Interdecadal Distribution of Persistent Organic Pollutants in Deep-Sea Chemosynthetic Bivalves

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Marine ecosystems are continuously subjected to anthropogenic environmental pollution. Understanding the spread of pollution and the potential risks it poses to deep-sea ecosystems is important for developing better conservation measures. Here, we identified non-negligible levels of persistent organic pollutants in deep-sea chemoautotrophic bivalves with limited or no filter feeding. The bivalves were collected from two sites: one located near a highly populated region and the other located relatively far from human activity. Analyses of samples collected nearly every decade in a period of 30 years suggested that environmental policy restrictions might be effective in reducing chemical pollution. However, the detection of contamination in deep-sea chemoautotrophic animals suggests that the pollution could be spreading globally to chemoautotrophic organisms with limited or no feeding. To protect these highly endemic and vulnerable deep-sea chemoautotrophic ecosystems, our findings indicate that further research on chemical contamination and its effects on these ecosystems is required.

Keywords: persistent organic pollutant, polychlorinated biphenyl, polybrominated diphenyl ether, bioaccumulation, deep-sea chemoautotrophic bivalve, Phreagena, Bathymodiolus

INTRODUCTION

Some organic compounds are resistant to environmental degradation and can accumulate in organisms, causing harmful effects. These chemicals are called persistent organic pollutants (POPs) and they have been of great academic and social interest since the late 20th century. Polychlorinated biphenyls (PCBs) are a group of substances obtained by the chlorination of biphenyls; they have 10 homologues and 209 congeners, theoretically, depending on the number and position of the chlorine atoms (Yuan et al., 2015). Additionally, coplanar PCBs are known to be extremely toxic (Safe, 1994). Owing to their advantageous electrical insulating, chemical resistant, and non-flammable properties, PCBs were manufactured globally during the mid-20th century for numerous applications including insulating oils for electrical transformers and capacitors, as well as additives in paints and plastics (Erickson and Kaley, 2011). However, large-scale PCB contamination of cooking oil eventually drew attention to their toxicity and potential for environmental pollution. The manufacturing of PCBs was banned in closed systems in the United States in 1979 and in the United Kingdom in 1981, but was not phased out until 1987 in European countries that border the Mediterranean Sea (Jepson et al., 2016). The international Stockholm Convention (2001), which aimed to eliminate all designated POPs, prohibited the manufacture of PCBs and severely restricted the use of remaining PCB stocks,
and a number of East Asian countries have signed the agreement. In Japan, The Chemical Substance Control Law (CSCL) was enacted in 1974 to prohibit the production, use, and import of PCBs (Koshiba et al., 2019). However, the disposal of PCB-containing products has not progressed sufficiently. The PCB Special Measures Law was enacted in 2001 for the secure and appropriate disposal of PCB waste by 2016 (later extended to 2027) based on the international Stockholm Convention. Additionally, polybrominated diphenyl ethers (PBDEs), which are brominated aromatic hydrocarbons heavily used as flame-retardants in plastics, possess structural variations similar to PCBs (Talsness, 2008). Due to their persistence in the human body, tetra-, penta-, hexa-, and hepta-BDEs were listed in the Stockholm Convention in 2009. In Japan, the use of PBDEs (other than deca-BDE) was stopped since the 1990s, owing to self-restrictions by the manufacturers. Additionally, deca-BDE was listed in the Stockholm Convention in 2017 and was banned from import and use in Japan in 2018. However, regardless of these efforts, environmental pollution caused by these substances continues to be detected in a wide range of biota collected worldwide (Domingo and Bocio, 2007; Lee and Kim, 2015).

