880**
730	Okinawa-Kadena (Hija River)	male	2.9	0.11	1.7	23	1100	13	170	29	41	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**1400**
801	Okinawa-Kadena (Hija River)	male	0.74	<0.05	2.0	38	2100	21	320	42	75	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**2600**
802	Okinawa-Kadena (Hija River)	male	2.5	<0.05	2.0	20	760	9.2	110	17	28	<0.05	<0.05	<0.05	2.2	3.2	2.0	**950**
601	Okinawa-Manko (Noha River)	female	0.19	<0.05	<0.07	6.1	140	<0.05	19	3.9	4.8	<0.05	<0.05	<0.05	3.6	<0.07	<1.5	**170**
612	Okinawa-Manko (Noha River)	female	0.90	<0.05	0.70	4.5	78	1.6	11	3.5	5.5	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**110**
628	Okinawa-Manko (Noha River)	female	1.0	<0.05	<0.07	4.6	67	1.5	8.4	3.9	8.7	<0.05	<0.05	<0.05	<0.07	0.29	<1.5	**94**
635	Okinawa-Manko (Noha River)	female	0.99	<0.05	1.5	11	320	23	38	15	11	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**420**
604	Okinawa-Manko (Noha River)	male	0.42	<0.05	<0.07	8.5	120	2.1	16	7.1	13	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**170**
605	Okinawa-Manko (Noha River)	male	0.24	<0.05	<0.07	5.1	89	2.5	12	4.0	7.8	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**120**
634	Okinawa-Manko (Noha River)	male	0.56	<0.05	2.0	18	750	23	96	21	22	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**930**
636	Okinawa-Manko (Noha River)	male	0.32	<0.05	<0.07	18	600	7.1	72	20	26	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**740**
239	Okinawa-Onna village (Shikaza River)	female	1.2	<0.05	<0.07	2.6	90	1.3	10	3.5	5.1	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**115**
212	Okinawa-Onna village (Shikaza River)	female	0.33	<0.05	<0.07	<0.06	31	<0.05	5.8	<0.05	<0.05	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**40**
213	Okinawa-Onna village (Shikaza River)	female	0.67	<0.05	<0.07	3.5	192	3.6	28	7.1	10	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**245**
215	Okinawa-Onna village (Shikaza River)	female	2.8	<0.05	<0.07	2.1	66	1.7	7.6	2.4	3.6	<0.05	<0.05	<0.05	<0.07	0.17	1.6	**85**
208	Okinawa-Onna village (Shikaza River)	male	1.0	<0.05	<0.07	2.5	76	1.7	8.4	2.8	4.3	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**100**
211	Okinawa-Onna village (Shikaza River)	male	0.21	<0.05	<0.07	<0.06	113	8.2	18	<0.05	8.7	<0.05	<0.05	<0.05	<0.07	0.8	<1.5	**150**
218	Okinawa-Onna village (Shikaza River)	male	0.67	<0.05	<0.07	<0.06	100	1.7	11	4.1	6.1	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**125**
220	Okinawa-Onna village (Shikaza River)	male	1.0	<0.05	<0.07	<0.06	152	1.8	21	3.5	6.7	<0.05	0.84	<0.05	<0.07	0.3	2.8	**190**
Mullet																		
Pool	Ishigaki Island	female	0.16	<0.05	<0.07	<0.06	<0.03	<0.05	1.5	<0.05	1.3	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**2.8**
702	Okinawa-Kadena (Hija River)	female	0.21	<0.05	2.3	17	740	18	99	22	28	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**930**
703	Okinawa-Kadena (Hija River)	female	0.18	<0.05	2.0	17	640	17	98	25	41	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**840**
707	Okinawa-Kadena (Hija River)	female	0.20	<0.05	<0.07	9.1	370	<0.05	73	3.7	20	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**480**
704	Okinawa-Kadena (Hija River)	male	0.17	<0.05	<0.07	7.5	200	<0.05	25	2.1	4.2	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**240**
705	Okinawa-Kadena (Hija River)	male	0.27	<0.05	1.5	15	720	18	103	22	39	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**920**
706	Okinawa-Kadena (Hija River)	male	0.24	<0.05	8.0	11	295	<0.05	48	3.3	12	<0.05	<0.05	<0.05	6.9	<0.07	<1.5	**380**
Pool	Okinawa-Manko (Noha River)	female	0.70	<0.05	<0.07	5.7	105	1.8	12	2.2	2.7	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**130**
Pool	Okinawa-Onna village (Shikaza River)	female	0.65	<0.05	<0.07	7.3	160	7.6	19	4.6	8.4	<0.05	<0.05	<0.05	<0.07	<0.07	<1.5	**210**

Table 6. Concentrations (ng/g lipid wt.) of organochlorine compounds in invertebrate species collected along the coastal areas of main Okinawa and Ishigaki Islands

Species	Sampling site	Lipid(%)	PCBs	DDT Compounds				HCH Compounds				CHL Compounds						HCB
				pp'-DDE	*pp'*-DDD	*pp'*-DDT	DDTs	α-HCH	β-HCH	γ-HCH	HCHs	Oxy	*t*-CA	*c*-CA	*t*-nona	*c*-nona	CHLs	
Tropical mussel	Okinawa-Onna village	0.24	**11**	< 2.4	3.1	3.9	**7.0**	< 2.2	< 3.7	< 2.1	**< 3.7**	4.8	7.7	5.1	**2.7**	2.7	23	**1.5**
	Ishigaki Island	0.52	**84**	6	< 1.6	5.4	**11**	< 2.2	< 3.7	< 2.1	**< 3.7**	7.0	4.2	6.5	**10**	< 0.9	28	**< 0.61**
Kanoko-gai	Okinawa-Onna village	1.1	**21**	32	5.3	6.0	**44**	< 2.2	< 3.7	< 2.1	**< 3.7**	< 0.5	13	19	**42**	11	85	**< 0.61**
	Ishigaki Island	1.4	**26**	7.4	3.8	1.9	**13**	< 2.2	< 3.7	< 2.1	**< 3.7**	3.2	4.9	7.8	**7.6**	4.5	28	**< 0.61**
Common spider conch	Okinawa-Onna village	0.98	**33**	2.1	5.9	15	**23**	< 2.2	< 3.7	< 2.1	**< 3.7**	6.3	3.2	< 1.9	3.9	1.7	**15**	**4.5**
	Ishigaki Island	0.44	**3.8**	< 2.4	< 1.6	< 1.7	**< 1.7**	< 2.2	< 3.7	< 2.1	**< 3.7**	2.8	< 1.9	2.4	**< 0.7**	< 0.9	5.2	**0.80**
Mud crab	Okinawa-Manko (Noha River)	0.23	**4900**	5600	630	55	**6300**	19	220	< 2.1	**240**	460	< 1.9	160	550	250	**1400**	**< 0.61**
Orange mud crab (male)	Ishigaki Island	0.51	**65**	100	11	< 1.7	**110**	< 2.2	120	< 2.1	**120**	27	5.9	10	**22**	14	80	**2.4**
Orange mud crab (female)	Ishigaki Island	0.64	**92**	120	8.6	< 1.7	**130**	< 2.2	< 3.7	< 2.1	**< 3.7**	20	3.3	4.7	**16**	7.6	51	**1.7**
Sentinel crab	Ishigaki Island	0.56	**97**	17	2.9	< 1.7	**20**	< 2.2	< 3.7	< 2.1	**< 3.7**	94	23	22	**99**	65	300	1.0

Table 7. Concentrations (ng/g lipid wt.) of PBDEs in invertebrate species collected along the coastal areas of main Okinawa and Ishigaki Islands

