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ORGANOCHLORINES IN INSHORE VERSUS OFFSHORE YELLOWTAIL FLOUNDER (*PLEURONECTES FERRUGINEA*): THE EFFECT OF A SMALL URBAN POPULATION ON THE ENVIRONMENT

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Levels of a series of organochlorine pesticides, including polychlorinated biphenyls (PCB) were determined in muscle, liver and gonad of yellowtail flounder, *Pleuronectes ferruginea*, collected in waters of the Northwest Atlantic, offshore the island of Newfoundland. Comparison was made to concentrations in tissues of fish maintained in tanks of the Northwest Atlantic Fisheries Centre, located in the city of St John's. PCB and DDE concentrations in liver of inshore females were more than ten times more elevated than in offshore females. Concentrations of several organochlorines were also significantly more elevated in liver, muscle and gonads of inshore versus offshore fish. The PCB congeners fingerprint displayed the predominance of PCB-153 > 138/163/164 > 118, in all samples. Twenty one PCB congeners were detectable in over 80% of liver samples, eight were detectable in muscle and 4 in gonads, while no fingerprint differences were apparent between sexes or inshore and offshore samples.

KEY WORDS: PCB, congeners, DDT, chlordanes, flounder, organochlorines.

INTRODUCTION

The Canadian Green Plan for Toxic Chemicals Program is a five year initiative which began in 1992 and is designed to assess the state of our environment. One project undertaken by the Toxicology Section of the Department of Fisheries and Oceans, in St John's, Newfoundland has been to determine the level of organic and inorganic contaminants in some commercial fish and invertebrate species¹⁻⁴. In the case of finfish, three tissues have been sampled: muscle, liver and gonad. These tissues were chosen because the level of contaminants in muscle tends to relate to long-term exposure to pollution and is of interest from a human health perspective⁵. Levels of lipophilic and hydrophobic contaminants in liver, an organ rich in lipid are usually higher than in other organs and are indicative of previous short-term exposure. Concentrations in gonads are important from a reproduction point of view and future of a species, since it is the early life stages of biota which are typically more susceptible to the toxic effects of pollutions⁶.

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The present manuscript compares the level of organochlorines in twenty two fish pools of yellowtail flounder, *Pleuronectes ferruginea*, collected in waters offshore the island of Newfoundland, to two pools of yellowtail flounder collected inshore and maintained in tanks, at the Northwest Atlantic Fisheries Centre, for the last two years of their lives. The offshore samples were equally divided into males and females and included animals ranging from 27 to 46 cm in length, while inshore animals were of a smaller size (21 to 26 cm).

MATERIALS AND METHODS

Two hundred and twenty six fish were caught from three locations within the Northwest Atlantic Fisheries Organization (NAFO) zones: 3Ps, 30 and 3N, in April-May 1993 (Figure 1). After dissection and examination of the morphometric data, pools containing 7 fish each, with an equal amount of tissue per fish were made with consideration to location, sex, size, organ type, liver index (ratio of liver to fish weight) and gonad index (ratio of gonad to fish weight). A total of 22 offshore pools were made for each tissue. These pools represented fish between 27 and 46 cm (expected 4 to 9 years old), with liver indices between 0.006 and 0.026 and gonad indices between 0.001 and 0.19, half the samples belonged to each sex. It was possible to obtain two inshore samples, one of females only and one of both sexes. The flounder maintained in the tanks were collected at Bellevue, Trinity Bay, in October 1991, fed capelin once a week and sacrificed in October 1993. The inshore fish were smaller, from 21 to 26 cm, with liver indices between 0.005 and 0.10 and gonad indices between 0.002 and 0.043.