Due to their hydrophobic and lipophilic natures, PCBs and PBDEs are only slightly soluble in water and readily associate with organic particles in aquatic environments, including algae, suspended sediment, and micro- or nano-plastics (Ghosh et al., 2003; Olencycz et al., 2015; Yamashita et al., 2019). Aquatic animals can be exposed to PCBs and PBDEs through two routes: uptake across the body surface (e.g., gills and/or epidermis), and dietary uptake through the digestive tract (Bjork, 1995). These pollutants can enter the adipose tissue and liver and bioaccumulate through the food chain (Matthews and Dedrick, 1984). Bivalve mollusks are generally filter feeders and are considered to be good indicators of environmental pollution because they accumulate various POPs from the surrounding water (Chiesa et al., 2018). There have been many reports of PCB and PBDE contamination in coastal bivalves worldwide, including in Japan (Ramu et al., 2007; Supplementary Table 1). These pollutants are transported for long distances by ocean currents, and therefore lead to transboundary problems that require the special attention and coordination of international efforts (Dewailly et al., 2007).

Persistent organic pollutants are considered to collect in the deep ocean via vertical transport through the various biogeochemical processes (Takahashi et al., 2014). Examining the behaviors of POPs provides insight into the extent of anthropogenic impacts on deep-sea ecosystems, in which organisms have long lifespans, slow growth, and late maturity, and are therefore particularly vulnerable to environmental disturbances. Recovery from such disturbances can be a long process, and in some cases can lead to the extinction of some organisms (Llodra and Billett, 2006). However, little is known about the distribution of POPs in deep-sea animals, except for fish (Takahashi et al., 2009). A recent study quantified PCBs and PBDEs in scavenging amphipods collected from the Kermadec and Marianas trenches at depths of more than 10,000 m (Jamiesson et al., 2017; Supplementary Table 1). Information on other benthic animals is limited; however, the PCB contents in epibenthic deep-sea invertebrates (sea anemones, sea cucumbers, sea pens, and sea lilies) from the northern Gulf of Mexico have been reported (Lawson et al., 2021; Supplementary Table 1).

Deep-sea endobenthic vesicomyid clams, including those of the genus Phreagena (formerly Calyptogena), and deep-sea epibenthic mussels belonging to the genus Bathymodiolus are endemic and dominant members of deep-sea chemosynthetic communities (Fisher, 1990). They harbor symbiotic chemosynthetic bacteria (gram-negative Gammaproteobacteria) in the epithelial cells of their gills (Kuwahara et al., 2007). The vesicomyid clams depend on these symbionts for nutrition, as their digestive tracts are non-functional (Childress and Fisher, 1992; Le Pennec et al., 1995). Bacterial symbionts are also the primary nutritional source for Bathymodiolus mussels, although they possess a functional gut (Page et al., 1991; Ponnudurai et al., 2017). Assuming that the contamination of bivalve mollusks by highly hydrophobic pollutants such as PCBs is predominantly due to dietary uptake through filter feeding, the pollution of chemosynthetic bivalves (that perform limited to no filter feeding) is expected to be negligible. However, as the non-dietary uptake of hydrophobic organic contaminants has been documented in bivalve filter feeders (Bjork, 1995), uptake across the body surface likely occurs, even in animals with limited filter feeding. In this study, we hypothesized that PCB contamination might be detected in chemosynthetic animals, and that the level of contamination would reflect the proximity of human activity. To test this and to understand the spread of POP pollution in marine ecosystems, we measured the concentrations of PCBs and PBDEs in deep-sea chemosynthetic Phreagena clams and Bathymodiolus mussels from Sagami Bay, which is located near a highly populated region that includes Tokyo (Statistics Bureau of Japan [SBJ], 2020; Nakajima et al., 2021; Figures 1A,B). For the Phreagena clams, we used 30 years of archived samples (collected from Sagami Bay in 1989, 1998, 2010, and newly in 2019) to investigate the changes in contamination over time. For comparison, we also analyzed Bathymodiolus mussels that inhabit Myojin Knoll, which is a relatively remote area located far from dense human activity (Nakajima et al., 2021; Figures 1A,C).