Species	Sampling site	Lipid(%)	BDE3	BDE15	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	ΣPBDEs
Tropical mussel	Okinawa-Onna village	0.24	<0.2	<0.2	0.33	1.4	1.1	1.0	<0.2	<0.2	<0.2	**3.8**
	Ishigaki Island	0.52	<0.1	<0.1	0.36	1.5	0.29	0.28	<0.1	<0.1	<0.1	**2.4**
Kanoko-gai	Okinawa-Onna village	1.1	<0.2	<0.2	0.25	7.5	1.7	0.30	<0.2	<0.2	<0.2	**9.7**
	Ishigaki Island	1.4	<0.1	<0.1	<0.1	0.26	0.71	<0.1	<0.1	<0.1	<0.1	**1.0**
Common spider conch	Okinawa-Onna village	0.98	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	**<0.2**
	Ishigaki Island	0.44	<0.2	<0.2	<0.2	0.33	<0.2	<0.2	<0.2	<0.2	<0.2	**0.47**
Mud crab	Okinawa-Manko (Noha River)	0.23	<0.2	<0.2	6.3	160	6.0	10	<0.2	<0.2	<0.2	**180**
Orange mud crab (male)	Ishigaki Island	0.51	<0.1	<0.1	0.27	1.7	0.40	0.31	0.41	0.42	0.82	**4.3**
Orange mud crab (female)	Ishigaki Island	0.64	<0.1	<0.1	0.20	0.79	0.36	<0.1	<0.1	0.28	0.90	**2.5**
Sentinel crab	Ishigaki Island	0.56	<0.1	<0.1	0.27	0.45	0.24	<0.1	<0.1	<0.1	1.5	**2.4**

Table 8. Concentrations (ng/g wet wt.) of organotin compounds in livers of skipjack tuna collected off Ishigaki Island

Sample ID	Sampling site	MBT	DBT	TBT	MOT	DOT	TOT	DPT	TPT
2005 PC-ST01	Pacific Ocean	<0.5	8.1	7.4	<0.8	<0.8	<0.8	<0.8	<0.8
2005 PC-ST02	Pacific Ocean	<0.5	2.8	6.3	<0.8	<0.8	<0.8	<0.8	<0.8
2005 PC-ST03	Pacific Ocean	<0.5	9.0	11	<0.8	<0.8	<0.8	<0.8	<0.8
2005 PC-ST04	Pacific Ocean	<0.5	2.7	5.0	<0.8	<0.8	<0.8	<0.8	<0.8
2005 PC-ST05	Pacific Ocean	<0.5	6.7	19	<0.8	<0.8	<0.8	<0.8	<0.8
2005 ECS-ST01	East China Sea	13	16	20	<0.7	<0.7	<0.7	<0.5	<0.5
2005 ECS-ST02	East China Sea	7.5	9.5	10	<0.7	<0.7	<0.7	<0.5	<0.5
2005 ECS-ST03	East China Sea	24	5.3	7.0	3.2	<0.7	<0.7	0.7	<0.5
2005 ECS-ST04	East China Sea	4.8	6.5	8.4	1.0	<0.7	<0.7	<0.5	<0.5
2005 ECS-ST05	East China Sea	7.5	4.5	6.8	5.5	<0.7	<0.7	<0.5	<0.5

Table 9. Concentrations (ng/g wet wt.) of organotin compounds in muscles of tilapia and mullet collected from main Okinawa and Ishigaki Islands

Sample ID	Sampling site	Sex	MBT	DBT	TBT	MOT	DOT	TOT	DPT	TPT
Tilapia										
5	Ishigaki Island	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
13	Ishigaki Island	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
19	Ishigaki Island	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
102	Ishigaki Island	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
7	Ishigaki Island	male	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
21	Ishigaki Island	male	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
43	Ishigaki Island	male	<5.3	<1.5	<0.3	<5.0	6.1	<0.5	<0.3	<0.3
44	Ishigaki Island	male	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
716	Okinawa-Kadena (Hija River)	female	<5.3	1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
722	Okinawa-Kadena (Hija River)	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
734	Okinawa-Kadena (Hija River)	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
737	Okinawa-Kadena (Hija River)	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
725	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
730	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	<0.3	<5.0	9.1	<0.5	<0.3	<0.3
801	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	<0.3	<5.0	4.0	<0.5	<0.3	<0.3
802	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	<0.3	<5.0	3.8	<0.5	<0.3	<0.3
601	Okinawa-Manko (Noha River)	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
612	Okinawa-Manko (Noha River)	female	14	1.5	<0.3	8.3	<3.4	<0.5	<0.3	<0.3
628	Okinawa-Manko (Noha River)	female	180	35	<0.3	5.8	<3.4	<0.5	<0.3	<0.3
635	Okinawa-Manko (Noha River)	female	27	2.5	<0.3	6.4	5.2	<0.5	<0.3	<0.3
604	Okinawa-Manko (Noha River)	male	<5.3	<1.5	<0.3	<5.0	4.0	<0.5	<0.3	<0.3
605	Okinawa-Manko (Noha River)	male	140	2.2	<0.3	13	<3.4	<0.5	<0.3	<0.3
634	Okinawa-Manko (Noha River)	male	82	28	<0.3	6.9	<3.4	<0.5	<0.3	<0.3
636	Okinawa-Manko (Noha River)	male	16	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
239	Okinawa-Onna village (Shikaza River)	female	<5.3	<1.5	<0.3	NA	5.6	<0.5	<0.3	<0.3
212	Okinawa-Onna village (Shikaza River)	female	<11	<1.5	0.8	NA	<3.4	<0.5	<0.3	<0.3
213	Okinawa-Onna village (Shikaza River)	female	<5.3	<1.5	<0.3	NA	<3.4	<0.5	<0.3	<0.3
215	Okinawa-Onna village (Shikaza River)	female	<5.3	<1.5	<0.3	NA	<3.4	<0.5	<0.3	<0.3
208	Okinawa-Onna village (Shikaza River)	male	<5.3	<1.5	<0.3	NA	<3.4	<0.5	<0.3	<0.3
211	Okinawa-Onna village (Shikaza River)	male	<5.3	<1.5	<0.3	NA	<3.4	<0.5	<0.3	<0.3
218	Okinawa-Onna village (Shikaza River)	male	18	<1.5	<0.3	NA	4.6	<0.5	<0.3	<0.3
220	Okinawa-Onna village (Shikaza River)	male	5.6	<1.5	<0.3	NA	<3.4	<0.5	<0.3	<0.3
Mullet										
Pool	Ishigaki Island	female	<5.3	<1.5	3.9	NA	<3.4	<0.5	<0.3	<0.3
702	Okinawa-Kadena (Hija River)	female	<5.3	<1.5	0.8	NA	<3.4	<0.5	<0.3	<0.3
703	Okinawa-Kadena (Hija River)	female	6.1	1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
707	Okinawa-Kadena (Hija River)	female	<5.3	<1.5	1.3	NA	<3.4	<0.5	<0.3	<0.3
704	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	0.6
705	Okinawa-Kadena (Hija River)	male	<5.3	<1.5	1.8	NA	<3.4	<0.5	<0.3	<0.3
706	Okinawa-Kadena (Hija River)	male	12.0	2.2	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
Pool	Okinawa-Manko (Noha River)	female	<5.3	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3
Pool	Okinawa-Onna village (Shikaza River)	female	5.5	<1.5	<0.3	<5.0	<3.4	<0.5	<0.3	<0.3

NA: Not analyzed

Table 10. Concentrations (ng/g wet wt.) of organotin compounds in invertebrate species collected along the coastal areas of main Okinawa and Ishigaki Islands

