Bull Environ Contam Toxicol (2009) 83:393–397
DOI 10.1007/s00128-009-9785-6

Concentrations of Persistent Organic Pollutants in Masu Salmon, *Oncorhynchus masou*

Mayuko Oka · Takaomi Arai · Yasuyuki Shibata · Nobuyuki Miyazaki

Received: 24 January 2009 / Accepted: 7 May 2009 / Published online: 22 May 2009

Abstract The levels of persistent organic pollutants (POPs) were analyzed in muscle, liver and gills of masu salmon caught in northern Japan. Liver is known to be a major pool site for POPs but there is not much information regarding accumulation of POPs in gills. The salmon were caught at the end of the feeding migration, and the average gonadosomatic index of masu salmon was 0.51 ± 0.16. The total amount of POPs (μg) accumulated in each organ was calculated in order to compare the accumulation potential of each organ. Even though the liver comprises only 1.8%–2.32% of the whole body mass it accumulated 16% ± 8.6% of the total POPs. Similarly, gills comprise only 1.4%–2.1% of the body mass but accumulated 9.1% ± 3.8% of the total POPs. Conversely, muscle comprises 72%–78% of the body mass and it accumulated 75% ± 12% of the total POPs. These results suggest that the gills, in addition to the liver, are an important site for the pooling of high levels of POPs at the end of the feeding migration.

Keywords POPs · Masu salmon

Persistent organic pollutants (POPs) such as DDTs and CHLs are chemicals that are ubiquitous in the environment and were mainly used as pesticides. They were widely used in Japan from the 1950s to the 1980s. Since these compounds are lipophilic and persistent, they are known to bioaccumulate in marine organisms (Jones and de Voogt 1999) specifically in fish (Hellou et al. 1993). Historically, liver has been most commonly used to study the accumulation of POPS in fish, due to its high lipid content. In addition, muscle is also frequently used for POPs analysis since fish are mostly comprised of muscle, and it is very easy to collect. Lastly, there are several studies analyzing the concentrations of POPs in gonad (eggs) to discuss maternal transfer of POPs to the fetus (Miller 1993; Svendsen et al. 2007). However, other organs are rarely used for POPs analysis.

If one addresses the possible routes of POPs intake in fish, (1) biomagnification – from prey, (2) bioconcentration – from water, and (3) maternal-fetal transfer – from mother, it is conceivable that gills play an important role of POPs intake in the case of bioconcentration. Nonetheless, POPs accumulation in gills has not been determined. In this study, we looked at this possibility by analyzing the concentrations of POPs in gills, together with muscle and liver of masu salmon. Masu salmon are anadromous fish and grow to about 60 cm in length. There are almost no studies regarding POPs distribution in various organs in masu salmon. Therefore, concentrations of POPs in muscle, liver, and gills of masu salmon were determined to understand the behavior of the POPs and to further elucidate the role of gills as a pool site of POPs.

Materials and Methods

Five samples of anadromous *Oncorhynchus masou* immediately prior to upstream migration were collected by a set net from Otsuchi Bay on April 18, 2003. Otsuchi Bay is

M. Oka (✉) · T. Arai · N. Miyazaki
Ocean Research Institute, The University of Tokyo,
1-15-1 Minamidai, Nakano, Tokyo 164-8639, Japan
e-mail: delphi19_@hotmail.com

Y. Shibata
National Institute of Environmental Studies,
16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

Table 1 Biological data of the masu salmons used for POPs analysis

Sample no.	Sex	Body length (mm)	Body weight (g)	Liver (g)	Gill (g)	Muscle (g)	GSI
MS1	Female	558.3	2,393	45.95	41.53	1,732	0.551
MS2	Female	526.2	1,916	35.14	27.38	1,489	0.613
MS3	Female	574.4	2,664	61.21	54.82	2,017	0.637
MS4	Female	549.8	2,848	56.09	40.75	2,200	0.249
MS5	Female	486.3	1,488	34.88	29.90	1,080	0.512

located in Iwate Prefecture in northeastern Japan. The length and weight of the masu salmon analyzed in this study was 539 ± 34 mm and 2,262 ± 557 g, respectively (Table 1). Gonadosomatic index (GSI) was used as an indicator of maturation and was calculated as shown:

$$\mathrm{GSI} = \mathrm{Gonad\,weight\,(g)/Body\,Weight\,(g)} \times 1000.$$

We determined the concentrations of hexachlorobenzene (HCB), Σhexachlorocyclohexanes (α-HCH + β-HCH + γ-HCH + δ-HCH), Σchlordanes (heptachlor + heptachlor epoxide + oxychlordane + *trans*-chlordane + *trans*-nona chlor + *cis*-nonachlor), and ΣDDTs (*p*,*p*′-DDD + *o*,*p*′-DDE + *p*,*p*′-DDE + *o*,*p*′-DDT + *p*,*p*′-DDT) by the method described below.