METHODS

Faunal Sampling

Phreagena clams were collected from the seep site off Hatsushima Island in Sagami Bay, Japan, at a depth of 857 m (Dive #6K1557) during cruise YK19-11 (August 28–September 14, 2019) using the HOV Shinkai 6500, operated by the R/V Yokosuka (Japan Agency of Marine-Earth Science and Technology; JAMSTEC). We also used the archived clams collected at a depth of 1,171 m (Dive #1074) during cruise NT10-01 (January 12–18, 2010) using the ROV Hyper-Dolphin operated by the R/V Natsushima (JAMSTEC), as well as at depths of 1205 m (Dive #2K0998) and 1,195 m (Dive #2K0450) during cruises NT98-06 (April 2–26, 1998) and NB9-08 (October 7–29, 1989), respectively, using the HOV Shinkai 2000 operated by the R/V Natsushima (Table 1). These clams were preserved as whole at JAMSTEC at
−80°C. At the seep site, two morphologically similar clam species (Phreagena okutanii and Phreagena soyoae) are known to form a mixed colony (Ikuta et al., 2018); however, we did not distinguish between these two species in this study. Bathymodiolus japonicus mussels were also collected from the seep site off Hatsushima Island at a depth of 901 m (Dive #6K1557), while Bathymodiolus septemdierum mussels were collected from hydrothermal vent sites at Myojin Knoll, Japan, at a depth of 1235 m (Dive #6K1556). All of the Bathymodiolus specimens were collected during cruise YK19-11 (Table 1). The bivalves were frozen and stored at −80°C. For each bivalve species and collection year, three individuals with a shell length of ~100 mm were used for subsequent analyses (Table 1).

The samples were processed on a clean bench. For dissection, the bivalve specimens were thawed in a dry-heat sterilized stainless steel tray at room temperature for several hours. Sterilized stainless steel scalpels were used to remove the shells. The entire soft bodies of the specimens were placed directly in sterile glass jars, covered with combusted aluminum foil, and stored at −30°C until analysis.

Chemical Analyses

Polychlorinated biphenyl and PBDE analyses were conducted in the laboratory of IDEA Consultants (Tokyo, Japan). The analytical method followed the procedures described by United States Environmental Protection Agency (US EPA) (United States Environmental Protection Agency [USEPA], 2003, 2010). Briefly, after internal standard solution was spiked into the homogenized bivalve samples (approximately 4 g wet), an acetone/hexane (1:2) mixture was added and two 2-min stirred extractions were conducted using a homogenizer. The extract obtained by centrifugation was treated with sulfuric acid and purified using multi-layer silica gel column chromatography after acetonitrile/hexane partitioning. After purification using gel permeation chromatography, the extract was divided for the PCB and PBDE analyses. For the PCB analysis, the sample volume was fixed at 30 µL, and syringe spikes were added. For the PBDE measurements, the sample was further purified using activated carbon dispersed silica gel column chromatography, the sample volume was fixed at 30 µL, and syringe spikes were added. The concentrations of PCBs and PBDEs in the samples were determined using gas chromatography–mass spectrometry with an Agilent 6890 GC (Agilent Technologies, Santa Clara, CA, United States) equipped with an AutoSpec Ultima mass selective detector (Waters Corporation, Milford, MA, United States). For the PCBs, 10 homologues (mono-CBs–deca-CB) and 14 coplanar PCBs were assessed. For the PBDEs, seven homologues (tetra-BDEs–deca-BDE) and six congeners listed in Annex A by the Stockholm Convention, were assessed. Procedural blanks were run at a rate of one in every ten samples and did not affect the sample concentrations. The method detection limits (MDLs) for each congener or...
TABLE 1 | Concentrations of PCBs and PBDEs in the chemosynthetic bivalves.

| Species  | Depth (m) | Shell Length (mm) | Lipid (%) | PCBs (ng/g lw) | Co-PCBs (ng/g lw) | PBDEs (ng/g lw) |
|----------|-----------|-------------------|-----------|---------------|------------------|-----------------|
| Phreagena spp. | 1195 | 97.2 | 1.4 | 0.02 | 4.6 | 0.3 |
| B. japonicus | 1205 | 88.8 | 1.2 | 0.38 | 5.0 | 0.2 |
| B. septemdierum | 1171 | 85.1 | 1.7 | 0.45 | 4.4 | 0.3 |
| 857 | 111 | 0.7 | 1.9 | 0.86 | 3.7 | 0.3 |
| 901 | 108.8 | 1.2 | 1.9 | 3.7 | 2.7 | 0.3 |
| 1235 | 107.1 | 1.5 | 1.9 | 4.4 | 2.7 | 0.3 |

* Detection limit.
** Not detected.
† Total amount of coplanar PCBs.

