Biomagnification process of total mercury in the aquatic ecosystem of an enclosed Isahaya Bay, Kyushu, western Japan

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A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy

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September 2018

Abstract

The studied of biomagnification process of total mercury (THg) in the coastal benthic ecosystem in Isahaya Bay, Kyushu, western Japan, where a part of mercury, was discharged by the volcanic activities of Mt. Unzen, which is being taken up by the primary producers (phytoplankton, cyanobacteria, benthic diatoms). Through environmental surveys and samplings of macro-benthic animals and fishes, the sediment contained 133 ± 23 ng/g d.w. (mean ± S.D.) of THg, which was about 4.9 times higher than that of particulate organic matter (POM) suspended in the overlying water on the sediment. This indicated that the sediment acted as if it was a concentrator of mercury, since the mercury was supplied continuously on the sediment by the deposition of POM. The biomagnification of THg tended to be accelerated significantly among the primary consumers of the macro-benthic communities that relied on the sediment for diets and/or habitats, which is referred to as “High THg content group”, and the secondary consumers that favored to prey on the primary consumers of “High total mercury content group” in the detritus food chain on the sea floor.

Among the fishes and mega-benthos such as cuttlefish, mantis shrimp, octopus, it was not able to recognize any significant relationship between the trophic position and THg content of the body tissues. The two species of the tertiary consumers of fish, *Lateolabrax japonicas* (Japanese seabass) and *Paralichthys olivaceus* (bastard halibut), contained 266 ± 99 ng/g d.w. (mean ± S.D., n = 5) and 249 ng/g d.w. (n = 1) of THg, while the THg contents of the six species of fishes of the secondary consumers or the intermediate consumers between the secondary and tertiary ones exceeded those of the tertiary consumers of fish. In particular, extremely high contents of THg were detected from *Hemitrygon akajei* (red stingray) (extra-large size class (72.0 cm, 5,150 g); 3,700 ng/g d.w., large size class (63.3 ± 4.9 cm, 1,847 ± 145 g, n = 4); 671 ± 340 ng/g d.w.), and *Acanthopagrus schlegelii* (blackhead seabream) (942 ng/g d.w.). The six species of fishes have a common feeding habit that favor benthic animals for diets, including bivalves, polychaetes, crabs, shrimps etc., and are referred to as “bentho-pelagic species”. Some of these benthic animals contain high levels of mercury, which are referred to as “High THg content group”, in both of the primary consumers and secondary ones. The extremely high contents of THg detected from the benthopelagic fishes seemed to be transferred from the sediment deposited on the sea floor via the detritus food chain of the benthic community and preferential predation of the benthic animals by the fishes.

Keyword: biomagnification, mercury, detritus food chain, macro-benthic animals, fish, mega-benthos, Isahaya Bay

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CHAPTER 1

General Introduction

1.1 Introduction

The contamination of mercury (Hg) in an environment is concerned to the negative effects to human health, while Hg has been continuously emitted (5,207 Mg/yr from natural sources such as volcanos and 2,320 Mg/yr from anthropogenic sources) to the global atmosphere (Pirrone et al. 2010), and deposited in the wet and dry forms on both of terrestrial and aquatic systems, and re-emits and/or re-mobilizes in various processes in the environment (Fig. 1-1). The coastal waters are large deposition and re-emission areas of Hg (AMAP/UNEP 2013). After Hg is discharged to the aquatic environment, its bioconcentration and bioaccumulation processes tend to occur in the primary producers (Arnot and Gobas 2006), and the Hg contents of the body tissues are apt to be biologically magnified in the organisms of higher trophic levels in the food web system (Gray 2002, Slin 2009). The biomaganification of Hg in the coastal aquatic ecosystem has brought serious negative impacts on the human health though the consumption of aquatic organisms as sea foods. Minamata disease that occurred around Minamata bay that faces Yatsushiro Sea, Kumamoto Prefecture, western Japan, in the 1950s, is one the most critical environmental incidents caused by the contamination of Hg to sea foods (Harada 1978). In this incident, the waste water containing methylmercury (MeHg) was discharged from a chemical plant to the bay. It was absorbed by phytoplankton in the water and the benthic organisms exposed to it in the sediment on the sea floor. The MeHg was transferred to the fishes along the food chains, increasing the contents of the bodies remarkably (NIMD 2014), and resulted in MeHg poisoning of various animals including birds, cats, and human.

In the aquatic systems, elemental mercury (Hg^0) is easily oxidized to InHg in sea water (Yamamoto, 1996), irrespective of its emitting sources, the volcanic activities or anthropogenic discharges, and apt to deposit on the sediment of the sea floor (Gill et al. 1999; Heyes et al. 2006, Hammerschmidt and Fitzgerald 2006). The InHg deposited on the sediment tends to be converted to poisonous MeHg (Obi et al. 2015), due to the biomethylation reaction by sulfate reducing bacteria, which often dominate in the microbial communities in the sediment (Weber et al. 1998, Clarkson and Magos 2006). It is very likely that the MeHg produced in the sediment is absorbed by the benthic organisms and its contents of the body tissues markedly increase in the organisms in the higher trophic levels through the predation in the aquatic animal communities (Fig. 1-2).

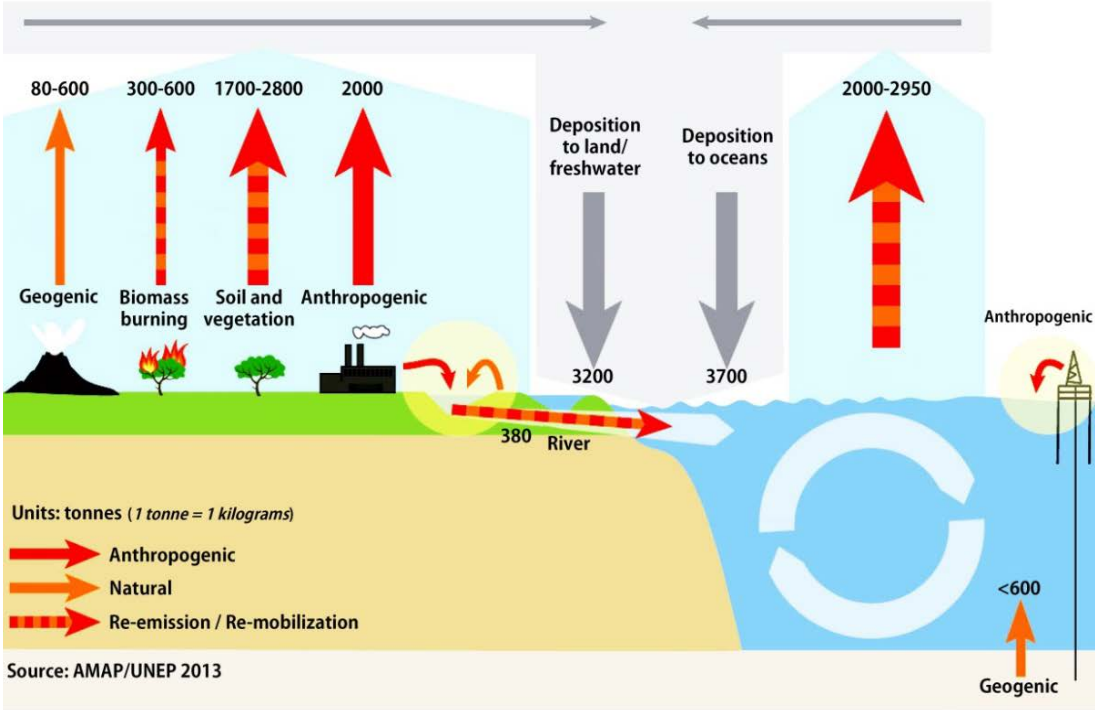
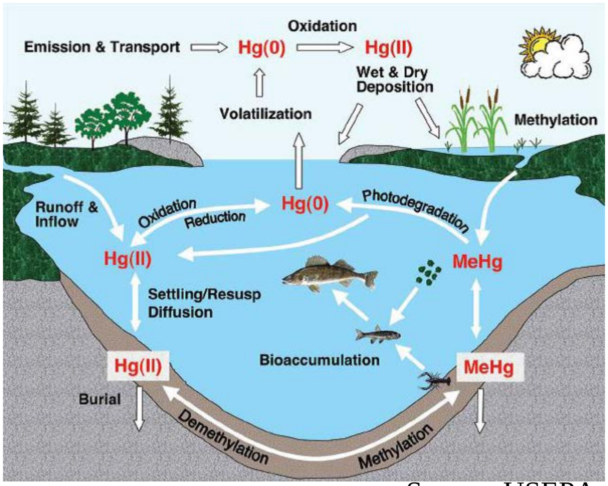


Fig. 1-1 Global Hg emission



Source: USEPA.

Fig. 1-2 Hg cycle in the aquatic system

Many previous studies on the Bioaccumulation of Hg and its transmission to the animals of the higher trophic levels in the aquatic animal communities have dealt with the phenomenon in lakes, rivers, and marine waters (cf. Atwell et al. 1998, Chen et al. 2000, Baeyens et al. 2003, Campbell et al. 2005, AL-Reasi et al. 2007, Pouilly et al. 2013, Clayden et al. 2014, Birgit et al. 2015, Ouédraogo et al. 2015, Poste et al. 2015). In these systems, even if the Hg was contained in the water with the concentration of less than 2 ng/L, it tended to be concentrated on ng/g order of the content in plankton, magnified to 2 to 25 times higher content in the bodies of the fishes that fed on the plankton and invertebrates, and further magnified by carnivorous fishes, birds, and mammals, known as biomagnification process.

In this study, I focus on the accumulation processes of elemental mercury in the aquatic organisms in an enclosed bay, Isahaya Bay, Kyushu, western Japan, where the mercury has been emitted from an active volcano continuously. In such coastal shallow waters, a part of Hg discharged to the aquatic environment deposit directly or indirectly as forms of contaminants of detritus, which are derived from POM (Particulate Organic Matter) mainly made up of dead bodies of phytoplankton and zooplankton and feces of zooplankton that predated on the phytoplankton, on the sediment of the sea floor (Li and Cai 2013), since the water is shallow. There, various benthic organisms occur due to extremely high primary productivity in the shallow waters. In the benthic system, the benthic animals tend to be exposed to the deposited Hg, and take in the Hg contaminated in the POM suspended in the overlying water and/or detritus deposited on the sediment through their feeding activities.These processes result in biomagnification of Hg in their bodies (Sizmur et al. 2013, Cardoso et al. 2014). Since the macro-benthic animals are ones of the main food items for many fishes occurring in the coastal shallow waters, the Hg settled on the benthic system is often taken up by the fishes occurring in the pelagic system (Eisler 1987, Atwell et al. 1998, Lavoie et al. 2010, Kim et al. 2012). In the previous studies conducted in the coastal shallow waters, however, the Hg contents were determined in limited members of dominant species of the benthic communities in their study areas (Fujiki and Tajima 1992, Bargagli et al. 1998, Trombini et al. 2003, Kim et al. 2004 Järv et al. 2013, Sadhu et al. 2015). Very few papers could report the whole scheme of the biomagnification process of Hg that proceeded in the coastal benthic system. Therefore, the characteristics of the biomagnification process of Hg in the benthic system in the coastal shallow waters and their linkages with those in the pelagic system are still not clear.

The purposes of this study are to collect the macro-benthic animals quantitatively in the subtidal areas and those occurring in the intertidal areas as much as possible, and catch the fishes and mega-benthos with several different nets in the inner part of Isahaya Bay, to determine their trophic positions in the aquatic animal communities in the bay using stable isotope analysis of carbon and nitrogen and their total mercury (THg) contents with a mercury analyzer, to describe the food web structure of the benthic animals and fishes and the relationship between their trophic positions (TP) and THg contents, and to clarify characteristics of the bioaccumulation processes of Hg in the aquatic animals occurring in the coastal shallow water.

In this doctoral thesis, Chapter 2 deals with the biomagnification process of THg in the macro-benthic communities in the bay, and Chapter 3 deals with the influence of benthic biomagnification process on the THg contents of fishes and mega-benthos in the bay. Finally, I conclude the whole scheme of the accumulation processes of THg in the aquatic organisms in the coastal shallow water.

CHAPTER 2

Biomagnification Process of Total Mercury in the Macro-benthic Communities in an Enclosed Bay, Isahaya Bay, Kyushu, Japan

Citation:

Jaingam W, Komorita T, Ishimatsu S, Takenaka R, Umehara A, Kobayashi J, Yamamoto M, Arizono K, Tsutsumi H. **Biomagnification process of total mercury in the macro-benthic animals in an enclosed bay, Isahaya Bay, Kyushu, Japan.** *Japanese Journal of Environmental Toxicology*. (in press).

2.1 Summary

In the inner part of Isahay Bay, Kyushu, western Japan, we assessed the biomaginification of total mercury (THg) in the benthic system. The sediment contained 133 ng/g d.w. of THg, which was about 4.9 times higher than particulate organic matter (POM) in the overlying water on the sediment. Both the primary and secondary consumers of the macro-benthic animals were divided into two groups in THg content, respectively. In the primary consumers, "High THg content group (101 ± 23 ng/g d.w., mean ± S.D.)" consisted of animals that relied on the THg-contaminated sediment for diets and/or habitats. Its mean THg content was about 4.0 times higher than that of "Low THg content group" that occurred on the sediment surface or outside the sediment, and depended on diets suspended in the water. In the secondary consumers, "High THg content group (215 ± 47 ng/g d.w.)" were made up of crabs and starfish, which favored the macro-benthic animals (mainly clams) of "High THg content group" of the primary consumers for diets. Two different biomagnification pathways of THg existed in a single food chain of the macro-benthic communities.

