

Biotransport of Organic Pollutants to an Inland Alaska Lake by Migrating Sockeye Salmon (*Oncorhynchus nerka*)

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ABSTRACT. Persistent organic pollutants such as polychlorinated biphenyls (PCBs) and the pesticide DDT, known to harm wildlife, have been shown to reach pristine Subarctic and Arctic areas by global atmospheric transport. Another transport route for pollutant entry into these ecosystems is provided by migrating salmon. Pollutant transport was studied in a population of sockeye salmon (*Oncorhynchus nerka*) in the Copper River, Alaska during their 410 km spawning migration. Pollutants accumulated by the salmon during their ocean life stage were not eliminated during migration, but were transported to the spawning lakes and accumulated in the freshwater food web there. The influence of the biotransported pollutants was investigated by comparing pollutant levels and compositions in atmospheric deposition as well as in two different populations of arctic grayling (*Thymallus arcticus*). One grayling population was in the salmon spawning lake and the other in a nearby lake not hosting anadromous fish, but receiving pollutants only via atmospheric deposition. The grayling in the salmon spawning lake were found to have concentrations of organic pollutants more than two times higher than those of the grayling in the salmon-free lake, and the pollutant composition resembled that found in salmon. Thus, in the studied Alaska river system, biotransport was found to have a far greater influence than atmospheric input on the PCB and DDT levels in lake biota.

Key words: biotransport, Pacific salmon, *Oncorhynchus nerka*, arctic grayling, *Thymallus arcticus*, organic pollutants, PCB, DDT, lipids, Copper River, Alaska

RÉSUMÉ. Il a été prouvé que des polluants organiques persistants comme les diphenyles polychlorés (PCB) et le pesticide D.D.T., dont on connaît les répercussions néfastes sur la faune, se répandent dans des zones subarctiques et arctiques vierges par le biais du transport atmosphérique global. Les saumons en migration offrent une autre voie de transport pour l'entrée des polluants dans ces écosystèmes. On a étudié le transport des polluants dans une population de saumon sockeye (*Oncorhynchus nerka*) remontant le fleuve Copper, en Alaska, durant la migration de frai de 410 km. Les polluants accumulés par le saumon au cours de sa vie océanique n'étaient pas éliminés durant la migration, mais étaient transportés dans les frayères des lacs où ils s'accumulaient dans le réseau trophique de l'eau douce. On a étudié l'influence des polluants bio-transportés en comparant les niveaux et les compositions de polluants dans les retombées atmosphériques ainsi que dans deux populations distinctes d'ombre arctique (*Thymallus arcticus*). Une des populations d'ombre se trouvait dans le lac où frayaient les saumons et l'autre dans un lac voisin, ne contenant pas de poissons anadromes, mais recevant des polluants uniquement par retombées atmosphériques. On a trouvé que l'ombre qui se trouvait dans le lac où frayaient les saumons avait des concentrations en polluants organiques deux fois plus grandes que l'ombre qui se trouvait dans le lac sans saumons, et la composition des polluants ressemblait à celle que l'on retrouvait dans le saumon. Ainsi, dans le réseau fluvial de l'Alaska qui faisait l'objet de cette étude, on a trouvé que le transport biologique avait une influence beaucoup plus importante que les retombées atmosphériques sur les niveaux de PCB et de D.D.T. dans le biote lacustre.

Mots clés: transport biologique, saumon du Pacifique, *Oncorhynchus nerka*, ombre arctique, *Thymallus arcticus*, polluants organiques, PCB, D.D.T., lipides, fleuve Copper, Alaska

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INTRODUCTION

In considering how persistent organic pollutants such as polychlorinated biphenyls (PCBs) and the pesticide DDT become distributed to pristine areas in temperate and arctic regions, the focus has been largely on atmospheric transfer.