The homologue concentration were calculated using the following equation:

$$\text{MDL} = t(n - 1, 0.05) \times s$$

where, \(t(n-1, 0.05)\) represents the Student's \(t\)-value at a \(\alpha\) level of 0.05 with \(n-1\) degrees of freedom, and \(s\) represents the standard deviation of the blank measurements in seven replicates. The total lipid contents of the samples were determined using a single-step extraction method, based on the conventional chloroform-methanol extraction method (Daugherty and Lento, 1983). The results are expressed either as ng/g lipid weight (lw) or ng/g dry weight (dw) calculated based on the weight of water loss from the freeze-dried samples. The Tukey–Kramer test (\(P = 0.05\)) was performed.
for statistical analyses using Excel 2011 (Microsoft, Redmond, WA, United States) and the add-on software Statcel4 (OMS publishers, Tokyo, Japan).

RESULTS

Table 1 presents the concentrations of PCBs (ng/g lw and ng/g dw) and PBDEs (ng/g lw) in Phreagena clams and Bathymodiolus mussels. PCBs were detected in all of the samples, and the total concentrations of PCBs (ΣPCBs) were 18–110 ng/g lw and 1.2–13 ng/g dw (mean = 44 ng/g lw and mean = 4.3 ng/g dw, SD = 3.9, respectively). Among the Phreagena clams, in terms of weight per lipid, the mean ΣPCB values of individuals collected in 1989 and 1998 were significantly higher than those of individuals collected in 2010 and 2019 (Figure 2A). However, there were no significant differences in the mean values per dw. Among the three bivalve species collected in 2019, the mean ΣPCB value of B. japonicus was higher than those of the Phreagena clams and B. septemdierum, for both the lw and dw (Figure 2A). There were no statistical differences between the mean ΣPCBs of the Phreagena clams and B. septemdierum collected in 2019.

In terms of the percentage of PCB homologues in the ΣPCBs of each individual, hexa- (14–40%, lw), penta- (25–35%, lw), and tetra-CBs (15–39%, lw) dominated the overall samples, followed by tri- (4–15%, lw) and hepta- CBs (4–12%, lw) (Figure 2B). The amounts of other homologues were notably small, and mono-CBs were not detected. Additionally, the percentages of the 14 coplanar PCBs in the ΣPCBs ranged from 8.6 to 17% (lw) for all samples (Supplementary Table 2).

No PBDEs were detected in the Phreagena clams collected in 2010 and 2019, although small amounts were detected in three clams collected in 1989 (average of total PBDEs = 0.2 ng/g lw) and in two clams collected in 1998 (average of total PBDEs = 0.9 ng/g lw) (Table 1). For the Bathymodiolus mussels, all of which were collected in 2019, small amounts of PBDEs were detected in two B. japonicus (0.2 ng/g lw) individuals and one B. septemdierum (0.3 ng/g lw) individual. Among the samples, most of the PBDEs detected were tetra-BDEs; deca-BDE was detected in one Phreagena clam individual collected in 1998 (Supplementary Table 2).