All materials used for sample collection and preparation were washed thoroughly with purified water and finally washed with acetone. All samples were dissected and muscle, liver, and gills were removed. Following dissection, they were wrapped in plastic bags and stored frozen at −20°C until chemical analysis. The procedure of chemical analysis is described bellow.

Approximately 5 g of homogenized samples were added to a 45 mL Dionex accelerated solvent extraction (ASE Dionex) cell. ^{13}C-labeled surrogate standards were added to the ASE cell. The sample was extracted with a mixture of hexane and acetone (1:1) (100°C, static 5 min, heating 5 min, purge 1 min, 2000 psi). The lipid content for each sample was determined gravimetrically. After the extract was concentrated, lipids were removed by gel permeation chromatography, using a teflon column (22–25 mm bore diameter, 50–70 cm length) filled with 50 g of resin (BioBeads S-X3) suspended with dichloromethane. The column was eluted with dichloromethane and cyclohexane (1:1) at a flow rate of 5 mL/min. The eluant was reduced in volume to ∼5 mL. The polar compounds were removed and separated by liquid chromatography on Florisil®. The first fraction, eluted with 100 mL of 5% diethylether in hexane, contained most of the compounds. The second fraction, eluted with 100 mL of 20% diethylether, contained endrin and dieldrin. After being reduced to approximately 5 mL, the first fraction was transferred to a silica gel column and was separated again into two fractions. The first fraction, eluted with 30 mL hexane, contained HCB and aldrin. The second fraction, eluted with 25% diethylether in hexane, contained the other POPs. All the fractions were reduced in volume to 0.5 mL by dry nitrogen. A syringe spike was added.

An Agilent 6890 series gas chromatography/negative chemical ionization mass spectrometer (Agilent 5973N) was used for identification and quantification. The separation was carried out with a HT-8 capillary column (SGE, 50 m length × 0.22 mm i.d., 0.25 μm film thickness) coated with 8% phenylpolycarborane-siloxane. The column temperature was programmed for 50°C held for 0.3 min, followed by an increase to 200°C at a rate of 20°C/min. It was further increased to a final temperature of 280°C at a rate of 2.5°C/min. The injector temperature and the ion source temperature were 260 and 150°C, respectively. Splitless injection (1 μL) of the sample was employed.

All the congeners were quantified using the isotope dilution method; therefore, ^{13}C-labeled surrogate standards corresponding to the target compounds were added to all the samples prior to extraction. ^{13}C-labeled surrogate standards were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). The recovery for each sample was between 60% and 120%.

Statistical differences in concentrations of compounds between liver, gills, and muscle of masu salmon were analyzed using Tukey HSD tests with a confidence range of $p < 0.05$. Statistical analysis was performed using R statistical software (R Development Core Team, 2007, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL; http://www.R-project.org).

Results and Discussion

The concentrations of POPs (wet wt.) in three organs of masu salmon are shown in Fig. 1. The highest concentration was detected in liver, followed by gills, and, lastly, muscle for most of the compounds analyzed (Fig. 1). Concentrations of four compound groups (ng/g wet wt.) in liver and gills were 3–26 times and 3–14 times higher than that in muscle, respectively (Tukey, $p < 0.05$). The lipid content in liver and gills was in the range of 12–66%,