2.2 Introduction

Discharging decomposable chemicals and heavy metals into the aquatic environment causes bioconcentration of these substances to the primary producers, being absorbed in the water (Arnot and Gobas 2006), and their contents tend to further remarkably increase in the bodies of the animals at higher trophic levels of the food chains in their communities, referred to as biomagnification (Gray 2002). In some cases hazardous substances such as PCB, mercury, etc. have caused serious unhealthy conditions or poisoning of the animals at the highest trophic level in the food chains of the communities, which include not only wild lives (eg. seals in the North Sea by PCB (Reijnders1980), harbor porpoise and whales in England by Hg, Se and Zn (Bennett et al. 2001), but also human beings (eg. Minamata desease by methylmercury (Harada 1995)). A number of studies have previously dealt with the bioaccumulation process of mercury in lakes, rivers, and marine waters (cf. Poste et al. 2015, Pouilly et al. 2013, Baeyens et al. 2003, AL-Reasi et al. 2007, Atwall et al. 1998). In these systems, even if the mercury was contained in the water with the concentration of less than 2 ng/L, it tended to be concentrated on ng/g order of the content in plankton, magnified to 2 to 25 times higher content in the bodies of the fishes that fed on the plankton and invertebrates, and further magnified by carnivorous fishes, birds, and mammals.

In this study, we focus on the bioaccumulation process of mercury on the sea floor in the coastal shallow waters. Mercury discharged to the aquatic environment is taken up by primary producers including phytoplankton, algae, microphytobenthos. These primary producers themselves, and their dead bodies including particulate of organic matter (POM) suspended in the water and detritus deposited on the sediment are exploited as main diets by various macro-benthic animals as primary consumers in the benthic community on the sea floor. Their feeding activities result in biomagnification of mercury in their bodies (Atwell et al. 1998, Cardoso et al. 2014, Sizmur et al. 2013). Since the primary consumers of the animals are preyed on by carnivorous benthic animals such as crab, starfish, and fish (NyBakken 1982), it is likely that the mercury once settled on the sea floor tends to transfer to the animals of higher trophic levels in the food chain of the benthic system, and is partly brought to the fishes in the pelagic system (Atwell et al. 1998, Lavoie et al. 2010). However, very few papers reported the whole scheme of the biomagnification process of mercury that occurred in the coastal benthic system. Its detailed process is still unclear.

Our study area, Isahaya Bay, is located in the western side of the inner part of Ariake Bay, Kyushu, western Japan (Fig. 2-1). Shimabara Peninsula with an active volcano, Mt. Unzen, is located beside the southern side of the bay. In general, the volcanic eruption is one of the principal natural sources of elemental mercury (Hg^0) in the atmosphere. At the last eruption of Mt. Unzen, about 2.95 tons of mercury was emitted to the atmosphere between 1990 and 1995 (Nriagu 2003). Furthermore, there are many hot springs and fumaroles around the volcano, where hot water and gases containing mercury (Sakamoto et al. 1988, 2003) are continuously emitting. They all seem to work as mercury discharging sources to the bay.

In this study, we conducted samplings of water and sediment and quantitative surveys of macro-benthic animals, and collected additional specimens of macro-benthic animals on the shore of the bay, and collected water samples with bloomed blue-green algae in the reservoir facing the bay. The purposes of this study are to determine carbon and nitrogen stable isotope ratios and total mercury (THg) contents of these samples and specimens, to describe the food web structure of the macro-benthic communities and the relationship between the trophic position and THg content of the macro-benthic animals, and to discuss how the primary consumers of macro-benthic animals take THg in their bodies, and it is transferred to the animals of upper trophic levels linked with the food chain in the benthic system.

2.3 Materials and methods

2.3.1 Study Area

Isahaya Bay is an enclosed bay located in the western side of the inner part of Ariake Bay, Kyushu, western Japan (Fig. 2-1). The total area of the bay is about 65 km^2, and its mean depth is about 10 m. Honmyo River passes through Isahaya City, and discharges into the bay. We established four stations (St. 1 to St. 4) in the subtidal area and St. 5 on the shore of the bay, and St. 6 at the center of the reservoir.

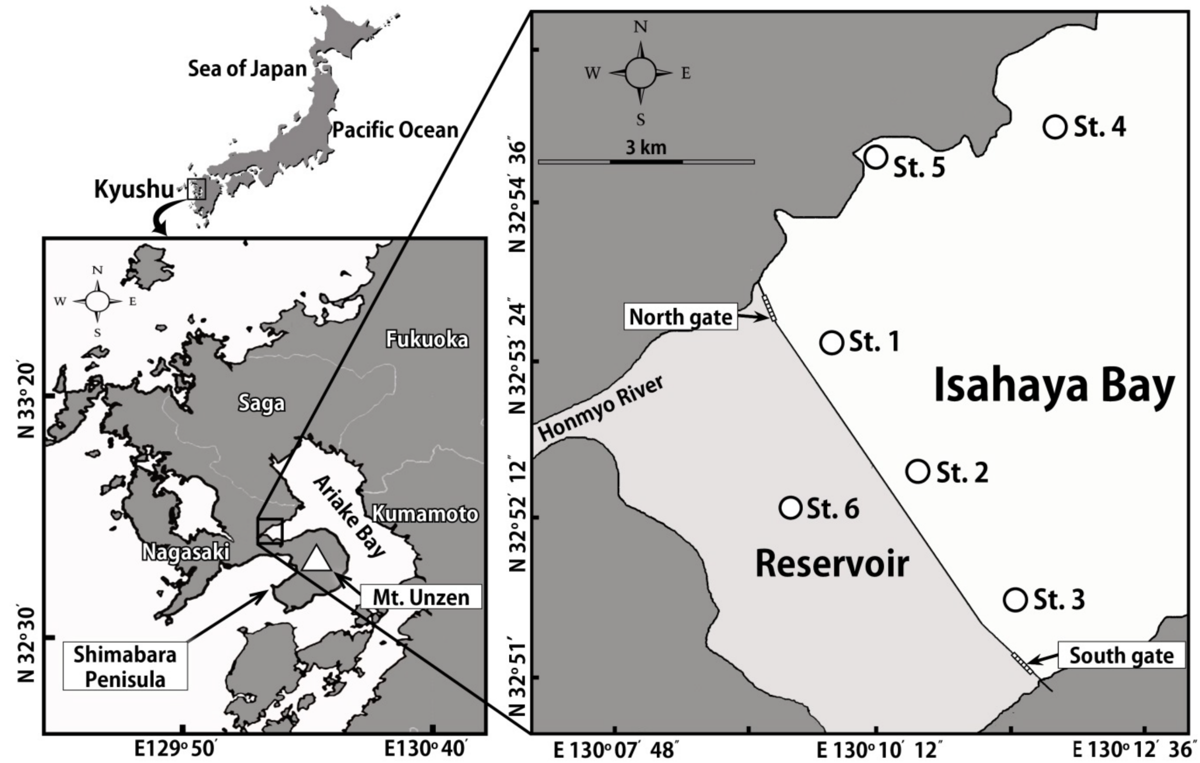


Fig. 2-1 Study area, ths inner part of Isahaya Bay, which is located in the western side of the inner part of Ariake Bay, Kyushu, western Japan.

2.3.2 Field Surveys

Table 2-1 shows the list of samplings and their dates conducted in this study. At the four stations (St. 1 to St. 4), we collected water and sediment, and quantitative samples of macro-benthic animals from a boat. At each station, we collected 2 L of the bottom water just above the sea floor with a Van Dorn water sampler, and six grab samples of the sediment with an Ekman–Birge grab sampler (20 × 20 cm). One of them was subsampled up to a depth of 1 cm using core samplers with the diameter of 29 mm ten times, kept in a plastic bag, and used for the chemical analyses. The remaining five samples were sieved on a sieve with 1 mm opening mesh, and the residues on the sieve were kept in plastic bags. Three of them were used for quantitative samples of macro-benthic animals. The remaining ones were used to collect the specimens of macro-benthic animals for chemical analyses. We collected phytoplankton with a plankton net at the three stations (St. 1, St. 3, and St. 4). We caught mussels, starfish, shrimps, and crabs with a fixed net and gill net at St. 4, collected bivalves and decapods by hand, and scraped microphytobenthos from the sediment surface with a spatula at St. 5, and took about 1 L of the surface water with a plastic bucket to sample cyanobacteria from a boat at St. 6.

Table 2-1 List of field surveys conducted in this study.

Stations	Samplings	Date
1 to 4	Water just above the sea floor, sediment for chemical analyses, quantitative samples of macro-benthic animals, and specimens of macro-benthic animals for chemical analyses	Nov. 5 in 2015, Feb. 1, May 28, and Aug. 9 in 2016
1, 3, 4	Phytoplankton with a plankton net	April 26 in 2017
4	Macro-benthic animals with a fixed net	Feb. 1, and May 28 in 2016
	Macro-benthic animals with a gill net	Nov. 27 in 2017
5	Macro-benthic animals by hand on the shore	Feb. 1, and May 28 in 2016
	Microphytobenthos on the sediment surface of the shore	May 24, and Aug. 3 in 2016
6	Water with bloomed cyanobacteria in the reservoir	Aug. 13, Sep. 9, and Oct. 19 in 2011

2.3.3 Treatment of the Samples

For the chemical analyses of stable isotope ratios of carbon and nitrogen and THg content, at the laboratory, we filtered 200 ml of the water samples with the pre-combusted 25 mm GF/F filter in order to collect POM in the water. 2 ml of the phytoplankton samples were also filtered with the GF/F filter. Colonies of cyanobacteria were sucked up with a pipette from the water samples until they filled up 5 ml plastic tubes. About 1 g and 5 g in wet weight of the sediment were subsampled with a spatula from each sediment sample for determination of carbon and nitrogen stable isotope ratios and THg content, respectively. They were kept in 5 ml of plastic vials.

To collect microphytobenthos, we put the surface sediment collected at St. 5 on a plastic tray, set a mesh cloth with 100 µm opening on it, and dispersed glass beads on the cloth. The tray was filled up with artificial seawater (salinity 30), and kept under a light for 24 hours. The glass beads were collected with a spatula, and washed with the seawater on a sieve with 63 µm opening, and the water was filtered with the GF/F filter to collect the microphytobenthos. To collect macro-benthic animals for chemical analyses, living ones were sorted from the residues after sieving the grab samples. The fleshy parts that exceeded 1 g in total wet weight as a single species were used for the chemical analyses. All samples were frozen at -20 °C, and freeze-dried for 48 hours with a freeze dryer.

For determination of stable isotope ratios of carbon and nitrogen, the freeze-dried samples were treated with 2N HCl to remove $CaCO_3$ in test tubes, centrifuged with 3,500 rpm for five minutes at 4 °C twice, and vacuum-dried for 48 hours. The samples of macro-benthic animal and cyanobacteria were delipidated with chloroform-methanol mixture solutions (2:1, v/v), centrifuged at 10,000 rpm for five minutes at 4 °C twice, and treated with 100 % methanol, centrifuged at 10,000 rpm for five minutes at 4 °C, and vacuum-dried for 48 hours. All these samples were ground to powder using a tissue grinder. The samples on GF/F filters (phytoplankton, microphytobenthos, POM) were treated with vapor of 12N HCl for 24 hours, rinsed with distilled water, and vacuumed-dried for 48 hours. The samples for quantitative surveys of macro-benthic animals were fixed with 10% formalin solution, and stained with Rose Bengal. Macro-benthic animals were sorted from the samples, identified, counted, and weighed by species.

2.3.4 Chemical Analyses

The stable isotope ratios of carbon and nitrogen of the samples were determined using an elemental analyzer (Flash Elemental Analyzer 1112 Series, Thermo Electron) and continuous flow isotope ratio mass spectrometer (Delta Plus, Thermo Electron). All isotopic data were reported in conventional delta notation (in ‰) as follows:

$$\delta^{13}\mathrm{C} = \left(\frac{^{13}\mathrm{C}/^{12}\mathrm{C}_{\mathrm{sample}}}{^{13}\mathrm{C}/^{12}\mathrm{C}_{\mathrm{standard}}} - 1\right) \times 1000\ (‰), \qquad \delta^{15}\mathrm{N} = \left(\frac{^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{sample}}}{^{15}\mathrm{N}/^{14}\mathrm{N}_{\mathrm{standard}}} - 1\right) \times 1000\ (‰)$$

Pee Dee Belemnite (PDB) and atmospheric nitrogen were used as references for ^{13}C and ^{15}N, respectively. Glycine was used as a working standard in this study.

THg contents of the samples were determined with an MA-3000 mercury analyzer (Thermal vaporization atomic absorption, Nippon Instruments). The NIMJ CRM 7302-a (marine sediment) and NIMJ CRM 7402-a (cod fish tissue) were used to test the accuracy of the method as standard reference for determination of THg content of the samples of POM and sediment and the specimens of the macro-benthic animals. In this study, we calculated biomagnification factor (BMF) of THg, which indicates an increase rate of THg content between the primary consumers (P.C.) and secondary consumers (S.C.) of macro-benthic animals, using the equation (1),

$$\mathrm{BMF} = \frac{[\text{mean THg}]_{\text{S.C.}}}{[\text{mean THg}]_{\text{P.C.}}} \text{ -------- } (1)$$

and trophic magnification slope (TMS) of THg, which indicates a biomagnification rate against $\delta^{15}N$ in the food chain, using the equation (2).

$$\mathrm{TMS} = \frac{\mathrm{Log}_{10}\left(\frac{[\text{mean THg}]_{\text{S.C.}}}{[\text{mean THg}]_{\text{P.C.}}}\right)}{\text{mean } \delta^{15}\mathrm{N}_{\text{S.C.}} - \text{mean } \delta^{15}\mathrm{N}_{\text{P.C.}}} = \frac{\mathrm{Log}_{10}(\mathrm{BMF})}{\text{mean } \delta^{15}\mathrm{N}_{\text{S.C.}} - \text{mean } \delta^{15}\mathrm{N}_{\text{P.C.}}} \text{ -------- } (2)$$

2.4 Results

2.4.1 Species Composition of the Macro-benthic Communities

Table 2-2 shows the densities and biomass of macro-benthic animals collected at the four stations (St. 1 to St. 4). The total density of the macro-benthic animals was 1,686.5 ind./m^2. 12 species occupied 90.3 % of the total abundance. *Theora lata* (bivalve) predominated the macro-benthic communities with the density of 901.0 ind./m^2 (53.4 % of the total abundance). *Raetellops pulchella* was another dominant bivalve (6.6 %). Four species of amphipods (*Crassicorophium* sp., *Ampelisca bocki*, *Photis longicaudata*, *Cerapus* sp.) occupied 19.2 % of the total abundance. In polychaete, *Sternaspis costata* and three *Pseudopolydora* species occupied 9.0 % of the total abundance. The total mean biomass of the macro-benthic animals was 57.5 g w.w./m^2. *T. lata* was the most predominant species also in biomass (39.8 g w.w./m^2, 69.2 % of the total biomass). *R. pulchella* and *Crassicorophium* sp. occupied 7.6 % and 4.1% of the total biomass, respectively. Table 2-3 shows the list of the specimens of 11 species of macro-benthic animals collected at St. 4 and St. 5, which included bivalves, shrimps, crabs, and starfish.