Species	Sampling site	MBT	DBT	TBT	MOT	DOT	TOT	DPT	TPT
Tropical mussel	Okinawa-Onna village	<5.0	<3.0	<0.5	NA	<5.0	<0.5	<0.5	<0.5
	Ishigaki Island	8.1	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Kanoko-gai	Okinawa-Onna village	<5.0	<3.0	<0.5	NA	<5.0	<0.5	<0.5	<0.5
	Ishigaki Island	5.7	<3.0	<0.5	7.7	<5.0	<0.5	<0.5	<0.5
Common spider conch	Okinawa-Onna village	5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Mud crab	Okinawa-Manko (Noha River)	<5.0	<3.0	<0.5	NA	<5.0	<0.5	<0.5	<0.5
Orange mud crab-5	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Orange mud crab-6	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Orange mud crab-7	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Orange mud crab-8	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5
Sentinel crab	Ishigaki Island	<5.0	<3.0	<0.5	<5.0	<5.0	<0.5	<0.5	<0.5

NA: Not analyzed

Table 11. Concentrations (μg/g dry weight) of trace elements in liver and muscle of skipjack tuna collected off Ishigaki Island

Tissue	Sampling site	Body length (mm)	Body weight (g)	V	Cr	Mn	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi
Liver	Pacific Ocean	370	1149	0.14	0.18	5.90	0.11	31.3	235	29	2.27	3.08	1.80	1.6	41.9	0.032	0.01	0.08	0.036	0.24	0.005	0.134	0.001
	Pacific Ocean	370	1143	0.12	0.25	4.82	0.08	18.0	156	30	2.16	1.28	1.79	0.40	21.7	0.017	< 0.01	0.06	0.028	0.14	0.004	0.073	< 0.001
	Pacific Ocean	360	1038	0.18	0.38	5.42	0.12	148	118	38	1.93	1.78	1.96	10	29.2	0.047	< 0.01	0.07	0.070	0.20	0.003	0.103	0.001
	Pacific Ocean	360	1191	0.089	0.17	6.91	0.10	28.3	318	43	2.33	1.72	1.78	0.65	41.8	0.014	< 0.01	0.08	0.018	0.17	0.003	0.081	< 0.001
	Pacific Ocean	375	1211	0.23	0.84	4.37	0.11	25.2	225	29	2.10	2.73	1.63	0.75	37.6	0.043	< 0.01	0.07	0.020	0.18	0.002	0.086	< 0.001
	East China Sea	365	1239	0.054	0.24	3.95	0.057	11.4	102	16	1.76	0.919	1.13	0.39	15.2	0.046	< 0.01	0.06	0.080	0.07	0.002	0.257	< 0.001
	East China Sea	365	1157	0.12	0.13	5.71	0.10	31.8	133	21	2.23	1.39	1.88	1.7	29.1	0.037	< 0.01	0.08	0.018	0.20	0.003	0.100	< 0.001
	East China Sea	375	1313	0.10	0.21	3.24	0.077	13.0	90.4	14	1.67	0.709	1.02	0.34	14.8	0.035	< 0.01	0.05	0.030	0.09	0.001	0.044	< 0.001
	East China Sea	355	1117	0.06	0.17	2.96	0.04	7.59	84.8	8.2	1.45	1.36	0.798	0.082	10.1	0.038	<0.01	0.05	0.012	0.06	0.003	0.025	0.001
	East China Sea	360	1139	0.11	0.20	5.28	0.10	27.5	121	21	2.19	0.724	1.55	1.3	34.1	0.026	< 0.01	0.07	0.036	0.13	0.002	0.061	< 0.001
Muscle	Pacific Ocean	370	1149	0.011	0.33	0.630	0.026	4.96	24.5	5.6	2.27	1.23	0.024	0.005	0.064	0.012	0.01	0.10	0.029	0.22	< 0.001	0.010	< 0.001
	Pacific Ocean	370	1143	0.012	0.25	0.622	0.029	6.03	21.9	3.8	2.62	2.07	0.018	0.002	0.028	0.012	< 0.01	0.10	0.028	0.14	< 0.001	0.011	< 0.001
	Pacific Ocean	360	1038	0.012	0.18	0.576	0.024	5.11	19.6	5.0	2.09	1.19	0.016	0.002	0.047	0.015	< 0.01	0.09	0.046	0.19	< 0.001	0.010	< 0.001
	Pacific Ocean	360	1191	0.014	0.28	0.569	0.020	5.53	22.3	4.3	2.28	0.839	0.018	0.003	0.053	0.011	< 0.01	0.10	0.026	0.12	< 0.001	0.013	< 0.001
	Pacific Ocean	375	1211	0.019	0.27	0.572	0.017	4.22	22.1	4.6	2.35	5.27	0.015	0.003	0.053	0.026	< 0.01	0.10	0.039	0.22	< 0.001	0.017	< 0.001
	East China Sea	365	1239	0.009	0.34	0.329	0.010	2.36	27.9	2.1	2.87	0.727	0.014	0.003	0.030	0.146	< 0.01	0.09	0.038	0.14	< 0.001	0.029	< 0.001
	East China Sea	365	1157	0.011	0.41	0.359	0.012	2.49	22.9	2.2	2.62	1.56	0.019	0.004	0.047	0.013	< 0.01	0.10	0.054	0.21	< 0.001	0.029	< 0.001
	East China Sea	375	1313	0.008	0.49	0.359	0.013	2.18	20.5	1.9	2.74	0.314	0.018	0.002	0.024	0.018	0.01	0.09	0.026	0.15	< 0.001	0.014	< 0.001
	East China Sea	355	1117	0.009	0.18	0.410	0.012	2.68	23.2	2.6	2.79	0.415	0.012	0.002	0.038	0.023	< 0.01	0.09	0.026	0.18	< 0.001	0.014	< 0.001
	East China Sea	360	1139	0.007	0.17	0.288	0.013	2.85	18.9	2.6	2.44	0.502	0.014	0.001	0.047	0.010	0.02	0.08	0.026	0.15	< 0.001	0.010	< 0.001

Table 12. Concentrations (μg/g dry weight) of trace elements in muscles of tilapia and mullet collected from main Okinawa and Ishigaki Islands