DISCUSSION

This study is the first detailed report of PCBs and PBDEs detected in deep-sea chemosynthetic animals, with ΣPCBs of 18–110 ng/g lw and 1.2–13 ng/g dw (mean = 44 ng/g lw and 4.3 ng/g dw). This range is well below the very high concentrations of PCBs detected in shallow water mussels collected from sites near highly populated regions of Japan (3,000 ng/g lw in Tokyo Bay and 2,000 ng/g lw in Osaka Bay). However, it is comparable to levels detected at some sites in other countries (Monirith et al., 2003; Ramu et al., 2007; Kimbrough et al., 2008, 2009; Olenczyz et al., 2015; Supplementary Table 1). Furthermore, this range is comparable to or slightly less than that in the other deep-sea organisms at lower trophic levels collected from Sagami Bay, including pelagic fishes, crustaceans, and zooplankton (Toyoshima et al., 2009). PCBs affect a number of biological and physiological processes in a variety of animals, and the deleterious effects of PCBs on reproduction is documented in aquatic organisms at various trophic levels, including bivalves (Chu et al., 2003; Lehmann et al., 2007). The biological and physiological effects of pollution on chemosynthetic bivalves and the amount of contamination that leads to these effects remain poorly understood; however, this study raises the potential possibility of additional anthropogenic disturbances to these highly endemic and vulnerable fauna; therefore, further attention and study are required. On a global scale, in addition to the seas around Japan, hydrothermal vents and seeps with chemosynthetic communities are distributed near relatively densely populated areas such as Monterey Bay, Tyrrhenian Sea, and the Gulf of Mexico (German et al., 2011). This study suggests that the pollution could be spreading to the non-feeding chemosynthetic organisms that inhabit these areas.

In this study, very small amounts of PBDEs were detected in the deep-sea chemosynthetic clams and mussels. In contrast, a previous study detected non-negligible amounts of PBDEs in deep-sea animals (fishes, crustaceans, and zooplankton) collected from Sagami Bay (Toyoshima et al., 2009), although in amounts less than PCBs. To understand the implications of our results, it is necessary to understand the contamination of the associated habitats, including sediments and seawater, by these pollutants.

The average total PCBs per g lw of B. japonicus was significantly higher than that in the Phreagena clams collected during the same cruise (2019) and from the same site. This could be because Bathymodiolus mussel reportedly filter feed to a slight extent (Page et al., 1991; Ponnudurai et al., 2017), and dietary uptake of the pollutant may increase the PCB contamination level. Alternatively, some biological or physiological differences between the two mollusks (e.g., differences in pumping and filtration rates, and an endobenthic or epibenthic habitat) could have affected the results. In any case, non-negligible amounts of PCBs were detected in the deep-sea chemosynthetic bivalves, which indicates that the contaminants were taken up completely or predominantly across the body surface without feeding. Lower-chlorinated PCBs (tetra-, penta-, and hexa-CBs) were dominant in all samples in this study, and similar results were obtained in previous studies of shallow-water mussels (Porte and Albaiges, 1994; Yamaguchi et al., 2000). Low-chlorinated compounds are relatively soluble in water (Bjork, 1995); therefore, it is possible that they were taken up by the Phreagena clams and Bathymodiolus mussels as dissolved materials. In contrast, there are two possible routes for the uptake of particle-bound substances from the body surface: cell membrane permeation due to the lipophilic nature of the contaminants and the direct uptake of particles into the cell. In the former route, contaminants must have sufficient time to desorb from the particles before diffusion across the body surface, such as through the gills (Klump et al., 1987; Gobas et al., 1993). The mucus present on the surface of the bivalve may contribute to this process. The direct uptake of organic particles, such as micro- or nano-plastics, across the body surface of bivalves, has been reported previously (Al-Sid-Cheikh et al., 2018; Ikuta et al., 2021).
FIGURE 2 | PCB concentrations measured in the samples. (A) Mean total PCB concentrations per g lw and dw. The mean values from three individuals are shown for each bivalve species and each collection year. Pink, light green, and light blue bars indicate mean total PCB concentrations per g lw for Phreagena clams, Bathymodiolus japonicus, and Bathymodiolus septemdierum, respectively. White bars are mean values per g dw. Red, green, and blue dots indicate the values for Phreagena clams, B. japonicus, and B. septemdierum, respectively. Red and black asterisks indicate statistically significant differences ($P < 0.05$) in the Phreagena clam samples and in the bivalves collected in 2019, respectively. Error bars represent the standard deviation ($n = 3$). (B) Congener compositions of PCBs in all samples. Concentrations are based on the weight per lipid. Mo, mono-; Di, di-; Tr, Tri-; Te, tetra-; Pe, penta-; Hx, hexa-; Hp, hepta-; Oc, octa-; No, nona-; De, deca-; PCB, polychlorinated biphenyl; lw, lipid weight; dw, dry weight.