2.4.2 Food Chain Structure of the Macro-benthic Communities

Figure 2-2 indicates the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of 18 species of macro-benthic animals collected in this study, and possible diets for the primary consumers (phytoplankton, POM, sediment with detritus, microphytobenthos, cyanobacteria contained in the water discharged from the reservoir). The possible diets had wide ranges of the values of $\delta^{13}C$ (-22.1 to -15.3 ‰) and $\delta^{15}N$ (6.3 to 9.9 ‰), since the microphytobenthos tend to discriminate ^{13}C much less than other primary producers in the process of carbon fixation in photosynthesis (Farquhar et al. 1989), and ^{15}N-rich cyanobacteria bloomed in the reservoir (Umehara et al. 2012).

The macro-benthic animals were divided into 11 species of primary consumers and seven species of secondary ones, according to the feeding habits described in the previous studies (Saito et al, 1998, Biernbaum 1979, Morton 2010, Carefoot 2010, Fauchald and Jumars 1979, Nakamura 2004, Morton 1974, Broom 1985, Gerdes 1983, Galimany et al. 2011, Omori 1975, Deshmukh et al. 2006, Okamoto and Kurihara 1989, Weimin et al. 1998, Allen 1983) and $\delta^{15}N$ values determined in this study. The primary consumers had wide ranges of the values of $\delta^{13}C$ (-18.7 to -15.9 ‰) and $\delta^{15}N$ (7.7 to 11.8 ‰), which reflected those of the possible diets. In general, the values of $\delta^{13}C$ and $\delta^{15}N$ of the animals tend to increase about 1.0 ‰ and 3.4 ‰ between prey and predator, respectively (Wada et al. 1991). In this study, the mean $\delta^{15}N$ values of the secondary consumers were 13.4 ‰, and 3.9 ‰ higher than that of the primary consumers. These facts indicate that the classification of the trophic levels of the macro-benthic animals in this study roughly followed the general trend on the isotopic shift of nitrogen stable isotope ratio.

Table 2-2 Carbon and nitrogen stable isotope ratios and THg contents of the macro-benthic animals collected in the quantitative surveys of macro-benthic communities at the four sampling stations (St. 1 to St. 4).

(a) **Species**	**Density**		**Biomass**		**Living mode**	**Feeding type**	**$\delta^{13}C$ (‰) mean ± S.D.**	**$\delta^{15}N$ (‰) mean ± S.D.**	**N_1**	**THg content (ng/g d.w.) mean ± S.D.**	**N_2**	**TL**	**References**
	(ind./m^2)	**Composition rate (%)**	**(g w.w./m^2)**	**Composition rate (%)**									
B: *Theora lata*	901.0	53.4	39.8	69.2	INF	D	-18.0 ± 0.5	7.7 ± 0.6	13	141	1	P.C.	(1)
M: *Crassicorophium* sp.	153.1	9.1	2.3	4.1	INF	SF/SD	NA	NA	-	NA	-	NA	(2)
B: *Raetellops pulchella*	112.0	6.6	4.4	7.6	INF	D	-18.2 ± 0.7	7.9 ± 1.2	12	122	1	P.C.	(3)
P: *Pseudopolydora achaeta*	88.5	5.3	-	-	EPI	D/F	NA	NA	-	NA	-	NA	(4)
M: *Ampelisca bocki*	77.6	4.6	1.6	2.8	EPI	SF/SD	-17.4 ± 0.5	8.1 ± 0.4	3	13.8	1	P.C.	(2)
M: *Photis longicaudata*	54.7	3.2	0.8	1.3	INF	SF/SD	-17.2 ± 0.2	9.3 ± 1.0	3	28.0	1	P.C.	(2)
M: *Cerapus* sp.	39.6	2.3	0.2	0.3	EPI	SD	-18.7 ± 0.3	9.8 ± 0.8	3	25.1	1	P.C.	(2)
P: *Sternaspis costata*	29.7	1.8	3.2	5.5	INF	D	-17.6 ± 0.2	8.6 ± 0.2	3	97.2	2	P.C.	(5)
P: *Glycera* sp.	20.8	1.2	1.8	3.1	INF	C	-15.8	12.6	2	23.7	1	S.C.	(5)
P: *Pseudopolydora kempi*	17.2	1.0	-	-	EPI	D/F	NA	NA	-	NA	-	NA	(4)
P: *Sigambra hanaokai*	15.1	0.9	0.1	0.1	INF	C	NA	NA	-	NA	-	NA	(5)
P: *Pseudopolydora reticulata*	14.6	0.9	-	-	EPI	D/F	NA	NA	-	NA	-	NA	(4)
Others	162.6	9.7	3.3	6.0									
Total	1,686.5	100.0	57.5	100.0									

B: Bivalvia, M: Malacostraca, P: Polycheata,

Living mode; INF: infauna, EPI: epifauna, TL: trophic level, P.C.: primary consumer, S.C.: secondary consumer,

Feeding type; D: deposit feeder, F: filter feeder, SF: suspension feeder, SD: surface detritivore, C: carnivore,

Data; NA: not analyzed, N_1: No. of data of the stable isotope analysis, N_2: No. of data of THg content analysis

References; (1): Saito et al, 1998, (2): Biernbaum 1979, (3): Morton 2010, (4): Carefoot 2010, (5): Fauchald and Jumars 1979

Table 2-3 Carbon and nitrogen stable isotope ratios and THg contents of macro-benthic animals caught with nets at St. 4, and/or collected on the shore at the St. 5, and possible diets for the primary consumers of the macro-benthic animals.

(b) Species	Weight (g w.w.) mean ± S.D.	No. of specimens	Living mode	Feeding type	δ^{13}C (‰) mean ± S.D.	δ^{15}N (‰) mean ± S.D.	N_1	THg content (ng/g d.w.) mean ± S.D.	N_2	TL	References
B: *Ruditapes philippinarum*	0.39 ± 0.18*	7	INF	F	-17.5 ± 0.3	9.7 ± 0.4	6	80.8	2	P.C.	(6)
B: *Arcuatula senhousia*	0.10 ± 0.03*	8	INF	F	-17.0 ± 0.4	10.4 ± 1.2	8	77.9	2	P.C.	(7)
B: *Tegillarca granosa*	4.83 ± 2.10*	6	INF	F	-15.9 ± 1.0	11.1 ± 0.5	6	91.7 ± 19.2	6	P.C.	(8)
B: *Crassostrea gigas*	4.10 ± 2.36*	8	EPI	F	-16.6 ± 0.4	11.8 ± 0.5	8	94.1 ± 22.3	8	P.C.	(9), (16)
B: *Mytilus galloprovincialis*	1.11 ± 0.38*	10	EPI	F	-18.0 ± 0.9	9.8 ± 0.4	6	34.1 ± 4.3	10	P.C.	(10)
M: *Metapenaeus* cf. *affinis*	4.75 ± 0.98	9	EPI	S/C	-16.1 ± 0.8	13.5 ± 0.7	9	102 ± 49	9	S.C.	(11)
M: *Metapenaeus* cf. *joyneri*	6.83 ± 2.06	5	EPI	S/C	-16.7 ± 1.1	14.4 ± 0.4	5	63.2 ± 20.0	5	S.C.	(12)
M: *Hemigrapsus* sp.	4.52 ± 0.41	3	EPI	C/S	-15.8 ± 0.3	12.6 ± 0.4	3	271 ± 22	3	S.C.	(13)
M: *Charybdis japonica*	125.7 ± 84.7	5	EPI	C/S	-15.9 ± 1.3	14.1 ± 0.6	5	183 ± 43	5	S.C.	(14)
M: *Portunus trituberculatus*	308.3 ± 61.3	4	EPI	C/S	-15.7 ± 1.7	14.0 ± 0.8	4	170 ± 48	4	S.C.	(14)
A: *Asterias* sp.	30.4 ± 6.4	4	EPI	C	-15.1 ± 0.6	12.8 ± 0.3	4	234	2	S.C.	(15)
Microphytobenthos					-15.3 ± 0.5	7.1 ± 0.9	5	NA	-		-
Phytoplankton					-19.2 ± 1.0	7.3 ± 0.8	3	NA	-		-
Cyanobacteria					-22.1 ± 2.4	9.9 ± 3.0	9	NA	-		-
POM					-21.9 ± 1.7	7.0 ± 1.0	9	27.2 ± 9.7	9		-
Sediment					-21.2 ± 1.3	6.3 ± 0.3	19	133 ± 23	19		-

B: Bivalvia, M: Malacostraca, A: Asteroidea, *Total wet weight without shells,

Living mode; INF: infauna, EPI: epifauna, TL: trophic level, P.C.: primary consumer, S.C.: secondary consumer,

Feeding type; F: filter feeder, S: scavenger, C: carnivore,

Data; NA: not analyzed, N_1: No. of data of the stable isotope analysis, N_2: No. of data of THg content analysis

References; (6): Nakamura 2004, (7): Morton 1974, (8): Broom 1985, (9): Gerdes 1983, (10): Galimany et al. 2011, (11): Omori 1975, (12): Deshmukh et al. 2006, (13): Okamoto and Kurihara 1989, (14): Weimin et al. 1998, (15): Allen 1983, (16): Iyooka et al. 2008

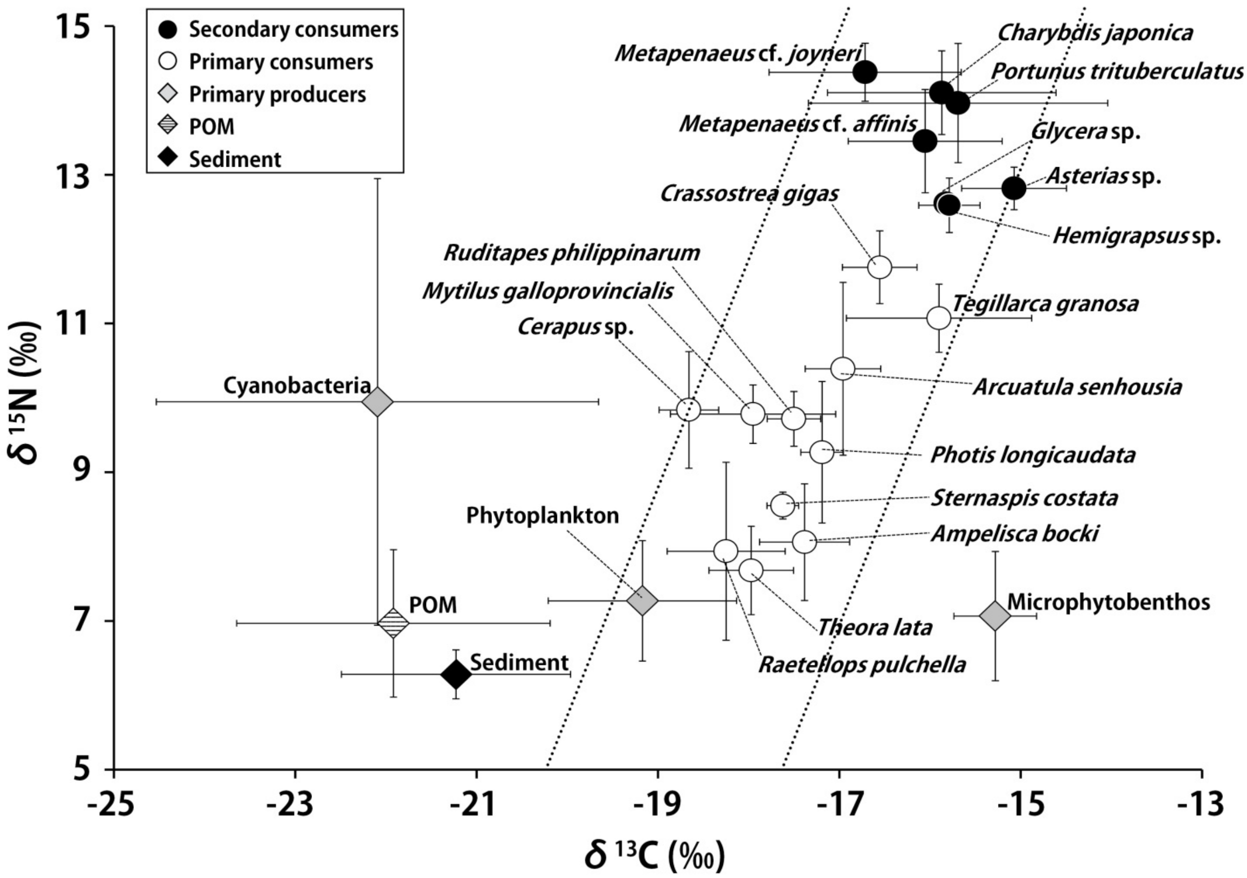


Fig. 2-2 Dual-isotope plots of the mean values of δ^{13}C and δ^{15}N of macro-benthic animals and their potential diets in the inner part of Isahaya Bay. Bars indicated standard deviations. The data of the plots are noted in Table 2-2 to Table 2-3. The dotted lines indicate the general trend of isotopic shift of carbon and nitrogen between prey and predator (Wada et al. 1991)

2.4.3 THg Contents of Sediment, POM, and Macro-benthic Animals

Figure 2-3 shows the relationship between δ^{15}N values and THg contents of POM, sediment and macro-benthic animals. The THg content of POM was 27.2 ng/g d.w., while that of the sediment was 133 ng/g d.w. (about 4.9 times higher than POM). The THg contents of the 11 species of primary consumers showed large variations between 13.8 and 141 ng/g d.w., and were roughly divided into “High" and “Low" THg content groups with common features in feeding habits and/or living modes (Table 2-2 to Table 2-3).