Sample ID	Sampling site	Sex	V	Cr	Mn	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi
Tilapia																						
5	Ishigaki Island	female	0.037	0.43	0.749	0.038	1.29	20.7	1.6	8.73	1.21	0.081	0.001	0.016	0.053	0.02	0.05	0.077	0.07	0.005	0.152	0.007
13	Ishigaki Island	female	0.019	0.42	0.542	0.041	2.53	20.3	1.7	9.80	0.508	0.038	0.001	0.017	0.139	0.02	0.09	0.069	0.20	0.007	0.222	0.013
19	Ishigaki Island	female	0.038	0.48	1.04	0.038	1.09	18.8	1.4	9.69	0.756	0.028	0.002	0.017	0.058	0.02	0.21	0.12	0.10	0.006	0.276	0.006
102	Ishigaki Island	female	0.015	0.24	0.624	0.043	0.957	16.7	1.1	8.51	0.628	0.025	0.001	0.002	0.016	<0.01	0.05	0.018	0.08	0.006	0.003	0.004
7	Ishigaki Island	male	0.016	0.38	0.546	0.040	1.16	22.3	2.1	9.89	0.557	0.054	0.003	0.010	0.047	0.02	0.05	0.025	0.14	0.007	0.123	0.005
21	Ishigaki Island	male	0.016	0.56	0.719	0.047	1.10	19.8	1.9	10.4	0.587	0.048	<0.001	0.020	0.061	0.02	0.06	0.032	0.34	0.006	0.257	0.008
43	Ishigaki Island	male	0.015	0.24	0.512	0.056	1.16	21.2	2.1	13.0	0.609	0.046	0.001	0.003	0.024	<0.01	0.07	0.051	0.18	0.009	0.036	0.011
44	Ishigaki Island	male	0.022	0.30	0.864	0.038	1.14	17.6	1.3	12.1	1.56	0.035	0.001	0.002	0.021	<0.01	0.06	0.045	0.07	0.006	0.030	0.006
716	Okinawa-Kadena (Hija River)	female	0.019	0.23	0.310	0.030	0.942	15.8	3.0	8.38	0.393	0.047	0.001	<0.001	0.018	<0.01	0.09	0.021	0.07	0.002	0.003	<0.001
722	Okinawa-Kadena (Hija River)	female	0.013	0.43	0.445	0.021	0.669	17.2	3.2	7.82	0.440	0.023	<0.001	<0.001	0.017	<0.01	0.05	0.014	0.06	0.001	0.003	0.001
734	Okinawa-Kadena (Hija River)	female	0.017	0.25	0.805	0.027	0.777	17.1	3.2	7.98	1.17	0.027	<0.001	0.002	0.012	<0.01	0.06	0.043	<0.05	0.002	0.005	0.001
737	Okinawa-Kadena (Hija River)	female	0.012	0.28	0.369	0.018	0.790	16.9	2.8	8.25	0.787	0.023	<0.001	<0.001	0.015	<0.01	0.07	0.017	<0.05	0.001	<0.001	0.001
725	Okinawa-Kadena (Hija River)	male	0.009	0.25	0.599	0.015	0.715	14.0	2.5	8.27	0.549	0.028	<0.001	0.001	0.019	<0.01	0.07	0.014	0.06	0.002	<0.001	0.001
730	Okinawa-Kadena (Hija River)	male	0.015	0.25	0.522	0.014	0.691	13.0	2.7	7.90	0.368	0.053	<0.001	0.002	0.011	<0.01	0.06	0.015	<0.05	<0.001	0.002	0.001
801	Okinawa-Kadena (Hija River)	male	0.011	0.24	0.357	0.021	0.757	17.6	2.4	10.0	0.752	0.034	<0.001	0.002	0.011	<0.01	0.06	0.011	<0.05	0.001	<0.001	0.001
802	Okinawa-Kadena (Hija River)	male	0.011	0.26	0.505	0.012	0.664	12.7	2.7	8.87	0.506	0.016	0.001	0.001	0.015	<0.01	0.08	0.020	0.06	0.002	<0.001	0.001
601	Okinawa-Manko (Noha River)	female	0.060	0.50	0.195	0.047	1.87	20.0	2.6	6.27	6.37	0.037	<0.001	<0.001	0.030	<0.01	0.04	0.035	<0.05	0.001	0.080	0.002
612	Okinawa-Manko (Noha River)	female	0.018	0.26	0.430	0.012	0.815	15.9	1.5	7.36	1.12	0.011	<0.001	<0.001	0.015	<0.01	0.03	0.012	<0.05	0.001	<0.001	0.001
628	Okinawa-Manko (Noha River)	female	0.014	0.16	0.418	0.021	0.736	15.4	1.3	8.16	1.24	0.013	<0.001	<0.001	0.013	<0.01	0.04	0.014	<0.05	0.001	<0.001	0.001
635	Okinawa-Manko (Noha River)	female	0.010	0.36	0.743	0.016	0.637	14.1	1.7	7.82	1.17	0.012	0.001	<0.001	0.031	<0.01	0.03	0.016	<0.05	0.002	0.002	0.001
604	Okinawa-Manko (Noha River)	male	0.052	0.47	0.484	0.014	0.645	13.9	1.5	9.68	0.862	0.017	0.002	0.002	0.011	<0.01	0.04	0.011	<0.05	0.002	0.002	0.002
605	Okinawa-Manko (Noha River)	male	0.018	0.17	0.230	0.011	0.707	14.5	1.1	11.3	1.08	0.012	<0.001	<0.001	0.014	0.01	0.05	0.008	<0.05	<0.001	0.118	<0.001
634	Okinawa-Manko (Noha River)	male	0.011	0.17	0.368	0.008	0.723	14.4	2.7	6.53	0.963	0.011	<0.001	<0.001	0.012	<0.01	0.03	0.006	0.12	<0.001	0.005	<0.001
636	Okinawa-Manko (Noha River)	male	0.015	0.42	0.409	0.017	0.830	12.5	1.9	7.73	1.23	0.015	0.001	<0.001	0.016	<0.01	0.04	0.009	0.10	<0.001	<0.001	<0.001
239	Okinawa-Onna village (Shikaza River)	female	0.062	0.40	0.612	0.043	0.702	17.0	1.3	6.32	1.30	0.057	0.022	0.047	0.052	0.03	0.09	0.048	0.06	0.036	0.057	0.033
212	Okinawa-Onna village (Shikaza River)	female	0.079	0.67	0.483	0.052	1.21	29.6	1.5	13.5	3.28	0.037	<0.001	0.012	0.033	0.02	0.10	0.035	0.06	0.001	0.189	0.007
213	Okinawa-Onna village (Shikaza River)	female	0.034	0.26	0.495	0.043	1.06	24.7	1.4	8.01	1.85	0.029	<0.001	0.003	0.021	<0.01	0.07	0.030	0.11	0.002	0.052	<0.001
215	Okinawa-Onna village (Shikaza River)	female	0.028	0.20	0.675	0.016	0.805	18.9	1.7	7.41	2.68	0.023	<0.001	0.002	0.037	0.02	0.05	0.018	0.07	0.003	0.529	0.001
208	Okinawa-Onna village (Shikaza River)	male	0.033	0.44	0.407	0.028	0.751	18.8	1.7	7.93	1.33	0.091	<0.001	0.002	0.053	<0.01	0.06	0.016	<0.05	0.004	0.041	0.002
211	Okinawa-Onna village (Shikaza River)	male	0.080	0.66	0.398	0.028	1.25	19.3	1.5	9.10	1.63	0.054	<0.001	0.003	0.025	0.01	0.09	0.028	<0.05	0.004	0.095	0.003
218	Okinawa-Onna village (Shikaza River)	male	0.030	0.32	0.520	0.014	0.709	17.0	1.5	7.47	1.54	0.025	<0.001	0.002	0.024	<0.01	0.06	0.015	0.07	0.002	0.015	0.001
220	Okinawa-Onna village (Shikaza River)	male	0.077	0.77	0.494	0.025	0.742	14.5	1.8	7.72	1.19	0.062	<0.001	0.007	0.024	0.01	0.06	0.021	<0.05	0.008	0.018	0.006
Mullet																						
702	Okinawa-Kadena (Hija River)	female	0.028	0.37	0.424	0.006	0.776	15.6	1.3	4.92	5.12	0.009	<0.001	0.001	0.048	<0.01	0.09	0.072	0.06	<0.001	0.017	0.001
703	Okinawa-Kadena (Hija River)	female	0.029	0.75	0.236	0.012	0.771	11.1	1.2	3.77	0.886	0.011	<0.001	0.012	0.077	<0.01	0.07	0.013	<0.05	0.004	0.010	0.004
707	Okinawa-Kadena (Hija River)	female	0.081	0.78	0.528	0.010	0.848	11.6	1.9	5.06	3.22	0.008	<0.001	<0.001	0.022	<0.01	0.10	0.061	<0.05	0.002	0.025	<0.001
704	Okinawa-Kadena (Hija River)	male	0.054	0.61	0.214	0.006	0.896	10.7	1.4	4.72	1.27	0.005	0.002	<0.001	0.015	<0.01	0.10	0.011	<0.05	0.002	0.015	<0.001
705	Okinawa-Kadena (Hija River)	male	0.017	1.0	0.299	0.008	0.952	12.6	1.1	4.92	1.18	0.013	<0.001	0.016	0.193	<0.01	0.08	0.021	<0.05	0.004	0.016	0.003
706	Okinawa-Kadena (Hija River)	male	0.015	0.32	0.457	0.006	0.724	15.3	1.3	5.49	1.18	0.007	<0.001	<0.001	0.052	<0.01	0.08	0.015	<0.05	0.002	0.009	<0.001
77	Ishigaki Island	female	0.033	0.43	0.543	0.026	0.842	12.7	2.0	11.4	0.536	0.019	<0.001	0.003	0.040	<0.01	0.06	0.027	<0.05	0.002	0.023	0.003
70	Ishigaki Island	female	0.062	0.68	0.829	0.022	0.927	13.1	1.8	10.4	2.31	0.021	<0.001	<0.001	0.024	<0.01	0.06	0.071	<0.05	0.002	0.028	0.001
80	Ishigaki Island	female	0.12	1.1	2.71	0.046	1.68	16.0	2.3	11.3	0.863	0.034	<0.001	0.004	0.156	<0.01	0.06	0.15	<0.05	0.003	0.073	0.002
78	Ishigaki Island	female	0.026	0.43	0.600	0.055	2.24	16.6	2.0	10.6	0.584	0.120	<0.001	0.009	0.020	<0.01	0.07	0.036	0.07	0.003	0.030	0.004
559	Okinawa-Manko (Noha River)	female	0.018	0.25	0.408	0.008	0.716	13.7	1.4	8.70	0.564	0.022	<0.001	0.007	0.043	<0.01	0.05	0.054	0.08	0.002	0.117	0.005
563	Okinawa-Manko (Noha River)	female	0.050	0.21	0.641	0.018	0.766	11.7	2.6	6.62	1.04	0.018	<0.001	0.009	0.034	<0.01	0.04	0.18	<0.05	0.002	0.063	0.003
558	Okinawa-Manko (Noha River)	female	0.029	0.14	0.512	0.012	1.16	19.8	1.5	5.18	2.96	0.023	<0.001	0.008	0.041	<0.01	0.04	0.11	0.10	<0.001	0.063	0.004
562	Okinawa-Manko (Noha River)	female	0.026	0.17	0.484	0.019	0.967	14.8	1.7	7.96	0.954	0.018	<0.001	0.002	0.029	<0.01	0.03	0.11	<0.05	<0.001	0.115	0.003
193	Okinawa-Onna village (Shikaza River)	female	0.018	0.16	0.298	0.006	0.987	22.5	1.5	6.49	2.54	0.014	<0.001	<0.001	0.066	<0.01	0.06	0.018	0.07	<0.001	0.031	0.002
203	Okinawa-Onna village (Shikaza River)	female	0.018	0.35	0.410	0.024	1.17	20.3	2.1	7.46	2.14	0.017	<0.001	0.002	0.037	<0.01	0.06	0.036	<0.05	<0.001	0.043	<0.001
192	Okinawa-Onna village (Shikaza River)	female	0.029	0.40	0.423	0.007	1.01	16.5	1.7	6.21	4.73	0.017	<0.001	<0.001	0.037	<0.01	0.05	0.020	<0.05	<0.001	0.050	0.001
175	Okinawa-Onna village (Shikaza River)	female	0.034	0.44	0.378	0.012	1.40	23.2	1.4	5.16	3.22	0.023	<0.001	0.001	0.035	<0.01	0.04	0.057	0.06	<0.001	0.109	0.002