The general direction of transport is believed to be towards the polar regions of the globe, since pollutants on the ground volatilize in areas of warmer climate, are transported by air mainly in the form of vapours, and "condense" or wash out in colder climate zones. The ambient temperature at which the "condensation point" is reached differs for the various

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compounds involved, indicating a global fractionation or global distillation (Wania and Mackay, 1993). As the pollutants reach aquatic and terrestrial ecosystems, they accumulate in biota because of their lipophilic and persistent properties. Several Arctic animals have been shown to contain high levels of organic pollutants, e.g., the polar bear (Norstrom et al., 1988), the arctic fox (Wang-Andersen et al., 1993), and the walrus (Muir et al., 1995). The effects of the pollutants on biota include reproduction disturbances, which have been discussed for various species of mammals (Reijnders, 1986), birds (Helander et al., 1982), and fish (Bengtsson et al., 1996).

Many species of animals shift their habitat during their life cycle. Birds migrate between foraging areas because of seasonal variations in prey abundance and the rearing of offspring. In anadromous fish such as salmon, spawning and rearing occur in freshwater, whereas the adult life stage is spent in the ocean. Catadromous fish such as eel (*Anguilla sp.*) spawn in the ocean, but spend the major part of their life cycle in freshwater. Before they migrate, such species generally accumulate lipids for both energy use and gonad development during migration. With this build-up of lipids occurs a concomitant accumulation of lipophilic pollutants (Spigarelli et al., 1983). Accordingly, migrating animals can act as vectors of organic pollutants between ecosystems, and fish have been shown to transport pollutants from the Great Lakes to the tributaries (Merna, 1986; Lum et al., 1987; Scudato and McDowell, 1989; Giesy et al., 1994).

During the summer and fall of 1994, we studied sockeye salmon (*Oncorhynchus nerka*) in the Copper River, Alaska, during their 410 km spawning migration from the Gulf of Alaska to one of the spawning lakes. The largely amphipod- and small fish-feeding sockeye salmon typically have a freshwater phase of 1 or 2 years, forage in the sea for 2 or 3 years, and then return to their native streams and lakes to spawn (Burgner, 1991). They die after spawning, and their degrading carcasses become a significant source of nutrients and organic matter to lake ecosystems (Kline et al., 1993).

Our hypothesis, that the organic pollutants that the salmon accumulate during their ocean life stage are not eliminated during the spawning migration, implies that the pollutants are transported to the spawning lake and possibly deposited there. We also investigated the availability of this proposed biotransported pollutant pool by examining the pollutant levels found in a stationary, aquatic top-predator, the arctic grayling (*Thymallus arcticus*), present in the salmon spawning lake, and comparing them with the levels found in grayling from a lake to which the salmon had no access. In the lakes that contain salmon, grayling obtain pollutants by feeding on salmon roe (Skopets and Prokopyev, 1990) and via transport through the food chain of material from degrading salmon carcasses. Grayling in salmon-free lakes obtain persistent pollutants as a result of atmospheric deposition only. We reasoned that the quantity and quality of persistent pollutants in an aquatic predator such as grayling would differ, depending upon the origin of the pollutants.

MATERIAL AND METHODS

Fish Samples

The salmon in the present study were of four groups: those caught up to 20 km outside the river mouth and about to start the freshwater migration ($n = 20$); fish at different stages of maturity caught at 16 sites along the migration route ($n = 67$, 0–388 km); mature fish caught 10 km below the spawning lake ($n = 20$); and spent fish caught in the spawning lake ($n = 10$, 410 km). At each site, both males and females were caught by dip-net, gill-net, or hook and line, and dorsal muscle tissue and gonads were excised from the fish.

In the spawning lake (Lower Fish Lake, N63°05', W145°25'), and in the reference lake nearby (Round Tangle Lake, N63°04', W145°58'), grayling were caught both before and after the salmon spawning period with hook and line or gill-net. The fish were aged and weighed, and their gender was determined. A sample of 23 fish was selected from each of the two lakes, selection taking account of age (2–7 years) and weight (120–730 g) so that the two samples would be comparable. Each fish was ground up whole and analysed individually for persistent pollutants.