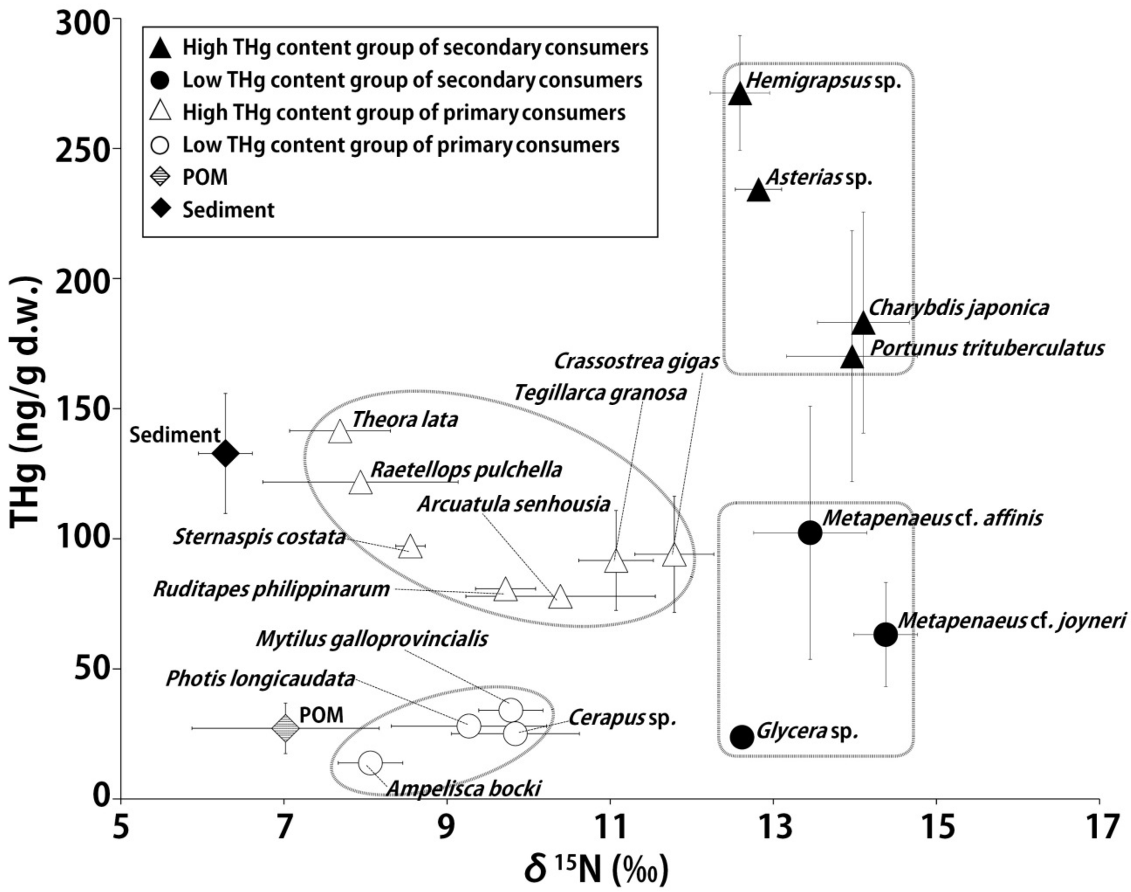


Fig. 2-3 Relationship between mean values of δ^{15}N and total mercury (THg) contents of POM, sediment, and body tissues of the macro-benthic animals collected in the inner part of Isahaya Bay. Bars indicate standard deviations. The data of the plots are noted in Table 2-2 to Table 2-3.

The former was made up of seven species with 101 ± 23 ng/g d.w. (mean ± S.D.) of THg contents. They included deposit-feeding bivalves (*T. lata*, *R. pulchella*) (Saito et al. 1998, Morton 2010) and polychaete (*S. costata*) (Fauchald and Jumars 1979) occuring in mud, and filter-feeding bivalves that burrow in sediment (*Ruditapes philippinarum*, *Arcuatula senhousia*, *Tegillarca granosa*) (Nakamura 2004, Morton 1974, Broom 1985), and create reefs on the muddy tidal flats (*Crassostrea gigas*) (Gerdes 1983, Iyooka et al. 2008). They are always exposed to the mercury-accumulated sediment, and feed it directly or filter the muddy water with re-suspended mud. The latter consisted of amphipods (*A. bocki*, *P. longicaudata*, *Cerapus* sp.) and mussel (*Mytilus galloprovincialis*). *A. bocki* and *Cerapus* sp. are epifauna, and all three species of amphipods are suspension feeders and/or surface detritivors (Biernbaum 1979). Therefore, they utilize the surface of the sediment for living space. The mussel attaches on the hard substrates, and filters the water to take in phytoplankton and POM as main diets (Galimany 2011). The THg content of these species was 25.2 ± 8.5 ng/g d.w., and about one fourth of "High THg content group". The differences of the THg contents between the two groups were statistically significant ($p<0.05$, Mann-Whitney U test).

The THg content of the seven species of the secondary consumers also showed larger variations ranged between 23.7 and 271 ng/g d.w., and they were divided roughly into "High" and "Low" THg content groups with common features of feeding habits. The former consisted of crabs (*Hemigrapsus* sp., *Charybdis japonica*, *Portunus trituberculatus*) and starfish (*Asterias* sp.), and contained 215 ± 47 ng/g d.w. of THg. They are all carnivores that favor bivalves (Okamoto and Kurihara 1989, Weimin et al. 1998, Allen 1983). The latter consisted of shrimps (*Metapenaeus* cf. *joyneri*, *Metapenaeus* cf. *affinis*) and carnivorous polychaete, *Glycera* sp., and contained 63.1 ± 39.3 ng/g d.w. of THg. The shrimps get foods mostly by scavenging (Omori 1975, Deshmukh et al. 2006), and *Glycera* feeds on small benthic animals (Fauchald and Jumars 1979). The THg content of "High THg content group" was about 3.4 times higher than that of "Low THg content group". The differences were statistically significant ($p<0.05$, Mann-Whitney U test).

2.5 Discussion

The results of this study indicated that the THg content of the sediment (133 ng/g d.w.) was about 4.9 times higher than that of POM (27.2 ng/g d.w.) (Fig. 2-3, Table 2-2 to Table 2-3). In the enclosed coastal shallow waters with the depth of less than 20 m, sinking POM in the water column is apt to reach the sea floor, and deposit as detritus on the sediment (cf. Wassmann 1983). If some mercury is contaminated in the POM, it remains in the sediment even after the organic matter of the detritus has been degraded by bacterial activities. The residence time of mercury in the ocean sediment is extremely long (Eisler 1987). Therefore, the sea floor with continuous deposition of mercury-contaminated POM seems to work as if it was an accumulator of mercury to the sediment, and a big reservoir of mercury. There, various benthic animals occur, exploiting the detritus and POM for their main diets. The THg content of the sediment in uncontaminated coastal waters without volcanic activities such as North Central U.S., Thailand, Finland, etc. ranged between 20 and 110 ng/g d.w., while those of the contaminated areas by the anthropogenic activities significantly exceeded 20.0 mg/kg d.w. (Eisler 1987). The macro-benthic animals occurring in this study were exposed to slightly elevated levels of THg of the sediment to the uncontaminated areas, presumably due to volcanic activities.

The relationship between the values of $\delta^{15}N$ and THg contents of macro-benthic animals in this study appears that the biomagnification of THg was distinct only in the four species of secondary consumers, which contained 170 to 271 ng/g d.w. of THg (Fig. 2-3), in the macro-benthic communities. However, the findings of "High" and "Low" THg content groups in both of the primary and secondary consumers revealed the presence of two different biomagnification pathways of THg in a food chain of the benthic system, which were hidden among the large variations of THg contents in each trophic level.

Previous studies on the biomagnification of mercury in the benthic system in the coastal waters reported that deep burrowing macro-benthic animals such as maldanid, capitelid, and nereid polychaes, and cockle bioaccumulated more MeHg or THg than other surface-dwelling invertebrates, and emphasized the relationship between the space utilization by the macro-benthic animals as primary consumers and their biomagnification of mercury (Sizmur et al. 2013). These facts coincide with the occurrence of deposit-feeding polychaetes and bivalves that burrow in sediment (saito et al. 1998, Morton 2010, Fauchald and Jumars 1979) and filter-feeding bivalves that inhabit the sediment (Nakamura 2004, Morton 1974, Broom 1985) or grow up on the muddy sediment (Gerdes 1983, Iyooka et al. 2008) as the members of “High THg content group” of the primary consumers in the macro-benthic communities in our study area. Here, a pathway that enhances the transfer of mercury discharged to the estuary to the primary consumers of the aquatic animals exists on the sediment.

The members of “High THg content group” of the secondary consumers of the macro-benthic communities included carnivorous crabs and starfish, which favor bivalves for diets (Okamoto and Kurihara 1998, Allen 1983). Their $\delta^{13}C$ and $\delta^{15}N$ values (-15.9 ‰ to -15.1 ‰, 12.6 ‰ to 14.1 ‰, respectively) indicated that at least four species of bivalves of “High THg content group” of the primary consumers (*Ruditapes philippinarum*, *Arcuatula senhousia*, *Tegillarca granosa*, *Crassostrea gigas*) were in the range of carbon and nitrogen stable isotope ratios of their prey animals (Fig. 2), according to the general trend of isotopic shift between prey and predator (Wada et al. 1991). The THg contained in the bodies of “High THg content group” of the secondary consumers seems to be mainly derived from these bivalves whereas the members of "Low THg content group" of the secondary consumers (shrimps and a carnivorous polychaete) are unlikely to prey on the bivalves of "High THg content group" of the primary consumers.

Previous studies on the biomagnification of THg of coastal marine organisms reported that the it was less distinct in benthic species than those in bentho-pelagic and pelagic species, and noted 0.111 (Lavoie et al. 2010) and 0.119 (Kim et al. 2012) as biomagnification rates against $\delta^{15}N$, which are equivalent to the trophic magnification slope (TMS) of THg in this study. BMF values of THg content between the primary and secondary consumers in this study were 2.13 in "High THg content group" and 2.50 in "Low THg content group", respectively. They indicate that the biomagnification of THg as the content became about twice occurred almost parallel between the primary and secondary consumers in these two different THg content groups (Fig. 2-4). The values of TMS of “High” and “Low” THg content groups were 0.086 and 0.092, respectively, and indicate that slightly lower levels of biomaganification of THg occurred among the macro-benthic animals. The THg contents among the members of “High THg content group” of the secondary consumers, however, reached the range between 170 and 271 ng/g d.w. (Table 2-2 to Table 2-3). If the fishes and mega-benthos classified as “bentho-pelagic species” fed these macro-benthic animals preferentially, their THg contents might exceed the provisional regulation value of THg, 400 ng/g w.w., which seems to be equivalent to about 2,000 ng/g d.w. for fish and shellfish in Japan (National Institute for Minamata Disease, Ministry of Environment 2013).

The two different biomagnification pathways of THg in the benthic system may actually fuse each other in the further higher trophic levels, and the members of "High THg content groups" of the primary and secondary consumers may work as key animals to transfer the mercury deposited on the sea floor to the pelagic system. Further study to clarify the mechanisms how the mercury is transferred to the bentho-pelagic animals will be necessary. The results will be reported elsewhere.