Table 13. Concentrations (µg/g dry weight) of trace elements in invertebrate species collected along the coastal areas of main Okinawa and Ishigaki Islands

Species	Sample ID	Sex	Samoling site	Tissue	V	Cr	Mn	Co	Cu	Zn	Se	Rb	Sr	Mo	Ag	Cd	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi
Tropical mussel			Okinawa-Onna village	Whole soft tissues	4.4	3.7	6.83	0.65	12.5	56.8	5.2	3.01	606	5.11	3.5	2.47	0.084	0.16	0.07	3.5	0.19	0.015	4.38	0.051
			Ishigaki Island	Whole soft tissues	2.6	2.6	19.9	1.3	12.6	50.4	9.0	6.58	184	12.7	0.13	0.551	0.088	0.06	0.08	1.8	0.23	0.005	2.66	0.049
Kanoko-gai			Okinawa-Onna village	Whole soft tissues	3.2	3.0	62.0	0.63	16.0	77.8	5.5	3.17	327	2.95	0.83	0.259	0.071	0.03	0.01	3.2	0.08	0.015	1.07	0.013
			Ishigaki Island	Whole soft tissues	1.3	3.1	85.2	0.50	48.0	111	3.6	3.69	145	1.63	0.30	0.253	0.254	0.10	0.09	2.7	0.07	0.009	4.85	0.016
Common spider conch			Okinawa-Onna village	Whole soft tissues	16	2.9	71.2	0.31	33.9	152	3.8	5.03	524	0.471	2.0	1.54	0.067	0.03	0.04	1.3	0.13	0.007	2.17	0.016
			Okinawa-Onna village	Whole soft tissues	11	2.2	167	1.3	106	284	8.4	5.47	275	0.558	2.1	1.00	0.103	0.02	0.07	0.85	0.23	0.007	2.30	0.025
			Ishigaki Island	Whole soft tissues	8.3	1.6	102	0.90	19.1	79.1	4.0	6.07	389	0.490	1.3	1.40	0.051	0.02	0.03	0.74	0.18	0.003	1.46	0.019
Mud crab	1	Male	Okinawa-Manko (Noha River)	Muscle	0.054	0.32	2.98	0.21	57.5	194	4.0	3.78	61.8	0.115	1.0	0.014	0.029	<0.01	0.02	0.14	0.11	0.003	0.010	0.007
	2	Male		Muscle	0.12	0.91	1.16	0.064	62.7	240	10	3.12	82.0	0.063	0.31	0.009	0.059	<0.01	0.02	0.078	0.17	0.002	0.086	0.007
	3	Male		Muscle	0.042	0.35	1.17	0.097	59.2	213	4.7	3.83	42.2	0.047	0.82	0.006	0.070	<0.01	0.02	0.096	0.09	0.002	0.109	0.007
	4	Female		Muscle	0.086	1.0	1.12	0.061	51.3	289	8.7	3.84	64.3	0.059	0.45	0.012	0.010	<0.01	0.02	0.063	0.16	<0.001	0.168	0.005
	5	Female		Muscle	0.043	0.40	1.54	0.093	104	304	8.8	4.00	134	0.043	0.26	0.002	0.047	<0.01	0.02	0.15	0.15	0.002	0.176	0.006
	6	Female		Muscle	0.055	0.64	3.46	0.13	53.1	220	6.5	3.56	54.6	0.043	1.8	0.002	0.029	<0.01	0.01	0.10	0.12	<0.001	0.054	0.006
Orange mud crab	2	Male	Ishigaki Island	Muscle	0.050	0.27	14.3	0.36	152	352	11	8.09	98.0	0.097	0.11	0.205	<0.05	<0.01	0.04	1.1	0.21	0.001	0.014	0.007
	3	Male		Muscle	0.076	0.56	17.3	0.38	80.0	258	8.2	5.89	111	0.115	0.050	0.142	<0.05	0.02	0.03	1.6	0.17	0.001	0.023	0.009
	4	Male		Muscle	0.068	0.39	20.9	0.49	89.2	229	6.5	6.20	45.9	0.108	0.17	0.332	<0.05	<0.01	0.04	1.1	0.20	0.003	0.016	0.009
	5	Male		Muscle	0.053	0.24	16.8	0.17	91.4	313	7.2	9.53	79.5	0.053	0.13	0.183	<0.05	<0.01	0.05	1.1	0.21	0.002	0.016	0.006
	8	Male		Muscle	0.11	0.40	8.57	0.21	132	274	8.0	7.47	73.8	0.135	0.17	0.341	<0.05	0.01	0.04	1.1	0.22	0.003	0.037	0.008
	9	Male		Muscle	0.10	0.41	27.7	0.09	68.4	393	7.3	5.71	133	0.097	0.55	0.740	<0.05	<0.01	0.05	4.2	1.4	0.002	0.018	0.003
	1	Female		Muscle	0.01	0.53	21.8	0.43	115	385	7.9	9.31	139	0.096	0.10	0.238	0.11	0.00	0.06	1.7	0.36	0.003	0.030	0.007
	6	Female		Muscle	0.093	0.42	33.9	0.56	108	342	5.8	8.00	150	0.215	0.65	0.954	<0.05	<0.01	0.04	1.6	0.27	0.003	0.025	0.015
	7	Female		Muscle	0.088	0.40	19.1	0.59	87.5	291	7.2	7.03	35.4	0.150	0.23	0.405	<0.05	<0.01	0.04	1.0	0.28	0.002	0.030	0.019