Atmospheric Deposition

Two samplers, modified after Agrell et al. (in press), were placed in the catchment area of each lake to sample the pollutants present in precipitation and dry fallout on polyurethane columns (PUC). Five fallout samples were collected from each of the four samplers in July–September 1994; each sampling period lasted from 6 to 20 days, depending on the precipitation amounts.

Lipid and Pollutant Extraction

The lipids and organic pollutants were extracted from the samples by use of chloroform:methanol 1:2 v/v. Chloroform and water were added to split the system into an aqueous and an organic phase (Bligh and Dyer, 1959). To obtain complete separation of the two phases, the total mixture was centrifuged and the chloroform phase transferred to a weighed tube. After the chloroform was evaporated at 40°C with N₂-gas, the lipid extract was freeze-dried to remove all solvent traces prior to gravimetric determination of total lipids.

Soxhlet Extraction of Organic Pollutants from the PUC

Adsorbed organic pollutants were removed from the PUC by extraction in a modified Soxhlet apparatus for 2 h by 34 ml acetone:*n*-hexane 10:7 v/v. The extract was concentrated in a vacuum centrifuge, and sample clean-up was performed as described below (Bremle et al., 1995).

Extract Clean-up Prior to PCB Analysis

Approximately 100 mg of the lipid extract was dissolved

in 400 μ l *n*-hexane and was eluted with *n*-hexane:dichloromethane 95:5 v/v through a column containing two layers of activated silica gel to which Na_2CO_3 and H_2SO_4 , respectively, had been added. Prior to gas-chromatographic analysis, the eluate was evaporated to dryness in a vacuum centrifuge and was redissolved in isooctane (Bremle et al., 1995).

Analysis of Organic Pollutants

Organic pollutants were analyzed by capillary gas chromatography, on column injection, using a 30 m DB-5 quartz capillary column and an electron capture detector on a Varian Star 3400 CX. The PCB components were identified and quantified as domains according to Mullin et al. (1984) and Schulz et al. (1989). Analytical performance was checked regularly by use of pesticide- and PCB standards (Clophen and Aroclor mixtures); pentachlorobenzene was used as a chromatographic standard, as described in Bremle et al. (1995).

Data Analysis

Means, confidence intervals, and other statistics were based on log-normalized data (Ott, 1990; Sokal and Rohlf, 1995), and calculations were carried out using Stat View and Microsoft Excel computer packages. The sum of PCBs includes all detected and unambiguously identified domains. The sum of DDTs includes p,p-DDT, o,p-DDT, p,p-DDE and p,p-DDD. The compositions of pollutants in grayling from the two lakes were compared by regression analysis to those in salmon and atmospheric fallout. We used the individual persistent pollutants in sources (salmon or atmospheric fallout) as the X-variate and their corresponding pollutants in receivers (the grayling in each of the two lakes) as the Y-variate (thus, the same pollutant in source and receiver comprise one X-Y-pair). This method allowed the compositions to be compared (Larsson and Okla, 1989). A complete match between the composition in the source and that in the receiver would result in a regression coefficient of 1. Total dissimilarity, in contrast, would result in a regression coefficient of 0.

RESULTS

As the salmon migrated upstream towards the spawning lake, the content of lipids in the muscle tissue decreased from 5.5% (95% confidence interval [C.I.] = 4.51–6.7, $n = 20$) initially to 2.17% (C.I. = 1.93–2.43, $n = 20$) at maturity. The lipid content correlated negatively with the distance of migration (Fig. 1, $r^2 = 0.50$, $p < 0.0001$, $n = 107$). The muscle lipid concentration of PCBs increased from 670 ng/g (C.I. = 608–738) to 2493 ng/g (C.I. = 2219–2801, $r^2 = 0.66$, $p < 0.0001$), and that of DDTs increased from 221 ng/g (C.I. = 195–251) to 1223 ng/g (C.I. = 1070–1398, $r^2 = 0.71$, $p < 0.0001$; see Figs. 2a, b).