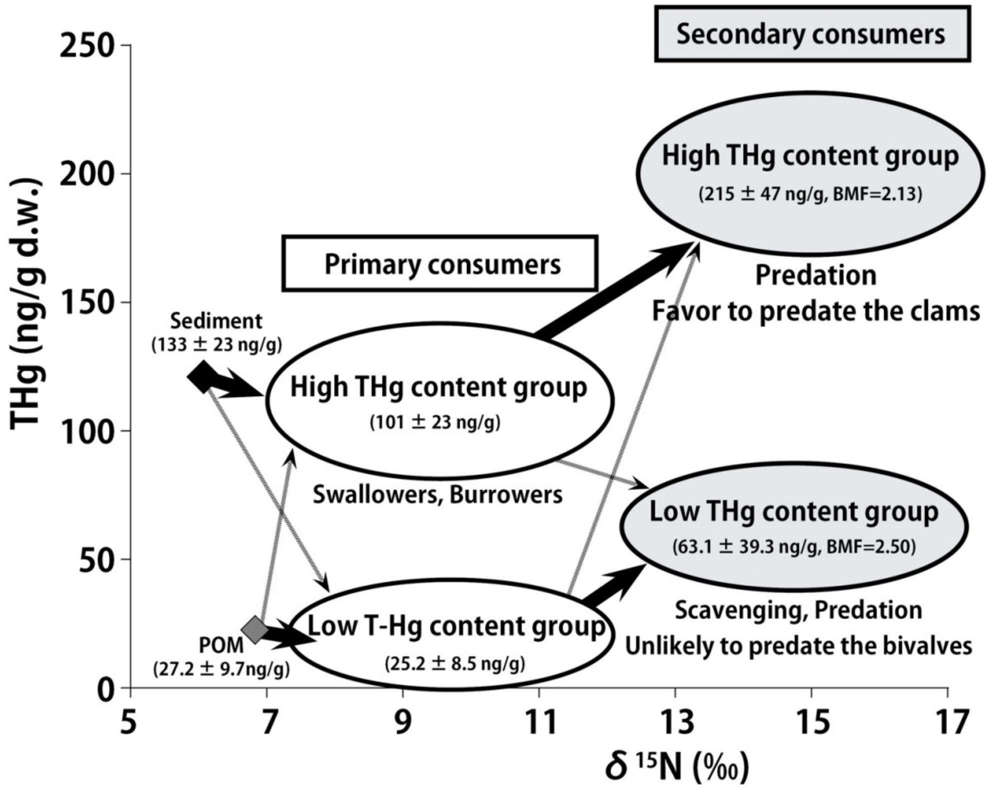


Fig. 2-4 Two different biomagnification pathways of total mercury (THg) along the food chain of the benthic system in the inner part of Isahaya Bay.

2.6 Conclusions

The results of the stable isotope analysis of carbon and nitrogen indicated that the macro-benthic communities in the present study area were formed by the organisms in three different trophic levels (primary producers, primary consumers, secondary consumers) linked in a single food chain. The determination of THg contents of POM, sediment, and macro-benthic animals noted that the sediment contained 133 ng/g d.w. of THg, which was about 4.9 times higher than that of POM. It indicates that the sea floor works like an accumulator of mercury to the sediment. The combined analysis with the results of stable isotope ratios of carbon and nitrogen and THg contents of macro-benthic animals revealed the presences of "High" and "Low" THg content groups with common living modes and/or feeding types in both the primary and secondary consumers, and "two different biomagnification pathways" of THg in a single food chain of the benthic system.

The primary consumers of "High THg content group" were made up of the animals that depended highly on the THg-contaminated sediment for habitats as burrowers, and/or diets as deposit-feeders, while those of "Low THg content group" occurred on the surface of the sediment or outside the sediment, and much less exposed to the THg-contaminated sediment. The BMF values of THg content between the primary and secondary consumers were 2.13 in "High THg content group" and 2.50 in "Low THg content group." The biomagnification of THg proceeded almost parallel between these two groups.

CHAPTER 3

Influence of benthic biomagnification process on the total mercury content of fish and mega-benthos in an enclosed bay

Citation:

Jaingam W, Komorita T, Ishimatsu S, Kobayashi J, Yamamoto M, Arizono K, Tsutsumi H. **Influence of benthic biomagnification process on the total mercury content of fish and mega-benthos in an enclosed bay.** *Journal of the Japanese Society of Shiranuikai & Kumagawa Regional Studies.* 2018; 12 (1): 3-14.

3.1 Summary

The contents of heavy metals and non-biodegradable chemicals of the body tissues tend to increase in the organisms of higher trophic levels of the biological community. However, we were not able to recognize any significant relationship between the trophic positions (TP) and total mercury (THg) contents of the body tissues among 24 species of fishes and mega-benthos collected in the inner part of Isahaya Bay, Kyushu, western Japan. The two species of the tertiary consumers of fish, *Lateolabrax japonicas* (Japanese seabass) and *Paralichthys olivaceus* (bastard halibut), contained 266 ± 99 ng/g d.w. (mean ± S.D., n = 5) and 249 ng/g d.w. (n = 1) of THg, while the contents of the six species of fishes of the secondary consumers, or the intermediate consumers between the secondary and tertiary ones, exceeded those of the tertiary consumers of fish. In particular, extremely high contents of THg were detected from *Hemitrygon akajei* (red stingray) (3,700 ng/g d.w., extra-large size class (72.0 cm, 5,150 g); 671 ± 340 ng/g d.w., large size class (63.3 ± 4.9 cm, 1,847 ± 145 g, n = 4)), and *Acanthopagrus schlegelii* (blackhead seabream) (942 ng/g d.w., n = 1). They have common feeding habits that favor macro-benthic animals for diets, including bivalves, polychaetes, crabs, shrimps, etc., and are referred to as "bentho-pelagic species". In the study area, the mercury discharged by volcanic activities was accumulated in the sediment, and the THg was concentrated on some macro-benthic animals through the biomagnification process in the detritus food chain in the benthic system. The high THg contents found in the bentho-pelagic fishes seem to be transferred from the preferential predation of the macro-benthic animals with high levels of THg contents.

3.2 Introduction

When mercury has been discharged from the surrounding lands to the coastal shallow waters, it is absorbed by primary producers such as phytoplankton, algae, and sea grass, and accumulates in their body tissues, which is referred to as "bioconcentration". In the pelagic system, the phytoplankton is preyed on by various organisms linked with a food chain, including zooplankton, fishes, marine mammals, birds, etc., increasing markedly the mercury contents of the bodies at higher trophic levels (Bargagli et al. 1998, Gray 2002, Campbell 2005, AL-Reasi et al. 2007). In parallel with the biomagnification process in the pelagic system, the mercury contents of the animals tend to increase along the detritus food chain in the benthic ecosystem. The primary producers that absorbed mercury in the water become directly or indirectly particulates of organic matter (POM), tend to sink down toward the sea floor, and deposit as detritus on the sediment. Benthic animals feed on the sediment with detritus, and/or filter POM suspended or re-suspended in the overlying water on the sediment, and this starts another biomagnification process in the benthic system (Lavoie et al. 2010, Kim et al. 2012).

Our studies have focused on the biomagnificaiton process of total mercury (THg) that proceeds in the benthic system of the coastal shallow waters. In the latest results of our studies in the benthic system in the inner part of Isahaya Bay (Jaingam et al (in press)), 27.2 ± 9.7 ng/g d.w. (mean ± S.D.) of THg was detected from POM suspended in the bottom water, while about 4.9 times higher THg content, 133 ± 23 ng/g d.w., was noted in the surface layer of the sediment. There, abundant various macro-benthic animals including bivalves, polychaetes, amphipods, etc. occurred, and worked as primary consumers, exploiting the detritus deposited on the surface sediment and POM in the overlying water on the sediment. Among these animals, we found a high THg content group with 101 ± 23 ng/g d.w. of THg contents, which consisted of deposit-feeding polychaetes and bivalves, and suspension-feeding bivalves burrowing in the sediment. The secondary consumers such as carnivorous crabs and starfish that favored the bivalves for diets had further about 2.1 times higher THg contents, 215 ± 47 ng/g d.w. than those of the primary consumers. The provisional regulation value of THg for fish and shellfish for foods is set 400 ng/g w.w in Japan (National Institute for Minamata Disease, Ministry of Environment 2013), which is equivalent to about 2,000 ng/g d.w. If some fishes and mega-benthos prey on these macro-benthic animals with high THg contents preferentially, it is likely that their THg contents exceed the provisional regulation value due to the effects of biomagnification.

In this study, we conducted sampling of fishes and mega-benthos with a gill net, fixed net, and cast net in the inner part of Isahaya Bay, which is an enclosed bay located in the western side of the inner part of Ariake Bay, Kyushu, western Japan. The purposes of this study were to determine the stable isotope ratios of carbon and nitrogen and the THg contents of the body tissues of fishes and mega-benthos, to clarify the relationship between their trophic positions in the community of aquatic animals and THg contents, and to discuss the influence of biomagnification of THg among the micro-benthic animals on the THg content of fishes and mega-benthos occurring in the bay.

3.3 Materials and methods

3.3.1 Study area

Isahaya Bay is an enclosed bay located in the western side of the inner part of Ariake Bay, Kyushu, Japan (Fig.3-1). The total area of the bay is about 65 km^2, and its average depth is 10 m. Shimabara Peninsula with an active volcano, Mt. Unzen, located on the southern side of the bay. In general, these volcanic eruptions were some of the principal natural sources of mercury in the atmosphere (Nriagu 1989). It has erupted at least three times (1663, 1792, 1990 to 1995) since the dawn of human history in Kyushu (Unzen Restoration Project Office 2007). At the last eruption of Mt. Unzen, about 2.95 tons of mercury was emitted to the atmosphere over 1,928 days (Nriagu 2003). Furthermore, there are many hot springs and fumaroles around the volcano, from where hot water and gases containing mercury are being continuously emitted (Sakamota et al. 1988, 2003, Ohsawa et al. 2002). They all seem to work as sources of discharging mercury to the bay. Here, we set three sampling stations in the inner part of the bay to collect fishes and mega-benthos with a gill net at St. 1, a fixed net at St. 2, and a cast net at St. 3.

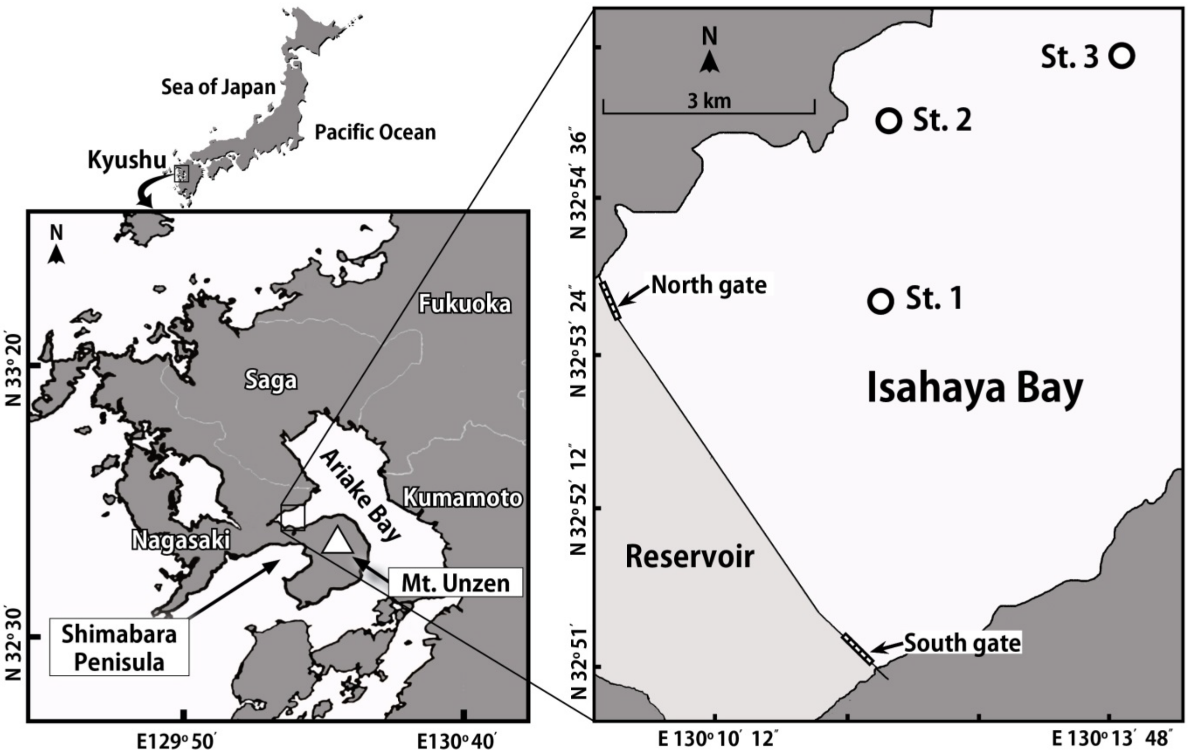


Fig. 3-1 Study area of this study, Inner part of Isahaya Bay, Kyushu, western Japan.

3.3.2 Sampling

On 17 October 2015, January 27, May 24, and August 3 in 2016, we collected fishes and mega-benthos, leaving a gill net in the water from a fishing boat for approximately 30 minutes, at St. 1, and with a fixed net which was permanently set at St. 2. At St. 3, we conducted samplings of a school of fish with a cast net from the fishing boat, searching for it with an echo sounder, on 27 November 2017. All of the collected specimens of the fishes and mega-benthos were kept in cooler boxes until they were brought back to the laboratory.

3.3.3 Treatment of the samples

At the laboratory, the specimens of the fishes and mega-benthos were identified, measured, weighed, and dissected with clean instruments to take the dorsal muscles without skin and bones of fishes and muscle of mega-benthos. About 6 g in wet weight of the muscles of each specimen was collected to use for the chemical analyses. These samples were washed with distilled water, put in 5 ml of plastic vials, and kept in a freezer at -20 °C. The frozen samples were freeze-dried for 48 hours with a freeze dryer, powdered with Agate mortar, and kept in the plastic vials.

Prior to determination of stable isotope ratios of carbon and nitrogen of the samples, about 10 mg d.w. of each sample was placed in the microtubes 1.5 mL, treated with 2N HCl to remove $CaCO_3$, vacuum-dried for 48 hours, delipidated with chloroform-methanol mixture solutions (2:1, v/v), and centrifuged at 10,000 rpm for five minutes at 4 °C twice. They were treated with 100 % methanol, centrifuged at 10,000 rpm for five minutes at 4 °C again, and vacuum-dried for 48 hours. Finally, they were ground to powder with pellet pestle. The remaining samples were used for determination of THg contents.