A series of 23 organochlorines: α -, β - and γ -HCH, HCB, oxychlordane, trans- and cis-chlordane, heptachlor, heptachlor epoxide, trans- and cis-nonachlor, methoxychlor, o,p'- and p,p'-DDE, p,p'-DDT, o,p'- and p,p'-DDD, mirex, α -endosulphan, dieldrin, endrin and aldrin, of polychlorinated biphenyls (PCB) measured in terms of three Aroclor standards, 1242, 1254 and 1260, as well as lipid content were analyzed in each sample. One hundred and eight PCB congeners were also analyzed: 5/8, 15, 16/32, 17, 18, 19, 21/28, 22, 24/27, 25, 26, 33, 40, 41/64/71, 45, 46, 47/48, 49, 52, 56/60, 66, 70/76, 74, 83, 84/89, 85, 87, 90/101, 91, 95, 97, 99, 105, 107, 110, 114, 118, 128, 129, 130, 131, 134, 136, 135/144, 137, 138/163/164, 141, 146, 149, 151, 153, 156, 157, 158, 170/190, 171, 172, 174, 175, 176, 177, 178, 180, 182/187, 183, 185, 189, 191, 194, 195, 196/203, 197, 198, 199, 201, 205, 206, 207, 208 and 209. Results are reported for those congeners detected in more than 80% of the samples. Those detected in less than 80% of samples, per tissue were present in a concentration lower than of 0.02 ng/g, wet weight and are not presented in Figure 3. The analysis of samples was performed by Axys Analytical Services, at Sidney, British Columbia and the analytical procedure has been described in Hellou *et al.* Blanks and duplicates were run with every 6 to 10 samples analyzed, recoveries of internal surrogate ^{13}C labelled standards of HCB, PCB 101, 180 and 209, and α -endosulphan were determined for each sample and concentrations adjusted accordingly. Labelled PCB 77, 126 and 169 were used as internal standards. Blanks rarely had any detectable levels of organochlorines, those present were accordingly subtracted from the samples belonging to the batch. In the case of duplicate analyses, the mean concentration was used in the calculations. The mean value of 5 recoveries, per sample, averaged over all the samples was of 77%, ranging from 36 to 140%. Six samples were analyzed in duplicate and displayed concentrations that were from 0 to 50% higher, with a mean of 4%. Six samples were pooled in duplicate and individual concentrations were from 0 to 400% higher, with a mean of 13%.