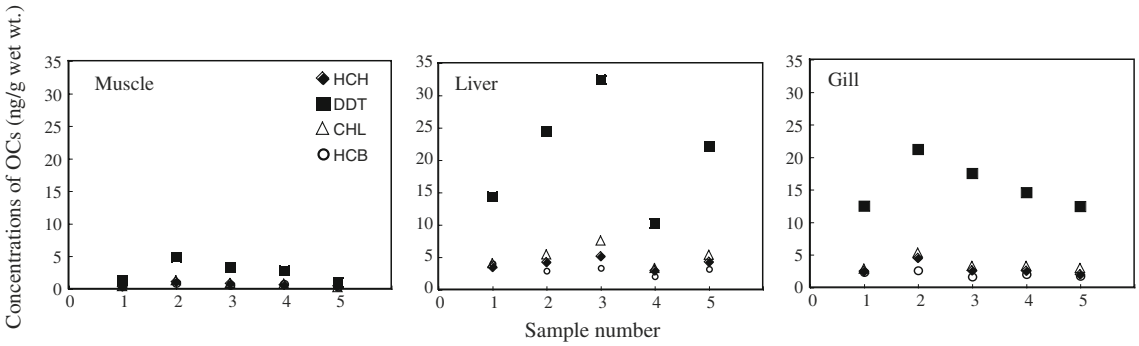

Fig. 1 Concentrations of POPs (wet wt.) in muscle, liver and gills of five masu salmon analyzed

Table 2 Lipid content (%) in liver, gills, and muscle of masu salmon

Sample number	1	2	3	4	5
Liver	20	49	55	12	66
Gills	29	17	31	15	28
Muscle	3.9	4.9	2.3	3.8	2.4

compared to 2.3–4.9% in muscle (Table 2). The concentrations of ΣDDTs were highest among the chemicals analyzed and were 4–5 times higher than the other compound groups (Fig. 1). When expressed in lipid weight basis, POPs in liver, muscle, and gills distributed homogenously (Fig. 2). There were no differences in the concentrations (lipid wt.) of pollutants between the three organs (Tukey, $0 > 0.05$) for all compound groups suggesting that the chemical equilibrium proposed by Russel et al. (1993) is at a steady state. In the present study, we analyzed the total lipid content (Table 2). Lipid contents and class compositions in fish have been studied to some degree (Henderson and Tocher 1987) and shows that the two main types of lipid in fish are neutral lipid and polar lipid. Polar lipid is composed primarily of phospholipids and fatty acids, whereas triglycerides are the main component of neutral lipid and account for most lipid in these three organs in salmonid fish (Henderson and Tocher 1987; Takeuchi et al. 1989). The accumulation of POPs in muscle, liver and gills has been shown to depend on the triglyceride content of these organs (Kawai et al. 1988; Jobling et al. 1998). Because the triglyceride content is almost the same in the three organs it follows that POPs accumulated in the three organs homogenously in lipid weight base.

It is known that relative proportions of the constituent lipid classes in salmonid fish change during seaward and spawning migration (Ando et al. 1985; Ota and Yamada 1974). Therefore, when discussing POPs accumulation and its dynamics in salmonid fish, it is essential to consider the growth stage (GSI) and/or lipid content of the fish. Although there are many reports of lipid content and GSI regarding chum salmon and other salmonid fish, there are few reports concerning masu salmon. To determine the growth stage of the salmon analyzed in the present study, GSI was calculated for all samples and the average GSI was 0.51 ± 0.16. This number is very small compared to that reported in Nomura (1984) which had an average GSI of four in masu salmon which were caught in the river in May. We assumed that the GSI were smaller in our study since the masu salmon were caught inside the bay in April, prior to entering the river. This suggests that the masu salmon analyzed in this study were either still in feeding migration or just entering spawning migration, and

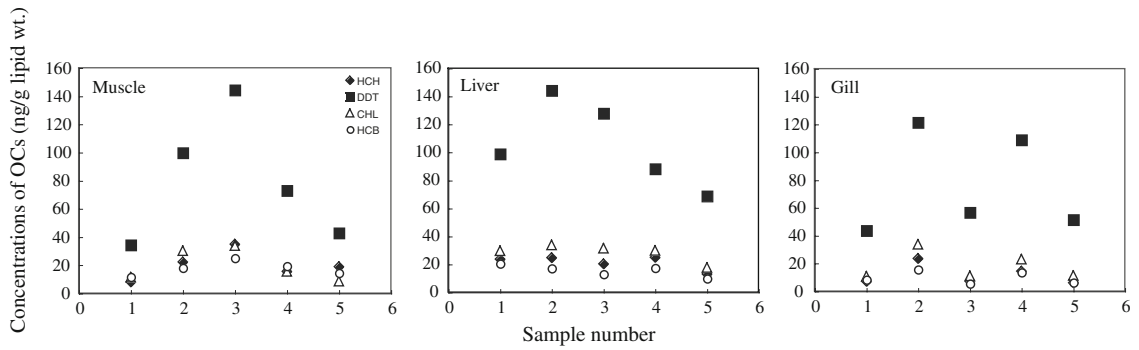

Fig. 2 Concentrations of POPs (lipid wt.) in muscle, liver, and gills of five masu salmon analyzed