3.3.4 Chemical analyses

The stable isotope ratios of carbon and nitrogen of the samples were determined using an elemental analyzer (Flash Elemental Analyzer 1112 Series, Thermo Electron) and continuous flow isotope ratio mass spectrometer (Delta Plus, Thermo Electron). All isotopic data were reported in conventional delta notation (in ‰) as follows:

$$\delta^{13}\mathrm{C} = \left(\frac{{}^{13}\mathrm{C}/{}^{12}\mathrm{C}_{\mathrm{sample}}}{{}^{13}\mathrm{C}/{}^{12}\mathrm{C}_{\mathrm{standard}}} - 1\right) \times 1000\ (‰), \qquad \delta^{15}\mathrm{N} = \left(\frac{{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{sample}}}{{}^{15}\mathrm{N}/{}^{14}\mathrm{N}_{\mathrm{standard}}} - 1\right) \times 1000\ (‰)$$

Pee Dee Belemnite (PDB) and atmospheric nitrogen were used as references for ^{13}C and ^{15}N, respectively. Glycine was used as a working standard in this study. The overall analytical error was within ± 0.2 ‰.

The THg contents of the samples were determined with an MA-3000 mercury analyzer (Thermal vaporization atomic absorption, Nippon Instruments). The NIMJ CRM 7402-a (cod fish tissue) was used to test the accuracy of the method as a standard reference for the determination of THg content of the specimens of the fishes and mega-benthos.

3.3.5 Analyses of trophic relationship and biomagnification of mercury

The trophic position of each species of the fishes and mega-benthos collected in this study was determined with its $\delta^{15}N$ value of the body tissues. The $\delta^{15}N$ value of the secondary consumers, 13.4 ± 0.7 ‰ (mean ± S.D.), was obtained with the data of seven species of the secondary consumers of the macro-benthic animals collected in the study area (Jaingam et al. (in press)). We assumed the isotopic shift of $\delta^{13}C$ and $\delta^{15}N$ values between the prey and predator as +1 ‰ and +3.4 ‰, respectively (Wada et al. 1991). Being based on these assumptions, the trophic position value of each species (TP_i) in the community of the aquatic animals was determined by the following equation (3).

$$TP_i = 3 + (\delta^{15}N_i - \delta^{15}N_{s.c.}) / 3.4\ (‰) \text{ --------(3)}$$

TP_i : trophic position of species i, $\delta^{15}N_i$: $\delta^{15}N$ value of species i, $\delta^{15}N_{s.\ c.}$: the mean of $\delta^{15}N$ values of seven species of the secondary consumers (S.C.) of the macro-benthic animals occurring in the study area (Jaingam et al. (in press)) = 13.4 (‰), 3.4 (‰): isotopic shift of $\delta^{15}N$ value between prey and predator (Wada et al. 1991).

In this study, the animal which has a TP value between 2.8 and 3.2 or one between 3.8 and 4.2 is regarded as a secondary consumer or a tertiary one, respectively, considering the range of the standard deviation of the $\delta^{15}N$ values (± 0.7 ‰) of the seven species of the secondary consumers of the macro-benthic animals collected in the study area (Jaingam et al. (in press)). The difference of 0.7 ‰ in $\delta^{15}N$ value is equivalent to that of 0.2 in TP value. The animal with a TP value between 3.3 and 3.7 is treated as “the intermediate consumer” between the secondary consumer and tertiary one, which is referred to “the intermediate consumer” in this paper.

3.4 Results

3.4.1 Isotopic signatures of the body tissues of fishes and mega-benthos

Fig. 3-2 indicates the relationship between $\delta^{13}C$ and $\delta^{15}N$ values of the body tissues of 24 species of animals collected by the samplings in the inner part of Isahaya Bay in this study, which were made up of 21 species of fishes, two species of cephalopoda, and one species of malacostraca, and their trophic positions in the food chain of the aquatic animal community in the bay. (The data on the stable isotope ratios of carbon and nitrogen of these species are noted in Table 3-1). The mean values of $\delta^{13}C$ and $\delta^{15}N$ of these animals ranged between -18.0 ‰ and -14.5 ‰, and 12.8 ‰ and 17.3 ‰, respectively. According to the general trend of isotopic shift between prey and predator[15)], the range of the $\delta^{13}C$ values, 3.5 ‰, indicates that they exploited the organic matters derived from multiple sources of the primarily producing organisms, and that of the $\delta^{15}N$ values, 4.5 ‰, which exceeded the general isotopic shift of $\delta^{15}N$ value between the predator and prey, 3.4 ‰, indicates that they preyed on the animals of different trophic positions.

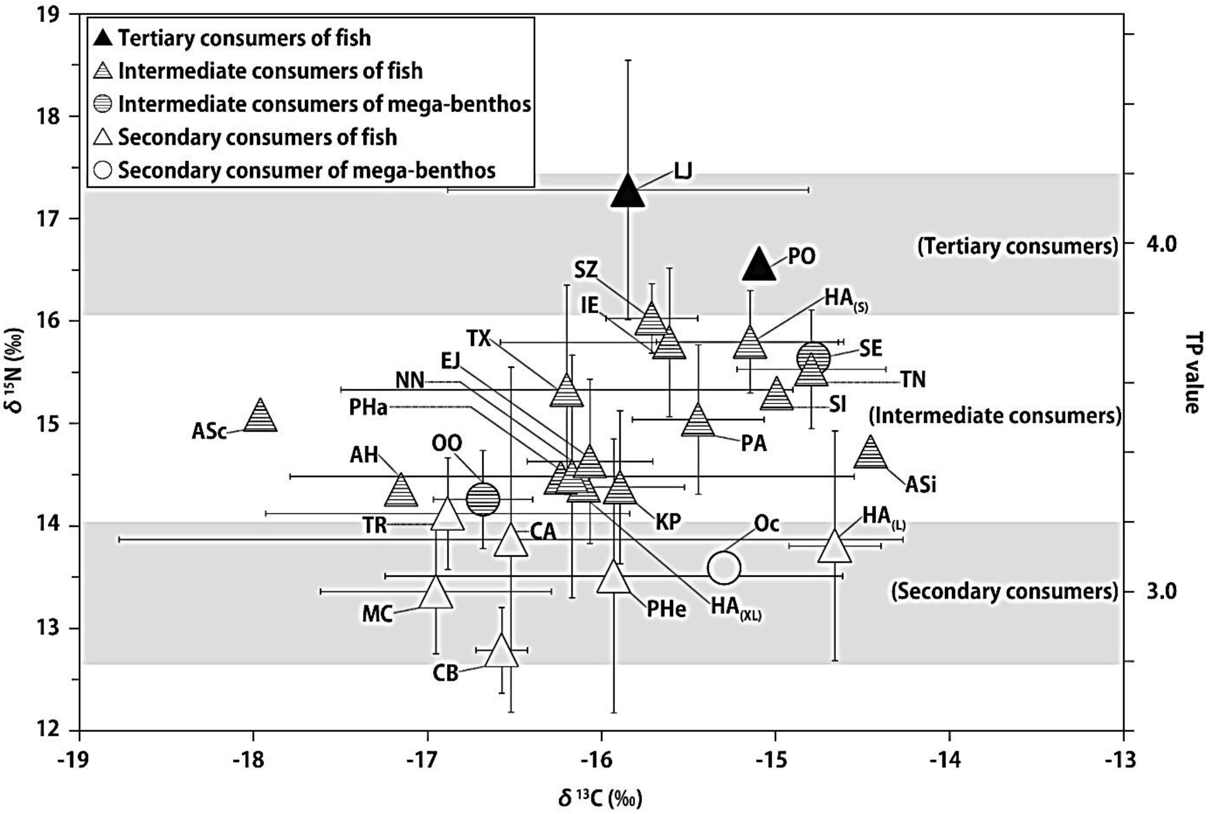


Fig. 3-2 $\delta^{13}C$ and $\delta^{15}N$ signatures of 24 species of the fishes and mega-benthos collected from the inner part of Isahaya Bay. The data of the plots are noted in Table 3-1. Error bars represent standard deviation of the mean values.

The fishes and mega-benthos collected in this study possess large mobility, and seem to feed on various diets in both of the food chains of the pelagic and benthic systems. It is, therefore, hard to define their trophic positions in the aquatic animal community simply from the observations of their feeding habits. However, judging from the TP values calculated with their $\delta^{15}N$ values (Table 3-1), two species of fishes, *Lateolabrax japonicas* (Japanese seabass, TP = 4.1) and *Paralichthys olivaceus* (bastard halibut, TP = 3.9), were regarded as tertiary consumers in the community of the aquatic animals in the bay. Six species of fishes, *Hemitrygon akajei* (large size (63.6 ± 4.9 cm, 1,847 ± 145 g, mean ± S.D., n = 4) of red stingray), *Callionymus beniteguri* (whitespotted dragonet), *Takifugu rubripes* (Japanese pufferfish), *Pseudopleuronectes herzensteini* (yellow striped flounder), *Cynoglossus abbreviates* (three-lined tongue sole) and *Mugil cephalus* (flathead grey mullet), and one species of cephalopoda, *Octopus* sp. (octopus) had TP values between 2.8 and 3.2, and were treated as secondary consumers.

The remaining 15 species of the aquatic animals were made up of 13 species of fishes, *Ilisha elongate* (elongate ilisha), *Pennahia argentata* (silver croaker), *Konosirus punctatus* (dotted gizzard shad), *Sebastes inermis* (dark-banded rockfish), *Takifugu niphobles* (grass puffer), *Takifugu xanthopterus* (yellowfin pufferfish), *Planiliza haematocheila* (so-iuy mullet), *Acanthopagrus schlegelii* (blackhead seabream), *Acanthopagrus sivicolus* (Okinawa seabream), *Nuchequula nuchalis* (spotnape ponyfish), *Amblychaeturichthys hexanema* (pinkgray goby), *Engraulis japonicas* (Japanese anchovy), and *Sardinella zunasi* (Japanese sardinella), two different size classes of red stingray (*H. akajei,* extral-large size; 72.0 cm, 5,150 g, (n = 1), small size; 30.6 ± 2.4 cm, 98.2 ± 29.1 g, (n = 4)), one species of cephalopoda, *Sepia esculenta* (cutttlefish), and one species of malacostraca, *Oratosquilla oratoria* (mantis shrimp), had TP values between 3.3 and 3.7. They were considered to be “intermediate consumers”, which fed on mixtures of the primary and the secondary consumers for diets.

3.4.2 THg contents of the body tissues of fishes and mega-benthos

Fig. 3-3 shows the relationship between the $\delta^{15}N$ values and THg contents of the body tissues of 24 species of fishes and mega-benthos collected in the inner part of Isahaya Bay. A general trend of biomagnification of THg content in the food chain of the animal community was not found in the THg contents of the fishes and mega-benthos collected in the study area. The values of two species of the tertiary consumers, *L. japonicas* and *P.*

olivaceus, were 266 ± 99 ng/g d.w. (mean ± S.D., n = 5) and 249 ng/g d.w. (n = 1), respectively, while five species of the intermediate consumers of fish, extra-large size class of *H. akajei* (TP = 3.3), *T. niphobles* (TP = 3.6), *A. schlegelii* (TP = 3.5), *P. haematocheila* (TP = 3.3) and *N. nuchalis* (TP = 3.3), and two species of the secondary consumers of fish, *T. rubripes* (TP = 3.2) and large size class of *H. akajei* (TP = 3.1), contained THg between 328 and 3,700 ng/g d.w. Although they were located at the lower trophic positions to the two species of the tertiary consumers in the community of aquatic animals, their THg contents exceeded those of the tertiary consumers.

Table 3-1 A species list of 24 species of the fishes and mega-benthos collected in the inner part of Isahaya Bay and the information on their body sizes, stable isotope ratios of carbon and nitrogen, THg contents, and trophic positions.