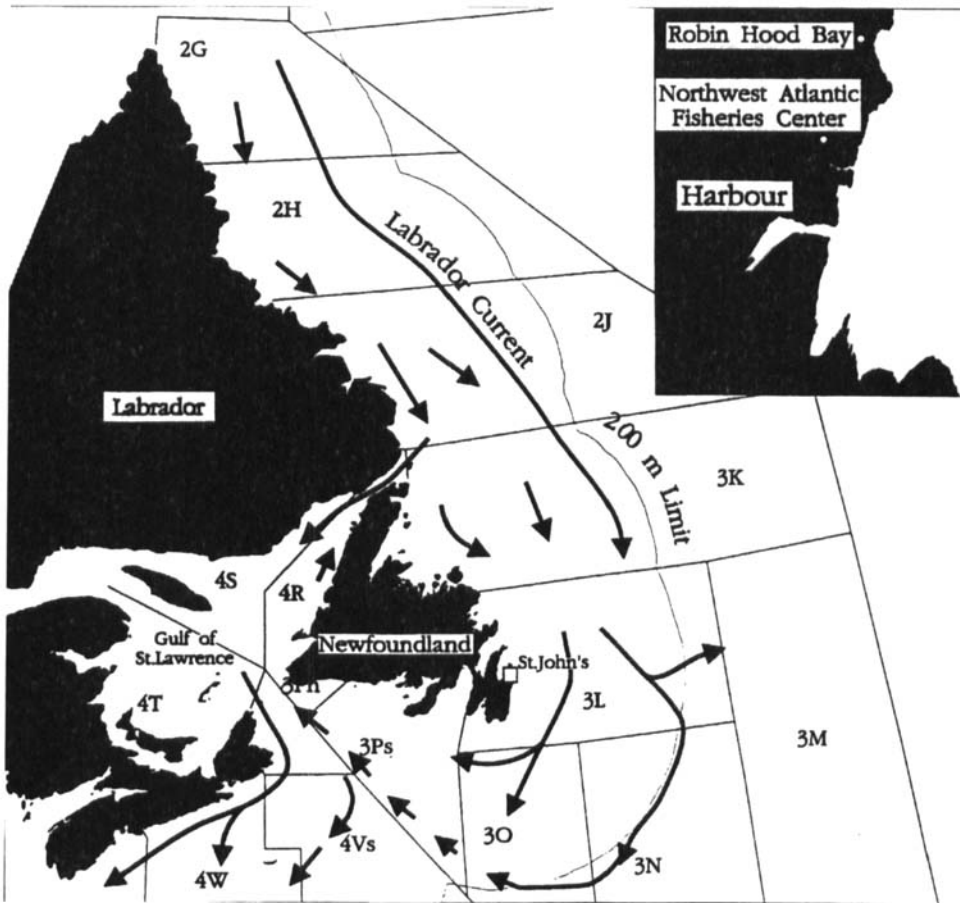


Figure 1 Map indicating the location of the sampling sites.

RESULTS AND DISCUSSION

Yellowtail flounder, *Pleuronectes ferruginea*, are known to inhabit the western North Atlantic, from southern Labrador to Chesapeake Bay. These benthic flatfish move to shallow waters in spring and to deeper waters in fall and early winter. Their diet is varied and includes polychaete worms, amphipods, shrimp and other invertebrates, sand lance and capelin, when available⁷.

Organochlorines are a group of contaminants derived from the use of pesticides, especially insecticides, earlier this century in the United States, Canada and Europe. The production and use of many of these ubiquitous compounds has been banned or restricted since the late 1970s, due to their recognized persistence in the environment, bioaccumulation and toxic effects⁸. Polychlorinated biphenyls (PCB) have been used for other industrial purposes, where heat-resistance, low vapour pressure, stability and non-flammability were important properties. For example, PCB have been used as dielectric fluids in transformers and capacitors, in antifouling paints and as industrial oil

additives⁹. More recently, it has been recognized that some organochlorines also derive from combustion processes^{10,11}.

Of the organochlorines analyzed (Figure 2) in yellowtail flounder, the polychlorinated biphenyls (PCB) concentration expressed in terms of the commercial PCB standard Aroclor 1254 displayed the highest level, in all analyzed organs. This same observation was made for a variety of organisms inhabiting the Atlantic Ocean, for surface water and air, over two decades ago¹². More recently the concentration of the same series of organochlorines was determined in tissues of cod (*Gadus morhua*) collected offshore Newfoundland¹, where PCB and DDT also predominated. Time trend analyses have shown that highly lipophilic and stable contaminants, such as the DDT and PCB will persist in remote areas, long after their restricted use¹³⁻¹⁶. In offshore samples, the mean concentration of Aroclor 1254 represented 29 and 27% of the sum of the mean organochlorine concentrations determined in liver; in muscle, it represented 28 and 24%; while in gonads, it represented 33 and 25%, in males and females, respectively. For the two inshore pools, PCB represented 41 and 43% of the liver concentrations, 45 and 50% of muscle concentrations, 46 and 51% of gonad concentrations with females having the highest concentration compared to the other group. Comparison of inshore and offshore results is provided in Table 1, for various groups of contaminants. As well, offshore female samples always had lower concentrations than males, regardless of tissue considered (Figure 2). When the vertical bars on the left hand side of the boxes are above the whisker, then concentrations of inshore samples can be regarded as statistically different.

The next highest single concentration in all tissues was that of p,p'-DDE, the major degradation product of p,p'-DDT (Figure 2). The sum of the chlordanes, DDT related compounds and PCB (Aroclor 1254) were present in a nearly similar ratio (1:1:1), in liver of offshore fish (Table 2). A different pattern was observed for the inshore samples, where PCB > DDT > chlordanes, in all examined tissues. As expected, concentrations of these three families of contaminants were always higher in liver than in muscle or gonads. Concentrations of chlordanes and DDT compounds were higher in muscle compared to gonads. Levels in female muscle were lower than in males, while levels in ovaries were higher than in the testes (Figure 2). Of the analyzed organochlorines, five were undetectable in all samples, regardless of tissue examined: heptachlor, methoxychlor, o,p-DDE, α -endosulphan and aldrin.