Kolandhasamy et al., 2018). PCBs and PBDEs are associated with micro- or nano-plastics in the ocean (Galloway and Lewis, 2016; Yu et al., 2019); therefore, the possibility of the latter route should be investigated further. Complex interactions between biological and non-biological factors govern the bioavailability and uptake of hydrophobic organic contaminants in filter feeding bivalves (Bjork, 1995). Therefore, the details of the contaminant uptake pathways in deep-sea chemosynthetic bivalves should be investigated in the future.

Comparing the total amounts of PCBs per g lw in the Phreagena clams approximately every 10 years since 1989, the level of contamination in 1989 and 1998 was significantly higher than that in 2010 and 2019. It was estimated that Phreagena clams with a shell length of $\sim$100 mm, as collected in this study, were alive for at least 10 years (Tada, 2009). Therefore, the samples collected may reflect pollution accumulation over a period of 10 years. However, the degradation of contaminants within the organism and differences in the degradation rates between the bivalves collected from deep-sea and those from the shallow waters needs further investigation and should be taken into account in future studies. PCBs were actively manufactured since the 1960s, and their production and use in Japan were prohibited in 1974. However, PCBs continued to leak into the environment after 1974, and strict disposal measures became mandatory in 2001. Applying this to the lifespan of the Phreagena clams, the relatively low amounts of PCBs observed in individuals after 2010 might reflect the effects of changes in environmental policies. However, the concentrations of pollutants in bivalves...
are dependent on both the pollutant concentration in the environments and the physiology of the organisms, such as body size and species (Björk, 1995). In the Phreagena clams (including the two species, P. okutanii and P. soyoae) used in study, older samples were collected from slightly deeper levels (Table 1). We cannot completely exclude the possibility that differences in the environmental concentrations or species variations, arising from the differences in the sampling depths, could have influenced the results.

Myojin Knoll is located relatively far from dense human activity (Statistics Bureau of Japan [SBJ], 2020). The B. septemderium individuals collected from this site, which has a very low amount of marine macro-debris (Nakajima et al., 2021), contained PCBs in amounts comparable to that in the Phreagena clams collected from Sagami Bay. This suggests that anthropogenic environmental pollution could be spreading over a wider area than expected. Myojin Knoll is located under the main current of Kuroshio; therefore, the pollutant could have been transported from the contaminated areas lying to the southwest of Myojin Knoll (Nakajima et al., 2021). Mussels enhance PCB contamination and bioavailability in their habitat by depositing PCB-enriched pseudofeces (Prince et al., 2021); hence, there are also concerns regarding the bioaccumulation of PCBs in co-existing crustaceans and other animals. Thus, to protect fragile deep-sea chemosynthetic ecosystems, it is necessary to continue monitoring these pollutants.

CONCLUSION

This study has suggested that environmental policy restrictions might be effective in reducing chemical pollution. However, the detection of POP contamination in deep-sea chemosynthetic animals suggests that the pollution may be globally spreading to vulnerable chemosynthetic organisms with limited or no feeding. Although the number of samples used in this study is small, this is the first report of POPs in a deep-sea chemosynthetic organism, and we provide an important baseline for future research. This study highlights the need for further detailed investigations and urgent actions to address POP contamination in deep-sea habitats, including that in non-feeding chemosynthetic animals.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author(s).

ETHICS STATEMENT

All animal experiments were conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan).

AUTHOR CONTRIBUTIONS

TI: conceptualization, methodology, formal analysis, investigation, writing – original draft, and visualization. RN: conceptualization, methodology, and writing – review and editing. MT: project administration and writing – review and editing. SC and KF: supervision and writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.751848/full#supplementary-material

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