Species name	Common name	Code	Total length (cm) mean ± S.D.	Body weight (g) mean ± S.D.	$\delta^{13}C$ (‰) mean ± S.D.	$\delta^{15}N$ (‰) mean ± S.D.	THg Content (ng/g d.w.) mean ± S.D.	THg Content (ng/g w.w.) mean ± S.D.	TP		No. of data
F: *Hemitrygon akajei* (Extra Large)	red stingray	$HA_{(XL)}$	72.0	5,150	-16.1	14.4	3,700	551	3.3	I.C.	1
F: *Hemitrygon akajei* (Large)	red stingray	$HA_{(L)}$	63.6 ± 4.9	1,847 ± 145	-14.7 ± 0.3	13.8 ± 1.1	671 ± 340	156 ± 82	3.1	S.C.	4
F: *Hemitrygon akajei* (Small)	red stingray	$HA_{(S)}$	30.6 ± 2.4	98.2 ± 29.1	-15.1 ± 0.5	15.8 ± 0.5	142 ± 76	28.4 ± 16.2	3.7	I.C.	6
F: *Lateolabrax japonicus*	Japanese seabass	LJ	30.1 ± 7.6	285 ± 201	-15.8 ± 1.0	17.3 ± 1.3	266 ± 99	55.0 ± 16.8	4.1	T.C.	5
F: *Paralichthys olivaceus*	bastard halibut	PO	45.0	1,000	-15.1	16.6	249	59.8	3.9	T.C.	1
F: *Sardinella zunasi*	Japanese sardinella	SZ	9.5 ± 0.4	7.4 ± 1.0	-15.7 ± 0.3	16.0 ± 0.3	232 ± 31.7	49.8 ± 7.3	3.7	I.C.	4
F: *Ilisha elongata*	elongate ilisha	IE	25.0 ± 4.4	112 ± 51	-15.6 ± 1.0	15.8 ± 0.7	175 ± 54	46.2 ± 14.9	3.7	I.C.	5
F: *Takifugu niphobles*	grass puffer	TN	11.2 ± 2.1	30.7 ± 15.2	-14.8 ± 0.4	15.5 ± 0.6	558 ± 226	103 ± 46	3.6	I.C.	4
F: *Sebastes inermis*	dark-banded rockfish	SI	15.5	73.3	-15.0	15.3	157	23.3	3.6	I.C.	1
F: *Takifugu xanthopterus*	yellowfin pufferfish	TX	17.7 ± 3.9	128 ± 85	-16.2 ± 1.3	15.3 ± 1.0	186 ± 74	41.0	3.6	I.C.	8
F: *Acanthopagrus schlegelii*	blackhead seabream	ASc	42.0	2,000	-18.0	15.1	942	256	3.5	I.C.	1
F: *Pennahia argentata*	silver croaker	PA	17.5 ± 3.5	65.2 ± 32.5	-15.4 ± 0.4	15.0 ± 0.7	225 ± 122	41.4 ± 21.9	3.5	I.C.	5
F: *Acanthopagrus sivicolus*	Okinawa seabream	ASi	19.5	142	-14.5	14.7	219	51.3	3.4	I.C.	1
F: *Engraulis japonicus*	Japanese anchovy	EJ	9.0 ± 0.6	4.5 ± 0.8	-16.1 ± 0.4	14.6 ± 0.8	70.9 ± 18.6	15.7 ± 4.2	3.4	I.C.	12
F: *Planiliza haematocheila*	so-iuy mullet	PHa	62.5	2,225	-16.2	14.5	328	51.6	3.3	I.C.	2
F: *Nuchequula nuchalis*	spotnape ponyfish	NN	10.1 ± 0.6	14.7 ± 2.5	-16.2 ± 1.6	14.5 ± 1.2	418 ± 68	103 ± 11	3.3	I.C.	4
F: *Konosirus punctatus*	dotted gizzard shad	KP	15.3 ± 0.4	31.2 ± 3.3	-15.9 ± 0.4	14.4 ± 0.7	109 ± 22	24.1 ± 5.0	3.3	I.C.	9
F: *Amblychaeturichthys hexanema*	pinkgray goby	AH	12.0	9.7	-17.2	14.4	41.1	8.2	3.3	I.C.	2
F: *Takifugu rubripes*	Japanese pufferfish	TR	21.1 ± 3.1	191 ± 55	-16.9 ± 1.0	14.1 ± 0.5	340 ± 208	64.4 ± 36.4	3.2	S.C.	4
F: *Cynoglossus abbreviatus*	three-lined tongue sole	CA	31.5 ± 3.5	187 ± 52	-16.5 ± 2.3	13.9 ±1.7	191 ± 50	41.8 ± 10.6	3.1	S.C.	3
F: *Pseudopleuronectes herzensteini*	yellow striped flounder	PHe	18.7 ± 7.0	103 ± 18	-15.9 ± 1.3	13.5 ± 1.3	134 ± 126	25.0 ± 26.7	3.0	S.C.	5
F: *Mugil cephalus*	flathead grey mullet	MC	53.7 ± 2.9	1,367 ± 388	-17.0 ± 0.7	13.4 ± 0.6	23.3 ± 14.1	6.1 ± 3.8	3.0	S.C.	3
F: *Callionymus beniteguri*	whitespotted dragonet	CB	10.8 ± 2.0	8.0 ± 3.1	-16.6 ± 0.1	12.8 ± 0.4	71.2 ± 29.5	13.0 ± 5.1	2.8	S.C.	3
C: *Sepia esculenta*	cutttlefish	SE	11.5	123	-14.8	15.6	168	27.0	3.6	I.C.	1
C: *Octopus* sp.	octopus	Oc	19.0	22.0	-15.3	13.6	167	26.1	3.0	S.C.	1
M: *Oratosquilla oratoria*	mantis shrimp	OO	9.6 ± 0.2	12.2 ± 0.8	-16.7 ± 0.3	14.3 ± 0.5	93.9 ± 10.6	21.4 ± 1.4	3.3	I.C.	3

F: fish, C: cephalopoda, M: malacostraca, THg: total mercury, TP: trophic position, Secondary consumers (S.C.): TP = 2.8 to 3.2, Intermediate consumers (I.C.): TP = 3.3 to 3.7, Tertiary consumers (T.C.): TP = 3.8 to 4.2

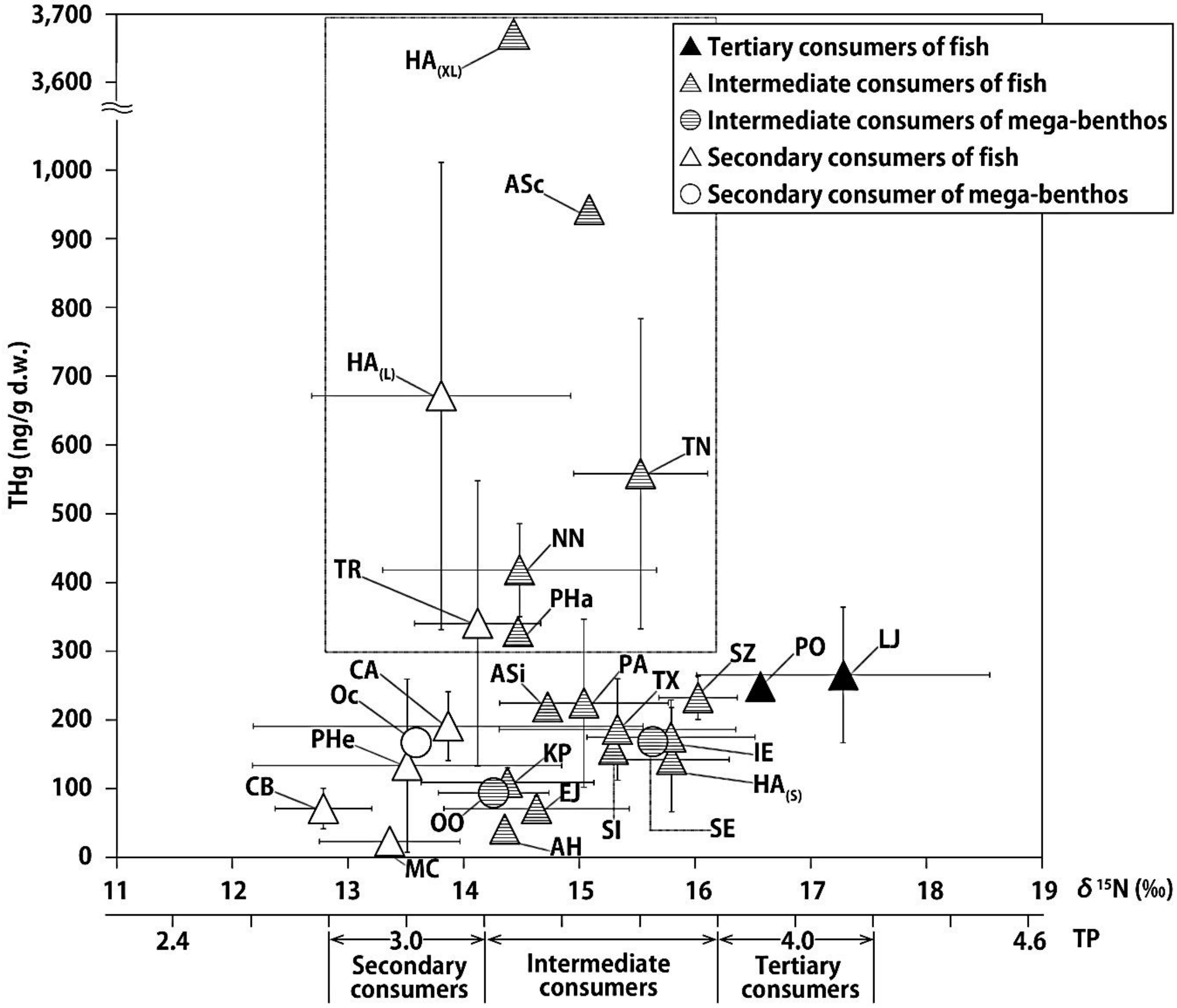


Fig. 3-3 The relationship between the δ^{15}N values and THg contents of the body tissues of the fishes and mega-benthos collected in the inner part of Isahaya Bay. Error bars represent standard deviation of the mean values. The data of the plots are noted in Table 3-1.

3.5 Discussion

According to the descriptions on the feeding habits of the two species of fishes as the tertiary consumers, *Lateolabrax japonicas* (Nakane et al. 2011) and *Paralichthys olivaceus* (Inoue et al. 2005), they prefer juveniles of fish and/or small species of fishes for main food items. The latter one is a demersal fish, but it swims up in the water to catch the pelagic animals such as Japanese anchovy, *Engraulis japonicas* during nighttime (Miyazaki et al. 2004). The small fishes as ones of their main food items seem to grow feeding on zooplankton in the water, which increase preying on phytoplankton. Therefore, their relatively low THg contents as the tertiary consumers (*L. japonicas*: 266 ± 99 ng/g d.w. (mean ± S.D.), *P. olivaceus*: 249 ng/g d.w.) seem to be responsible for their feeding habits that mainly rely on the foods produced in the food chain of the pelagic system.

Among the six species that exceeded the THg contents of the tertiary consumers, *Hemitrygon akajei* (red stingray) with extra-large body size (72.0 cm, 5,150 g, n = 1) as the intermediate consumer (TP = 3.3) and large ones (63.3 ± 4.9 cm, 1,847 ± 145 g, n = 4) as the secondary consumer (TP = 3.1) had conspicuously high THg contents, 3,700 ng/g d.w. and 671 ± 240 ng/g d.w., respectively, while the THg content of the small ones (30.6 ± 2.4 cm, 98.2 ± 29.1 ng/g d.w., n = 6) was 142 ± 76 ng/g d.w, and had a much higher TP value (3.7) than those of the extra-large and large individuals. These facts indicate that the trophic position of *H. akajei* went down significantly as it grew up to adults, increasing the THg contents markedly. This species favors venerid bivalves as one of their main diets (Nakane et al. 2011 and Tsutsumi et al. (in press)), but the results of this study indicate that main food items of this species tends to change from the nearly tertiary consumers to the secondary ones such as the venerid bivalves, as it grows. In the study on the feeding habits of this species collected in the inner part of Ariake Bay (Kanazawa 2003), the majority of the stomach contents were occupied by crabs, and the bivalves were not found at all in them. These results are, however, not contradictory to the results of this study, since most of the specimens were less than 50 cm (Kanazawa 2003), which are classified to small individuals of this study. In the study area, the inner part of Isahaya Bay, the macro-benthic animals were divided into two groups in THg content, “High THg content group” and “Low THg content group” in both members of the primary and secondary consumers (Jaingam et al. (in press)). The “High THg content group” of the primary consumers consisted of deposit feeding bivalves and polychaetes and bivalves burrowing in the sediment, which included a venerid bivalve, *Ruditapes philippinarum,* with the THg content of 80.8 ng/g d.w, and that of the secondary consumers was made up of the predators of the primary consumers of “High THg content group” such as crabs and starfish (Jaingam et al. (in press)). It is, therefore, very likely that *H. akajei* depended on *R. philippinarum* for diets more preferentially as it grew up, and the preferential predation resulted in the accelerated accumulation of THg in its body although its trophic position went down.

Acanthopagurus schlegelii had the second highest THg content (942 ng/g d.w., n =1), and was one of the intermediate consumers (TP = 3.5). It is a demersal fish with a restricted migration habit (Tsuyuki and Umino 2017), and feeds predominantly on mollusks, crustaceans, and polychaetes (Vahabnezhad et al. 2016, Froese and Pauly 2018). In particular, it prefers oysters (*Crassostrea gigas*) for diets exclusively (Tsuyuki and Umino 2017), which was also a member of “High THg content group” of the primary consumers (94.1 ± 22.3 ng/g d.w., n = 8) of the macro-benthic communities in the study area (Jaingam et al. (in press)).

The remaining four species with high THg contents between 328 and 558 ng/g d.w. were two species of carnivorous pufferfish (*Takifugu niphobles* and *Takifugu rubripes*), *Planiliza haematocheila,* and *Nuchequula nuchalis*. The pufferfishes favor macro-benthic animals such as bivalves and crabs for diets (Inoue et al. 2005, Froese and Pauly 2018, Takita and Intong 1991, Nakajima 2011), which were included in the members of “High THg content groups” of the primary and secondary consumers of the macro-benthic communities in the study area, respectively (Jaingam et al. (in press)). *P. haematocheila* belongs to Mugilidae. In general, the fish of this family feeds on the sediment with detritus, microphytobenthos and small benthic invertebrates (Cardona 2016). *N. nuchalis* has a mouth with a downward opening to suck up the food items from the bottom, and favors macro-benthic animals including bivalves, polychaetes, crustaceans, etc., and small fish for diets (Tiews and Divino 1972).

Thus, the six species of the fishes of the secondary and intermediate consumers of which the THg contents exceeded those of the pelagic fishes of the tertiary consumers prefer commonly macro-benthic animals for diets. They are referred to as bentho-pelagic fishes (Lavoie et al. 2010), and their preys include the members of “High THg content groups” of the primary and secondary consumers (Jaingam et al. (in press)). On the sea floor in the present study area, the sediment contained 133 ± 23 ng/g d.w. of THg due to deposition of POM with 27.2 ± 9.7 ng/g d.w. of THg contents (Jaingam et al. (in press)). These results emphasize the strong influence of the THg accumulation process on the sediment and its biomagnification by the macro-benthic animals to the THg contents of the bentho-pelagic fishes in the coastal shallow water.