It has been recognized that organochlorines tend to correlate with the level of lipids in tissues and the age of the organisms¹⁷⁻¹⁹. The extent of the correlation and whether accumulation in females takes place at a different rate than in males, due to spawning, is still debatable and may depend on species⁸. The present inshore samples were from smaller finfish than the offshore samples and therefore, relatively lower concentrations could be expected. Lipid content of inshore samples was within the range of offshore samples. In liver: 2.5-8% vs 5.3 and 6.8%; muscle: 0.3-2.1% vs 0.3% in both cases; gonad: 0.6-3.2% vs 0.6 and 2.0%, range of offshore samples vs inshore female and male yellowtail flounder, respectively. Therefore normalising concentrations for the lipid content had a minor effect on the comparison of offshore to inshore levels (Table 1 and 3)^{19,20}. The ratio of the total concentration of organochlorines, expressed in terms of lipid weight and wet weight, for inshore versus offshore samples is presented in Table 1. Results indicate a major difference in the bioaccumulation of contaminants in liver, muscle and gonad tissue (Table 1 and Figure 2). A difference in the tissue distribution of organochlorines has also been observed in ducks and mammals^{21,22}. The distribution of total organochlorines and specific groups of contaminants in tissues of offshore males was unlike that of females (Table 3).

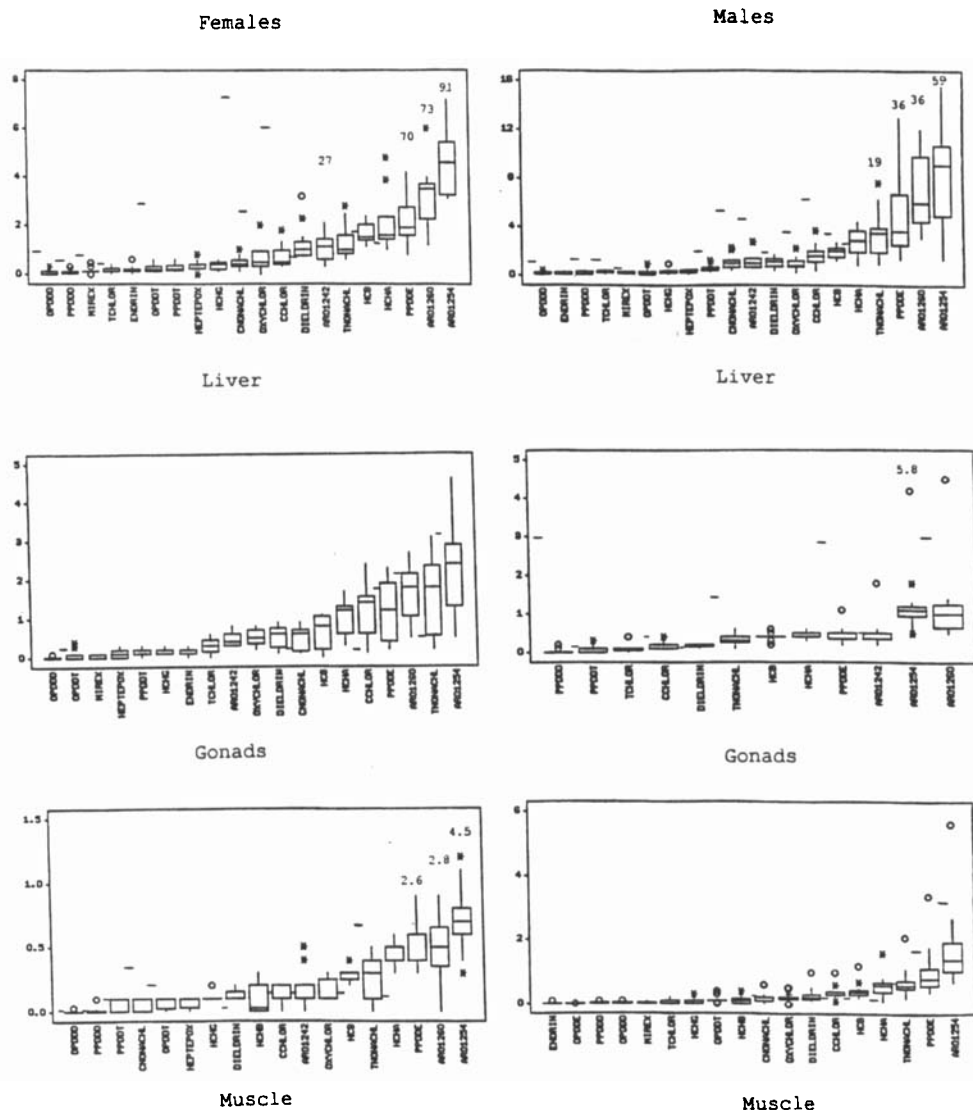


Figure 2 Box and whisker plots displaying median concentration (vertical bar within box, ng/g, wet weight) of contaminants in offshore samples, where boxes enclose the middle half of the data, whiskers show the range of concentrations, stars and circles indicate possible and probable outliers, respectively*. Concentrations of inshore samples are indicated by either a vertical bar to the left-hand side of the box, or a number above the box.

TCHLOR refers to trans-chlordane, HEPTEPOX is heptachlor epoxide, HCHG is γ -HCH, CNONACHLOR is cis-nonachlor, CCHLOR is cis-chlordane, TNONACHL is trans-nonachlor, HCHA is α -HCH and OXYCHLOR is oxychlordane. The y axis units are in ng/g, wet weight.