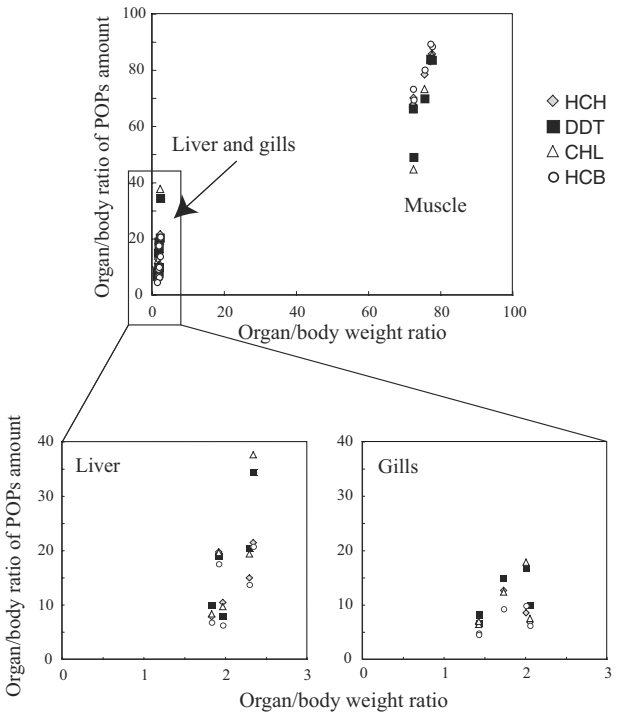

Fig. 3 Relationship between organ/body ratio of POPs amount and organ/body weight ratio in muscle, liver, and gills of masu salmon. The relationship in liver and gills were extended and shown separately

maturation was not yet in process. In this last stage of feeding migration, enough nutrients have been taken into the body and lipid content is predicted to be highest throughout the life cycle. Thus, lipid was accumulated highly in liver and gills, and POPs which bind to lipid, accumulated heavily in these two organs. To elucidate the accumulation potential of each organ, the organ/whole body ratio (%) of the total amount of POPs and the organ/whole body weight ratio (%) were determined (Fig. 3). Although the weight percentage of liver and gills was in the range of 1.4%–2.3%, the percentage of total POPs was 4.5%–37.6%. Thus, in spite of their small size, the liver and gills accumulated large amounts of POPs compared to the muscle (Fig. 3). This study revealed that not only liver, which is already known as an important pool site for POPs, but also gills accumulated POPs at high levels during feeding migration and clearly indicated that gills should also be considered as a new pool site. High concentrations of POPs in these organs also suggest that salmon at this stage are at a risk of toxic effects.

Anadromous salmon go on a fast during spawning migration and are known to lose lipid as GSI gets higher (Henderson and Tocher 1987; Ando et al. 1985) suggesting that the lipid transfers to the eggs as the fish matures. Maternal transfer of POPs to eggs has been investigated in some salmonid fish (Miller 1993; Miller and Amrhein 1995), however, the detailed mechanism of maternal transfer of POPs to eggs has not yet been revealed. It is well known that liver plays an important role in maturation, and that most of the nutrients are transferred to the eggs from liver (Idler and Tsuyuki 1958). Therefore, it is easily predicted that POPs in liver will be mobilized with the lipid but the dynamics and fate of POPs from gills and muscle is still unknown. Salmon species are known to play an important role in the river ecosystem (Naiman et al. 2002) and if the carcasses of salmon hold high amounts of POPs, its predators, such as bears and birds are likely to be exposed to these compounds as well. Although there are many studies regarding salmon species and POPs accumulation, there are still very few studies addressing the dynamic accumulation in organs other than liver and muscle throughout the life cycle of the salmon. Further investigation regarding POPs accumulation in salmon is essential in order to estimate its influence on the ecosystem, and to precisely understand the amounts of POPs accumulated to assess the toxic effects of POPs to masu salmon.

Acknowledgments This study was supported by a Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (No. 12NP0201) and JSPS Research Fellowships for Young Scientists (Y.W.).

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