Table 14. Body weight, gonad somatic index (GSI), and vitellogenin (VTG) concentrations in blood of tilapia and mullet

Sample ID	Sampling site	Sex	Body weight (g)	Gonad weight (g)	GSI*	VTG (µg/g)	Histological finding in gonad
Tilapia							
5	Ishigaki Island	female	304	1.560	0.514	91.1	
13	Ishigaki Island	female	430	2.516	0.585	554.6	
19	Ishigaki Island	female	470	1.200	0.255	70.6	
102	Ishigaki Island	female	360	3.430	0.953	-	
7	Ishigaki Island	male	480	0.939	0.196	-	Agglutination
21	Ishigaki Island	male	600	0.979	0.163	-	
43	Ishigaki Island	male	830	2.764	0.333	-	
44	Ishigaki Island	male	470	1.928	0.410	-	
716	Okinawa-Kadena (Hija River)	female	516	2.269	0.440	130.8	
722	Okinawa-Kadena (Hija River)	female	265	0.378	0.143	N.D.	
734	Okinawa-Kadena (Hija River)	female	333	0.857	0.257	N.D.	
737	Okinawa-Kadena (Hija River)	female	270	0.489	0.181	65.6	Discoloration
725	Okinawa-Kadena (Hija River)	male	469	0.438	0.093	N.D.	
730	Okinawa-Kadena (Hija River)	male	650	0.418	0.064	N.D.	
801	Okinawa-Kadena (Hija River)	male	612	0.679	0.111	N.D.	Blister
802	Okinawa-Kadena (Hija River)	male	504	0.386	0.077	N.D.	Blister
601	Okinawa-Manko (Noha River)	female	437	0.665	0.152	N.D.	Discoloration
612	Okinawa-Manko (Noha River)	female	459	0.920	0.200	N.D.	
628	Okinawa-Manko (Noha River)	female	397	2.294	0.578	244.3	
635	Okinawa-Manko (Noha River)	female	340	1.098	0.323	N.D.	Induration (surface)
604	Okinawa-Manko (Noha River)	male	458	2.559	0.559	N.D.	
605	Okinawa-Manko (Noha River)	male	628	0.947	0.151	N.D.	
634	Okinawa-Manko (Noha River)	male	550	4.991	0.907	N.D.	Tumor (base)
636	Okinawa-Manko (Noha River)	male	538	3.558	0.661	N.D.	
239	Okinawa-Onna village (Shikaza River)	female	164	5.567	3.395	142.7	
212	Okinawa-Onna village (Shikaza River)	female	146	0.655	0.449	N.D.	
213	Okinawa-Onna village (Shikaza River)	female	142	2.498	1.759	250.0	
215	Okinawa-Onna village (Shikaza River)	female	157	0.781	0.497	24.5	
208	Okinawa-Onna village (Shikaza River)	male	368	0.285	0.077	N.D.	
211	Okinawa-Onna village (Shikaza River)	male	300	0.953	0.318	N.D.	
218	Okinawa-Onna village (Shikaza River)	male	335	0.380	0.113	N.D.	
220	Okinawa-Onna village (Shikaza River)	male	379	0.519	0.137	N.D.	
Mullet							
702	Okinawa-Kadena (Hija River)	female	303	0.423	0.140	N.D.	
703	Okinawa-Kadena (Hija River)	female	361	0.700	0.194	N.D.	
707	Okinawa-Kadena (Hija River)	female	336	0.569	0.169	N.D.	
704	Okinawa-Kadena (Hija River)	male	276	0.189	0.068	N.D.	
705	Okinawa-Kadena (Hija River)	male	508	0.070	0.014	N.D.	
706	Okinawa-Kadena (Hija River)	male	371	0.100	0.027	N.D.	

*GSI = gonad weight / body weight x 100
N.D.: not detected
–: no available data

Report on a study of toxicological effects of harmful chemical substances on juveniles of a reef-building coral

with special emphasis on their acquisition of zooxanthellae

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1. Introduction

Mass mortality of reef-building (or hermatypic) corals and deterioration of coral reef ecosystems have been observed recently in many coral reefs of the world. The main causes are thought to be ‘bleaching’ (loss of the brown-colored symbionts) of corals due to the rise in the sea surface temperature, outbreaks of coralivorous animals (e.g. crown-of-thorns starfishes), and pollution of coastal marine seawaters. The main pollutants are terrestrial run-offs (sediments and nutrients in the sewage) and residual chemical substances that are used as anti-foulants and biocides. There have not been many studies on the eco-toxicological effects of those chemicals on hermatypic corals, mainly due to the difficulties of mass-culturing and experimentally manipulating corals in laboratories.

I have studied toxicological effects of several chemicals on juvenile corals (0.5 - 2.5 months after fertilization), that are more easily cultured in laboratories than colonial adult corals. In 2005, susceptibilities of aposymbiotic (lacking symbionts) and symbiotic juveniles of the hermatypic coral *Acropora tenuis* to the following three chemicals were examined and compared: tributyltin-chloride (TBT-Cl, widely used as an anti-foulant), diuron (DCMU, widely used as a herbicide and anti-foulant) and dichlorvos (DDVP, insecticide). Juveniles were exposed to the chemicals for 10 days, and morphological abnormalities and changes in the symbiont density in tentacles were studied.

A. tenuis juveniles were more sensitive in the symbiotic state than the aposymbiotic condition to DCMU and DDVP. By contrast, their sensitivities were comparable in the two conditions to TBT-Cl. In symbiotic juveniles exposed to TBT-Cl at nominal concentrations of 1 μg/L or higher, abnormalities such as detachment of soft tissues from the exoskeleton and decrease in the symbiont density were observed. In exposure to DCMU at various concentrations, reduction in the symbiont density was observed at 10 μg/L, and detachment of soft tissues at 100 μg/L. Specimens exposed to DDVP exhibited tissue detachment and reduced symbiont population at concentrations at or higher than 100 μg/L. These results have already been published (Watanabe et al. 2006), and the details are not described here.