It is well known that levels of contaminants, in air, sediments, water and biota are higher near industrialised or more populated centres, than in remote areas, although these remote areas are not free of contaminants²³⁻²⁶. The presence of contaminants in several matrices collected in remote areas, such as the Arctic has been explained by their

Table 1 Ratio of organochlorines in inshore versus offshore samples in terms of wet weight and (lipid weight).

	<i>Liver</i>	<i>Muscle</i>	<i>Gonads</i>
<i>Total organochlorines</i>			
Males	4.9 (3.8)	1.2 (3.5)	0.9 (1.3)
Females	13 (10)	3.1 (5.2)	0.2 (0.6)
<i>PCB (Aroclor 1254)</i>			
Males	6.9 (5.4)	1.9 (5.5)	4.5 (6.5)
Females	20 (16)	6.4 (11)	1.4 (4.2)
<i>DDTs</i>			
Males	6.4 (5)	1.6 (4.6)	> 6.0 (8.7)
Females	26 (20)	2.1 (3.6)	> 3.5 (11)
<i>Chlordanes</i>			
Males	4.8 (3.7)	0.8 (2.3)	> 5 (7.2)
Females	17 (13)	2.0 (3.4)	< 0.2 (0.6)

-Ratios compare females to females and offshore males to a group of mixed sex.

-The > and < signs are used when the concentration of at least one organochlorine was undetectable.

physical-chemical properties, which dictate their environmental behaviour²⁷. Briefly, it can be stated that the volatilisation of persistent contaminants with a high Henry's law constant (ratio of solute partial pressure in air to equilibrium concentration in water) takes place in tropical waters and after atmospheric transport, there is a preferential loading of these chemicals to colder water bodies. The spatial distribution of PCB and DDT has been summarized by Lagonathan and Kannan¹⁵.

Table 2 Ratio of concentration of different organochlorines observed in the three tissues.

<i>Location</i>	<i>Liver</i>	<i>Muscle</i>	<i>Gonads</i>
<i>DDE:DDD:DDT</i>			
Offshore M	25:1:3	11:ND:1	5:ND:1
Inshore M and F	40:1:2.3	11:ND:1	30:ND:1
Offshore F	22:1:3	ND	12:1:3
Inshore F	117:1:4.8	ND	ND
<i>PCB:DDT:Chlordanes</i>			
Offshore M	1:1.2:1.6	2:1:2	1:1:1
Inshore M and F	2.1:1.7:1	5:3:1	3:2:1
Offshore F	1.6:1:1	1:1:1	2:1:4
Inshore F	1.6:1.1:1	3:2:1	6:4:3
<i>t-nonachlor:t-chlordane</i>			
Offshore M	11	7	3
Inshore M and F	21	ND	7
Offshore F	6.5	ND	5.3
Inshore F	54	ND	ND

-ND: non detectable.

Table 3 Distribution of lipids (%) and ratio of contaminants in tissues (liver: gonad: muscle).