As we predicted from the results of the study on the bioaccumulation of THg in the macro-benthic animals occurring in the bay (Jaingam et al. (in press)), one species of the bentho-pelagic fishes, *H. akajei* (extra-large size), which favors macro-benthic animals of “High THg content groups” for diets (in particular, venerid bivalve*, R. philippinarum*), had a THg content, 3,700 ng/g d.w., which exceeded the Japanese provisional standard value of THg for fish and shellfish for food, 400 ng/g w.w., about 2,000 ng/g d.w. (National

Institute for Minamata Disease, Ministry of Environment 2013). The extremely high THg contents was almost equivalent to those of the marine mammals and sea birds occurring in unpolluted areas by the anthropogenic factors and far from the active volcanos (Campbell et al. 2005, Lavoie et al. 2010, Atwell et al. 1998, Clayden et al. 2014). Therefore, the results of this study indicate that mercury discharge by the volcanic activities of Mt. Unzen and its bioconcentration by the phytoplankton and accumulation on the sediment of the sea floor in the bay and biomagnification process in the benthic system have a great potential for pushing up the THg contents of some bentho-pelagic fishes to the levels equivalent to those of the animals located at the top of the food web system of the animals in the pelagic system.

3.6 Conclusions

We collected 24 species of aquatic animals with nets from a fishing boat in the inner part of Isahaya Bay between 17 October 2015 and 27 November 2017. They were made up of 21 species of fishes, two species of cephalopoda, and one species of malacostraca, and determined their trophic positions in the food chain of the aquatic animal community using the results of stable isotope ratios of carbon and nitrogen. They included two species of the tertiary consumers of fish, seven species of the secondary consumers including six species of fishes and one species of cephalopoda (octopus), and 16 species of the intermediate consumers between the secondary and tertiary ones including 14 species of fishes, one species of cephalopoda (cutttlefish), and one species of malacostraca (mantis shrimp).

The results of the analysis of THg content of these animals revealed that a general trend of biomagnification of mercury in the food chain of the animal community was not found in the fishes and mega-benthos collected in the study area. The two species of the tertiary consumers, *Lateolabrax japonicas* (Japanese seabass) and *Paralichthys olivaceus* (bastard halibut) contained 266 ± 99 ng/g d.w. (mean ± S.D., n = 5) and 249 ng/g d.w. (n = 1) of THg, respectively, while five species of the intermediate consumers of fish, an extra-large size class of *Hemitrygon akajei*, *Takifugu niphobles*, *Planiliza haematocheila*, *Acanthopagrus schlegelii*, and *Nuchequula nuchalis* , and two species of the secondary consumers of fish, *Takifugu rubripes* and a large size class of *H. akajei*, contained 328 to 3,700 ng/g d.w. of THg. The secondary and intermediate consumers of the fishes, of which the THg contents exceeded those of the tertiary consumers, have common features in feeding habits in that they preferentially prey on macro-benthic animals including bivalves, polychaetes, crabs, and shrimps as main diets. They are referred to as “bentho-pelagic species”. Some of the diet species of the macro-benthic animals contain high levels of THg through the deposition of mercury discharged by the volcanic activities on the sediment of the sea floor and biomagnification process in the detritus food chain in the macro-benthic communities. Thus, the high contents of THg detected from the bentho-pelagic fishes seemed to be transferred from the mercury deposited on the sediment via biomagnification process in the detritus food chain of the macro-benthic communities and their preferential predation on the macro-benthic animals.

CHAPTER 4

Conclusions

4.1 Conclusions

The conclusions of this study are illustrated in Fig. 4-1. The Hg discharged to an enclosed shallow water, Isahaya Bay, seems to be mainly emitted from the volcanic activities of Mt. Unzen. It is partly absorbed by the phytoplankton, and its dead bodies and the feces and dead bodies of zooplankton that preyed on the phytoplankton tend to be contaminated by the Hg discharged to the bay. These substances were suspended as POM in the water sinking down in the water column, and deposited as detritus on the sediment of the sea floor. The Hg contents of POM collected from the overlying water on the sea floor were noted as 27.2 ± 9.7 ng/g d.w (mean ± S.D.) at the sampling stations located in the inner part of the bay, while those of sediment were about five times higher than the POM (133 ± 23 ng/g d.w.). These results indicate that the sea floor acts as an accumulator of Hg to the sediment, since one of the heavy metals, Hg, remains in the sediment even if the organic matter of POM was decomposed by the bacterial activities. Therefore, the results of this study emphasize the presence of two steps of the concentration process of Hg, which involve the bioconcentration by the phytoplankton in the water and the concentration followed by the bacterial decomposition of the detritus on the sea floor, in the enclosed bay with shallow water.

According to the previous knowledge on the feeding behaviors of the macro-benthic animals and the results of the stable isotope analysis of carbon and nitrogen of the body tissues of the specimens, 18 species of the macro-benthic animals collected from the sea floor and shore in the bay were made up of the members of two different trophic levels, primary and secondary consumers. The primary consumers were roughly divided into two different groups by the levels of THg contamination, High and Low THg content groups. The “High THg content group” contained 101 ± 23 ng/g d.w. of THg, and included the deposit-feeding bivalves (*Theora lata, Raetellops pulchella*) and polychetes (*Sternaspis costata*) that inhabit in the sediment and swallow the sediment with detritus and suspension-feeding bivalves burrowing into the sediment (*Ruditapes philippinarum, Arcuatula senhousia, Tegillarca granosa*) or creating the reefs on the sediment (*Crassostrea gigas*). They were always exposed to the mercury deposited on the sediment. The “Low THg content group” contained 25.2 ± 8.5 ng/g d.w. of THg, and consisted of the amphipods (*Ampelisca bocki, Photis longicaudata, Cerapus* sp.), which are suspension feeders and/or surface detritivores, and inhabit on the surface of the sediment, and a filter-feeding bivalve (mussel; *Mytilus galloprovincialis*) attached on the hard substrates. These species were much less exposed to the mercury deposited on the sediment

The secondary consumers of the macro-benthic animals were also roughly divided into the two different groups of THg content, “High THg content group” and “Low THg content group”. The “High THg content group” contained 215 ± 47 ng/g d.w. of THg, and was made up of carnivorous carbs (*Hemigrapsus* sp., *Charybdis japonica*, *Portunus trituberculatus*) and sea star (*Asterias* sp.) that favor the infaunal bivalves and polychaetes involved in the “High THg content group” of the primary producers. The “Low THg content group” contained 63.1 ± 39.3 ng/g d.w. of THg, and consisted of shrimps (*Metapenaeus* cf. *affinis*, *Metapenaeus* cf. *joyneri*) and a carnivorous polychaete (*Glycera* sp.). The shrimps are epifauna, and mainly scavenge the dead animals remained on the sediment surface, and the polcyaete favor the small organisms occurring in the sediment. They all seem to be unlikely to prey on the bivalves of "High THg content group" of the primary consumers. The THg content of "High THg content group" was about 3.4 times higher than that of “Low THg content group”, but a small difference was found in the biomagification factors (BMF) of THg between the primary and secondary consumers in each of the “High THg content group (2.13)” and “Low THg content groups (2.50)”, respectively. Therefore, the difference of THg content between the “High and Low content groups” reflected the THg contents of their main foods.

The THg contents of fishes and maga-benthos collected in the bay were significantly influenced by the THg contents of their main foods rather than their trophic positions. In this study, two species of tertiary consumers of the fishes (*Leteolabrax japonicus* and *Paralichthys olivaceus*) were collected, and their THg contents were 266 ± 99 ng/g d.w. and 249 ng/g d.w., respectively. The THg contents of six species of fishes, which were secondary consumers (*Hemitrygon akajei*; large size class (63.6 ± 4.9 cm, 1,847 ± 145 g) and *Takifugu rubripes*) or intermediate consumers between the secondary and tertiary consumers (*Hemitrygon* akajei; extra-large size class (72.0 cm, 5,150 g), *Acanthopagrus sivicolus*, *Takifugu niphobles*, *Nuchequula nuchalis*, *Planiliza haematocheila*), however, exceeded those of the tertiary consumers of the fishes (328 and 3,700 ng/g d.w.). The tertiary consumers of the fishes mainly feed on the secondary consumers of small fishes and other animals occurring in the water, which are produced in the pelagic system, while the six species of the fishes favor to feed on the macro-benthic animals as main diets, including bivalves, polychaetes, crabs, shrimps, etc. that were included in the “High THg content groups” of the primary and secondary consumers. They are referred to as “bentho-pelagic fishes” that mainly depend on the foods, in particular bivalves, produced in the benthic system.

These relationships between the THg contents of the fishes and their main foods are characterized by the changes of THg contents following the growth of red stingray (*Hemitrygon akajei*). The small individuals (30.6 ± 2.4 cm, 98.2 ± 29.1 g) contained 142 ± 76 ng/g d.w of THg, while the THg contents markedly increased as its grew. The large ones (63.6 ± 4.9 cm, 1,847 ± 145 g) and extra-large ones (72.0 cm, 5,150 g) contained 671 ± 340 ng/g d.w. and 3,700 ng/g d.w., respectively. It feeds on mainly small crustaceans as the secondary consumers, while the large individuals favor bivalves as the primary consumers, in particular, *Ruditape philippinarum*, which was included as the members of the “High THg content group” of the primary consumers in this study. Therefore, the trophic position of this species tends to descend toward the secondary consumer as its growth, but the THg content markedly increased due to the THg contents of the main foods. The highest value of THg content of the extra-large individual was equivalent to those of marine mammals and sea birds which are recognized as the fourth consumers in the food chain.

Thus, in the coastal shallow waters such as an enclosed bay, Isahaya Bay, the mercury deposited and concentrated on the sediment of the sea floor has a great effect on the acceleration of its biomagnification of the macro-benthic animals and bentho-pelagic fishes that feed the macro-benthic animals.

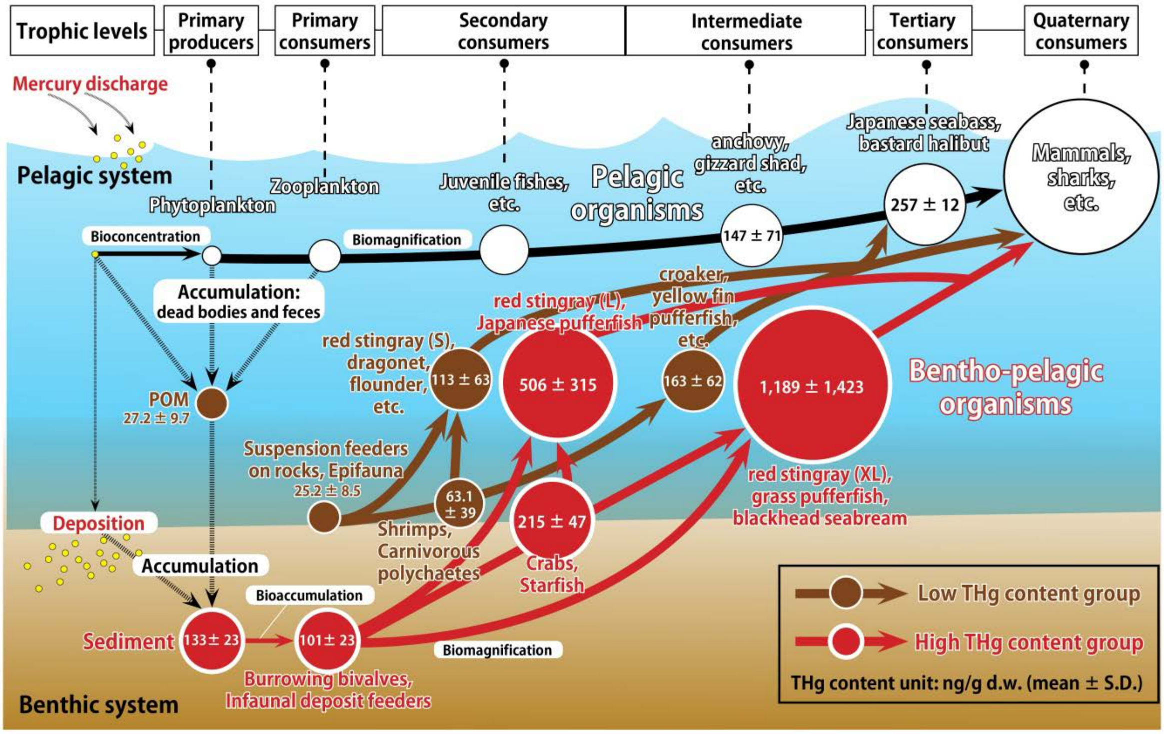


Fig. 4-1 Whole scheme of the bioaccumulation and biomagnification process of THg that proceeded in the benthic ecosystem of the inner part of Isahaya Bay.

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my supervisor, Dr. Hiroaki Tsutsumi for all support to conduct this study, more especially for all the constructive and exclusive discussions. I got the enormously valuable guidance for my life and opening my mind to the scientific thinking way. I also really appreciate to Dr. Tomohiro Komorita for great support all of the valuable suggestions during my study and the good opportunities to learn about the Japanese culture.

I would like to thankfully Dr. Jun Kobayashi and Dr. Megumi Yamamoto for their generous suggestion and giving me much knowledge on chemical analyses. Also, I deeply appreciate to Dr. Tetsuro Agusa, Dr. Koji Arizono, Dr. Daizhou Zhang, and Dr. Yasuhiro Ishibashi for their criticism to the results and valuable suggestion on this study, and Dr. Jeffrey Stewart Morrow for his critical reading, correction, and great recommendation of the English text.

I am most grateful to Dr. Chittima Aryuthaka, Dr. Suriyan Tunkijjanukij, Dr. Thon Thamrongnawasawat, Dr. Napakhwan Whanpetch, Mr. Teerapong Duangdee, Ms. Punthip Wisespongpun, Mr. Shobu Ishimatsu, Dr. Umehara Akira, Dr. Risa Takenaka, Mr. Tasuku Nishioka, Dr. Kazumasa Yamada, Dr. Rei Somiya, and Ms. Yukiko Uchiyama for their great help in this study, as well as thank you for all my friends in Thailand and Japan for their encouragement. Finally, I would like to thank my family for all support.

This research was financially supported by Kumamoto Prefectural Government Office Prefectural University of Kumamoto, Japan with the program of the Prefectural University of Kumamoto International Postgraduate Scholarship for Research on Mercury.

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