In the present study, effects of TBT-Cl and DCMU on juveniles of *A. tenuis* were studied again. In 2005, *Symbiodinium* strain PL-TS-1 (clade A3, originally isolated from a zooxanthellate giant clam) was used to render *A. tenuis* juveniles symbiotic. This time, strain CCMP2467 (hereafter referred to as 2467), clade A1 cells originally isolated from the coral *Stylophora pistillata*, was used, because the latter strain was found to maintain symbiosis with *A. tenuis* juveniles longer than the former strain in a laboratory culture condition (Watanabe et al. 2007). In 2006, the periods of exposure to TBT-Cl and DCMU were extended to 48 and 50 days, respectively. Negative impacts of these chemicals were observed at lower concentrations than in the former study.

Impacts of 'red clay' (a typical red surface soil in Okinawa, Japan), and two chemical substances (metribuzin, and deaminated metribuzin or metribuzin DA) were also studied using *A. tenuis* juveniles inhabited by 2467 cells (10 day exposure). No deleterious effects of red clay were observed in an experiment in 2005 at concentrations lower than 100 mg/L. Thus, very high concentrations up to 10 g/L were tested in the present study. Metribuzin [CAS#21087-64-9] is a triazin-type herbicide commonly used in Okinawa. Metribuzin DA is a degradation product, and its inhibitory effect on photosynthesis is presumed to be weaker than that of metribuzin.

2. Materials and methods, results and discussion

Toxicological effects of chemical substances and red clay on symbiotic juveniles of the hermatypic coral *Acropora tenuis* were studied in the following two aspects:

a. Conspicuous morphological abnormalities such as detachment of soft tissues from and skeleton and complete lysis of tissues (organismal death)

and

b. growth of juveniles (increase in the height and basal area).

Experimental procedures were the same as described in Watanabe et al. 2006, except that strain 2467 was used instead of PL-TS-1 and liquid food was added to the coral culture (see Watanabe et al. 2007 for details). The following studies were carried out in 2006, except for exposure to metribuzin, which was done in 2007 (the reagent was temporarily unavailable in 2006).

In the present study, we tested five substances. As described below in a-f, negative impacts on coral juveniles were observed with all of them. However, effects of red clay, metribuzin and metribuzin DA were observed only at very high concentrations as compared to TBT-Cl and DCMU.

a) TBT-Cl (tributyltin chloride) [CAS#1461-22-9]: TBT-Cl has been widely used as an anti-foulant. Its use has been regulated or prohibited in developed or advanced countries (including Japan), but in some developing countries it seems to be still in use. In this study, coral juveniles were exposed for up to 48 days to the following nominal concentrations: 0.1, 0.4, 1, 2.5 µg/L.

As shown in Table 1, in the group exposed to 2.5 µg/L of TBT-Cl for 10 days, abnormalities were observed in all of the exposed individuals (Table 1). About 1/3 were found dead, and in the remaining individuals partial detachment of soft tissues from the skeleton or expulsion of aggregates of deteched tissues ('bail-out') was observed. Partial detachment of soft tissues was seen in more than half of the juveniles exposed to 1 µg/L for 10 days.

No abnormality could be seen in the groups exposed to 0.1 and 0.4 µg/L for 10 days. The toxicity test was extended, therefore, to 48 days. After the long exposure period, no morphological abnormalities were obvious in the majority of the juveniles (Table 1). In some individuals, partial detachment of tissues was observed, and some others became overgrown by algae (probably diatoms) as they were still alive (Fig. 1B). In the control group that was not exposed to TBT-Cl, growth of similar algae was seen over the petri dishes used for the culture, but not over coral juveniles (Fig. 1A). Presumably healthy corals can repel such algae, whereas individuals highly stressed by TBT-Cl lost such ability and became covered by algae.

After 48 days, juveniles in the control group and the groups exposed to 0.1 and 0.4 μg/L were removed from the petri dishes and their height was measured to evaluate inhibitory effect of TBT-Cl on coral growth (Table 2). In the group exposed to 0.4 μg/L, significant delay in vertical growth was observed as compared to the control group.

b) DCMU [CAS#330-54-1] (3-(3,4-dichlorophenyl)-1,1-dimethylurea): DCMU, also called diuron, is widely used as a herbicide in agriculture and also as an anti-foulant. In this study, *A. tenuis* juveniles were exposed to nominal concentrations of 0.3, 1, 3 and 10 μg/L for 50 days.

No morphological abnormalities (such as tissue detachment and death) were observed at any concentration. However, in comparing the average juvenile heights between the control group (not exposed to DCMU) and the exposed groups, juveniles exposed to 1, 3 and 10 μg/L were significantly lower than the control individuals. This observation indicates that DCMU delays the vertical growth of *A. tenuis* juveniles at concentrations above 1 μg/L.

c) Preliminary simultaneous exposure to TBT-Cl and DCMU: *A. tenuis* juveniles are exposed simultaneously to these two chemicals to find whether TBT-Cl and DCMU shows synergistic effects. An experiment was carried out, together with the above analyses, using a group of 35 juveniles exposed for 10 days to 0.4 μg/L of TBT-Cl and 1 μg/L of DCMU. Partial detachment of soft tissues from the skeleton was observed in 11% of the specimens. This value was higher than the proportion of the individuals that exhibited partial detachment (3%) when exposed only to 0.4 μg/L of TBT-Cl, though the difference was not statistically significant. No treated juvenile became covered by algae, probably because DCMU inhibited algal growth. The height of these individuals was not measured.

d) metribuzin [CAS#21087-64-9] : Metribuzin is a triazin-herbicide and commonly used in Okinawa, Japan, although its use in agriculture is not as high as glyphosate or DCMU. In the present study, *A. tenuis* juveniles were exposed to the following six nominal concentrations of metribuzin for 10 days: 0.3, 1, 3, 10, 30, 100 μg /L. No abnormalities were observed in the groups exposed to the three lower concentrations. Partial tissue detachment or bail-out was observed in 2% (n=46) of the treated individuals at 10 μg /L, 21% (n=34) at 30 μg /L and 21% (n=28) at 100 μg /L. Photographs of the specimens were taken before and after the exposure to compare the symbiont density. Visual inspection of these pictures did not reveal significant change in the symbiont populations even at high metribuzin concentrations such as 30 and 100 μg /L.

e) metribuzin DA (deaminated metribuzin) : A degradation product of the herbicide metribuzin. In the present study, *A. tenusi* juveniles were exposed to the following three nominal concentrations of metribuzin DA for 10 days: 1, 10, and 100 mg/L. At the two lower concentrations, no abnormalities were observed. At 100 mg/L, 56% (n=34) of the individuals exhibited partial tissue detachment.

f) Red clay : Typical surface soil in Okinawa, Japan. In this study, “Kunigami-maaji” collected in a mountainous area in Oogimi village was used. Symbiotic juveniles of *A. tenuis* were exposed to the clay at 0.1, 1, 10 and 100 g/L for 10 days. In the group exposed to 100 g/L, 29% (n=31) of the individuals exhibited bleaching or necrosis (or possibly detachment) of soft tissues (Fig. 2). No abnormalities were observed at lower concentrations.

The petri dishes containing coral juveniles and red clay were constantly shaken to agitate the clay. However, most of the clay remained at the bottom. In the presence of 100 g/L of red clay, lower parts of the juveniles were constantly under the clay. The abnormalities were seen in the peripheral regions of juveniles – parts that are constantly buried under the clay. Even in the juveniles that exhibited abnormalities, the central or apical parts (including tentacle rings) remained apparently healthy (the symbiont density did not seem to be decreased), and none of the treated juveniles were dead at the organismal level. These observations suggest that the abnormalities observed in the limited parts of the treated juveniles were caused by suffocation or stress due to constant contact with the clay.

The effect concentration (100 g/L) is extremely high, and is expected to seldom occur in nature. It was, therefore, surprising that the parts of the treated juveniles that were above the sediment remained apparently healthy. This observation may suggest that substances in “Kunigami-maaji” that are soluble in seawater are not harmful to coral juveniles.