	Total	Lipids	PCB	DDT	Chlordanes
Offshore M	8:1:2	5.3:2.7:0.9	7:1:1	10:1:2	13:1:3
Offshore F	6:3:1	4.3:1.5:0.5	7:3:1	5:1:2	4:7:1
Inshore F	33:1:1	5.3:0.6:0.3	28:1:1	35:1:1	43:1:1
Inshore M+F	19:2:1	6.8:2.0:0.3	17:2:1	19:2:1	28:2:1

The present concentrations of organochlorines in tissues of yellowtail flounder allowed the comparison of the baseline levels deriving from exposure to contaminants from atmospheric transport sources to the immediate contribution of a small Canadian population. Our PCB and DDT muscle results, for offshore and inshore fish are somewhat similar to Muir's *et al.*²⁸ comparison of pelagic fish ($n = 19$, PCB = 333 and DDT = 200 ng/g, lipid weight) to abyssal fish ($n = 1$, PCB = 2328, DDT = 1465 ng/g, lipid weight) inhabiting Arctic waters. There is only limited information on levels of organochlorines in biota from the Northwest Atlantic (reviewed by Wells and Rolston²⁹) and especially flatfish^{e.g.12,30}. A comparison has previously been made between cod from various locations¹, where those from around Newfoundland displayed the lowest levels.

Levels of different organochlorine contaminants in muscle and liver of finfish species have been reported to vary by more than a factor of 10–100, in different areas of the world, nearer and further away from point sources of pollution^{19,26,31–33}. As expected, organochlorines concentrations varied most in liver of offshore versus inshore fish, while differences were still significant for PCB and DDT, in muscle and gonad of these two groups of fish (Table 3), in agreement with their higher stability.

Two other factors can play a role in the level of contamination of inshore versus offshore samples. The time of sampling: spring for offshore and fall for inshore finfish, as well as diet. Seasonal variations in the level of a variety of contaminants have been reported in air, water and biota. Higher concentrations are observed in the environment, due to temperature and snow melt in winter and spring, compared to summer and fall^{15,34}. This expected variation is opposite to that presently observed. Water and diet represent uptake sources of contaminants, for the finfish. The yellowtail flounder held in the tanks had a uniform diet of capelin (fed once a week), somewhat representative of the diet of the feral finfish population. Therefore, it appears reasonable to conclude that the level of contaminants in tissues were affected mainly by the level of contaminants present in seawater pumped into the aquaria. This conclusion is in agreement with work suggesting that uptake of PCB is predominantly governed by an equilibrium partitioning process between water and organisms (reviewed by Kalmaz and Kalmaz⁹), that also lead to the formulation of models to predict the bioconcentration of chemicals from water^{e.g.35}. However, some models do incorporate a food chain component^{e.g.36}.

Information was also obtained on the distribution of a large number of PCB congeners in tissues (Figure 3). These include PCB-28, 52, 101, 138, 153 and 180 (IUPAC) recommended by the International Commission for the Exploration of the Sea³⁷ and PCB-105, 118, 128, 149, 156 and 170 recommended by the Community Bureau of Reference³⁸ (plus the preceding 6). PCB-153, 138/163/164 and 118 were the three most predominant peaks, present in this decreasing order, in all analyzed samples. Congener 153 is one of five highly bioaccumulative members, while 138 and 118 are members of the ten most toxic coplanar PCB³⁹. Congeners possessing chlorine substituents that lead to a coplanar configuration are generally thought to be more toxic than non-coplanar

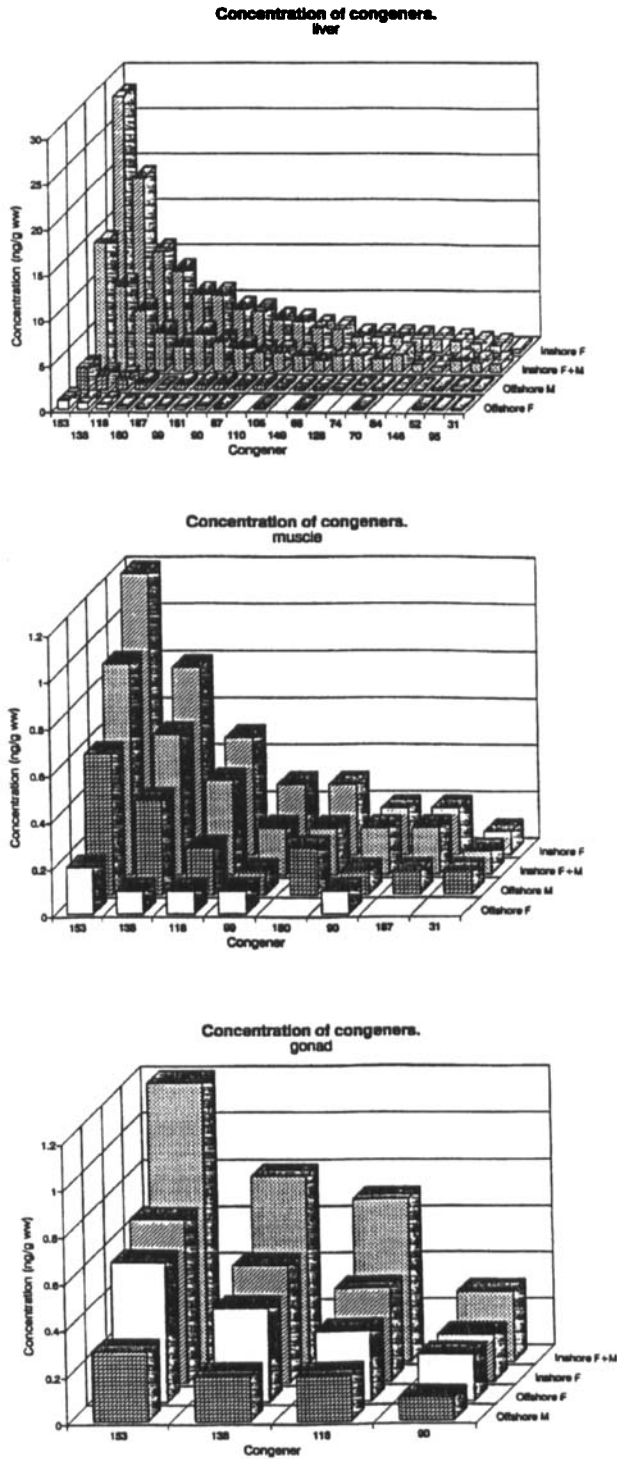


Figure 3 PCB congeners detected in over 80% of each group of liver, muscle and gonad samples.

ones. Liver had the largest number of congeners (21 detectable in over 80% of the samples). Gonad had the lowest number of detectable congeners (4), while muscle had 8 detectable. As displayed in Figure 3, the same general fingerprint was observed for males and females, inshore and offshore finfish.

St John's, the capital of Newfoundland is a relatively small city by North-American standards, with a population of 130,000. The harbour, situated at the centre of town is used as a direct sewer outlet for the city and its surrounding population (Figure 1). As well, the water run-off from the city garbage dump (Robin Hood Bay, Figure 1) is relatively close to our laboratory's seawater intake. Even without the presence of major industries, there are higher levels of pollution within the proximity of the city. The present inshore-offshore results regarding the concentration of organochlorines in yellowtail flounder put into perspective the effect of humans on their environment. As reviewed by Hamdy and Gooch⁴⁰, the bioaccumulation of organochlorines is governed by many variables, but will take place within days of exposure, while depuration is a much slower process, with half-lives of up to 175 days (reviewed by Niimi⁴¹).

The Canadian guidelines for consumption of food products are of 5.0 ppm for the DDT group of contaminants, 2 ppm, for PCB and 0.1 ppm, for HCB, heptachlor/heptachlor epoxide, aldrin/dieldrin, endrin and mirex. These contaminants were present in muscle tissue, at levels much below these limits. It should also be mentioned that Germany and Holland have guidelines for several individual chlorobiphenyls. Concerns regarding the effect of organochlorines have led to studies on egg and larval viability⁴²⁻⁴⁷. A threshold level of 120 ng/g was proposed for PCB⁴³, while concentrations of up to 2000 ng/g of PCB did not appear to affect fertilisation success in English sole⁴⁸. Presently, mean PCB levels in ovaries of inshore and offshore fish were of 3.3 and 2.3 ng/g, much lower than those expected to affect larval viability.

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