Supplement: attempts to use juveniles of *Galaxea fascicularis* in eco-tox study

All of the works described above were done using juveniles of *Acropora tenuis*. The larvae/juveniles of *Acropora* spp. have advantages in experimental studies, in that their settlement and metamorphosis can be artificially induced. It is, therefore, easy to produce large numbers of juvenile corals of this species. However, sensitivities to chemicals may vary in different coral species, and it is necessary to perform toxicological studies on multiple coral species. For this reason, I attempted to grow azooxanthellate fertilized eggs of *Galaxea fascicularis* into symbiotic juveniles, and use them in eco-tox studies.

In 2005, very few *G. fascicularis* juveniles could be obtained, due to failure in the fertilization step. In 2006, the fertilization procedure was improved, and many larvae could be obtained and subsequently transported to Tokyo successfully. Effects of peptide Hym248, which can induce metamorphosis in *Acropora* spp., were tested on *G. fascicularis*, but induction of settlement or metamorphosis could not been observed within 5 hours. Spontaneous settlement and metamorphosis was observed, such that about half of the larvae became polyps in a week. However, the majority of the *G. fascicularis* polyps were devoured by minute coralivorous animals (ciliates etc). Infection of *Symbiodinium* strain 2467 was tested on a small number of remaining polyps. The *G. fascicularis* polyps became densely inhabited by 2467 cells, and maintained symbiosis for at least two months.

Similar minute animals were observed in the culture of *A. tenuis* juveniles. It seems, however, that the polyps of *A. tenuis* are somehow more resistant to these pests, and none of the polyps was killed. Such minute animals seem to be ubiquitous in coastal seawater in Okinawa and adhere to coral larvae/polyps. These pests are, therefore, very hard to remove from the culture even after washing extensively with filtered seawater. Thus, it was not possible to collect enough *G. fascicularis* juveniles for eco-toxicological studies.

3. Collection of coral larvae, and laboratory experiments

Fertilization of *A. tenuis* eggs and culture of larvae were done by members of Akajima Marine Science Laboratory. I went to Akajima on June 15th, 2006, and took larvae back to Tokyo on June 21st. In 2007, larvae were brought back to Tokyo on June 10th. Subsequent experiments were done at Ocean Research Institute, the University of Tokyo (Fig. 4).

To perform fertilization of *G. fascicularis* and collect larvae, trips were made in July and August, 2006 to Sesoko Station, the Tropical Biosphere Research Station, University of the Ryukyus. In June, very few larvae could be obtained, because spawning occurred in a very small scale, and crosses were not highly successful. In August, a large number of larvae could be obtained.

4. Eco-toxicological risks of the chemicals used in this study

Organotins including TBT have been widely used as anti-foulants in paints for ships and fishing devices, but in Japan its use is prohibited. According to Japan Ministry of the Environment, recent levels of TBT dissolved in coastal seawaters are generally around 0.01 μg/L or lower. However, a large number of ships from countries that do not have regulation on its use visit Okinawa, and a relatively high level of 0.16 μg/L was reported in Naha port in late 1990's. This value is about half of the effect level (0.4 μg/L) of TBT-Cl on juvenile growth in the present study. Hydrophobic substances like TBT not only are dissolved in seawater, but also attach to detritus (minute organic particulate matters either suspended in seawater or accumulated in sediments), and so corals may also be exposed to TBT through ingestion of contaminated particles. Thus, the possibility cannot be excluded that corals are affected in semi-closed areas such as ports and marinas where TBT levels are relatively high.

DCMU is used as a herbicide in farming, as well as an alternative anti-foulant. In Okinawa, it is commonly used in sugar cane and pineapple farming, and its consumption as an agricultural herbicide in this prefecture is the highest in Japan. No studies have been reported on the concentrations of TBT in aquatic environments in Okinawa. In western mainland Japan, concentrations up to 3 μg/L (three times higher than the effect level in this study) were observed. Thus, DCMU may be present at similar levels in coastal seawaters of Okinawa, negatively impacting the growth of hermatypic corals. After heavy rainfalls, large amounts of herbicides will flow into coastal seawater together with red soil, and may thereby be harmful to corals in coastal areas. Accumulation of data is needed on residual levels of herbicides at many sites in Okinawa.

In 2006, the duration of exposure experiments could be extended, as compared to the previous year, by changing the *Symbiodinium* strain and culture condition (addition of liquid food) (Watanabe et al. 2007). Using this culture system, toxicological effects of chemical substances that are suspected to have negative impacts on corals can be studied easily in laboratories.

With the possible exception of semi-closed environments such as ports and marinas, it is unlikely that single chemical substances are present at concentrations above the effect levels for corals. However, multiple potentially dangerous chemicals may be present at low levels in wide ranges of coastal areas. It is necessary, therefore, to study cumulative effects of multiple chemical substances, and investigate contamination levels of seawaters in coral habitats. Furthermore, combinatorial (or possibly synergistic) effects of chemical stresses and other stresses (e.g. anomalous temperature and salinity) also need to be studied in the future.

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Table 1. Numbers of individual juveniles that exhibited abnormalities after exposure to TBT-Cl for 10 and 48 days. Numerals in parentheses indicate percentages.

10 days					
TBT-Cl conc.	**Total number of individuals**	**Normal individuals**	**Partial detachment**	**Bail-out**	**Dead**
0 μg/L	36	36(100)	0(0)	0(0)	0(0)
0.1 μg/L	39	39(100)	0(0)	0(0)	0(0)
0.4 μg/L	35	35(100)	0(0)	0(0)	0(0)
1 μg/L	35	17(49)	18(51)	0(0)	0(0)
2.5 μg/L	32	0(0)	19(59)	2(6)	11(34)

48 days				
TBT-Cl conc.	**Total number of individuals**	**Normal individuals**	**Partial detachment**	**Covered by algae**
0 μg/L	34	34(100)	0(0)	0(0)
0.1 μg/L	39	37(95)	2(5)	0(0)
0.4 μg/L	35	31(89)	1(3)	3(9)
1 μg/L	N.D.	N.D.	N.D.	N.D.
2.5 μg/L	N.D.	N.D.	N.D.	N.D.

Table 2. Average heights of juveniles exposed to various concentrations of DCMU and TBT-Cl. Asterisks indicate significant difference to the control groups

(*: $P < 0.05$, **: $P < 0.01$).

DCMU conc.	Average height (mm)
0 μg/L	0.66+0.11
0.3 μg/L	0.65+0.09
1 μg/L	0.61+0.08*
3 μg/L	0.57+0.08**
10 μg/L	0.58+0.06**

TBT-Cl conc.	Average height (mm)
0 μg/L	0.64±0.10
0.1μg/L	0.59±0.10
0.4μg/L	0.56±0.06**

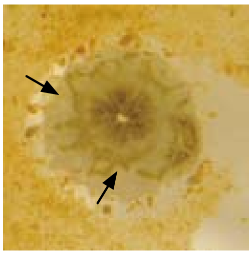
Figure1. Rare abnormality observed in juveniles exposed to 0.4 μg/L of TBT-Cl

A. A normal juvenile cultured in the absence of TBT-Cl.

B. An individual covered by algae during exposure to 0.4 μg/L of TBT-Cl for 48 days.

Figure 2. Abnormalities observed in juveniles cultured in the presence of 100 g/L of red clay for 10 days.

A. Partial bleaching in the periphery (arrow)



B. Necrosis (or possibly detachment) seen in the periphery (arrows)

Figure 3. Juveniles of *Galaxea fascicularis* inhabited by *Symbiodinium* cells strain 2467 (brown color)

Figure 4. Inside of an incubator in which exposure to chemicals was done.

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