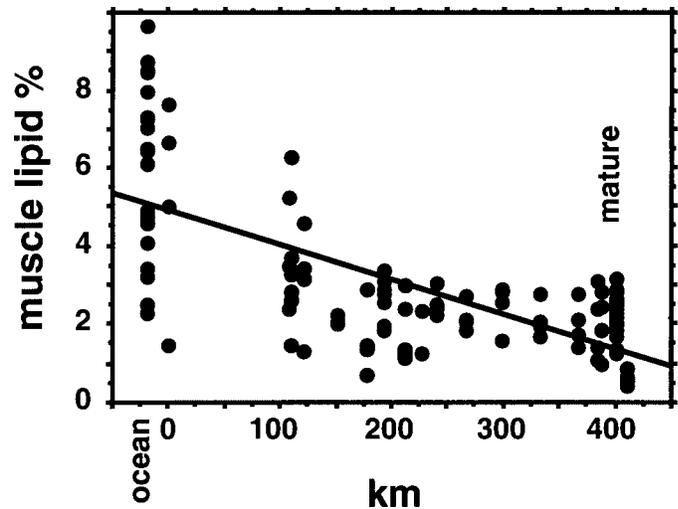


FIG. 1. The muscle lipids in sockeye salmon are utilized for energy and allocated to the gonads during the maturation process. The decrease is correlated with the length of upstream migration, which extends from outside the river mouth (in the ocean), to entering the river at 0 km, to the fish maturing after 400 km, and finally to their becoming spent in the spawning lake ($n = 117$).

The spent fish also had elevated concentrations of both PCBs (7910 ng/g, C.I. = 7022–8911) and DDTs (4863 ng/g lipid, C.I. = 4265–5544). These data were excluded from the regression analysis, since the processes responsible for the elevated concentrations in the dying fish are probably not a part of the maturation process of the salmon.

Lipids were reallocated from muscle tissues to the female gonads in connection with maturation; the gonad weight increased from 143 g initially (C.I. = 113–182, $n = 10$) to 394 g (C.I. = 350–445, $n = 10$) at maturity. The persistent pollutants were transferred to the gonads at a still higher rate, their concentrations in roe lipids approximately doubling. The concentration of PCBs increased from 361 ng/g lipid (C.I. = 306–426) to 759 ng/g (C.I. = 645–894, $r^2 = 0.54$, $p < 0.0001$) and that of DDTs increased from 147 ng/g lipid (C.I. = 123–177) to 363 ng/g (C.I. = 301–439, $r^2 = 0.59$, $p < 0.0001$; see Figs. 2c, d).

The grayling in the salmon spawning lake contained significantly higher concentrations of both PCBs and DDTs than those in the salmon-free lake ($p < 0.001$, t-test, $n = 46$): the mean concentrations in lipids were 1024 ng/g (C.I. = 786–1332) and 548 ng/g (C.I. = 452–664), respectively, for PCBs, and 239 ng/g (C.I. = 193–296) and 41 ng/g (C.I. = 36–46) for DDTs. These differences indicated the strong influence of biotransported pollutants on the salmon spawning lake.

To investigate the effect of biotransport on the pollutant levels further, we measured the atmospheric deposition of pollutants in the two lakes. During the five periods studied, atmospheric deposition ranged from 10–250 $\text{pg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for PCBs and from 0.6–1 $\text{pg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for DDTs. There were no significant differences in fallout between the two catchment areas ($p = 0.32$ for PCBs and 0.49 for DDTs, $n = 20$).

By means of regression analysis, the composition of persistent organic pollutants in atmospheric fallout, salmon, and grayling (Fig. 3) was compared for the two lakes (Fig. 4),

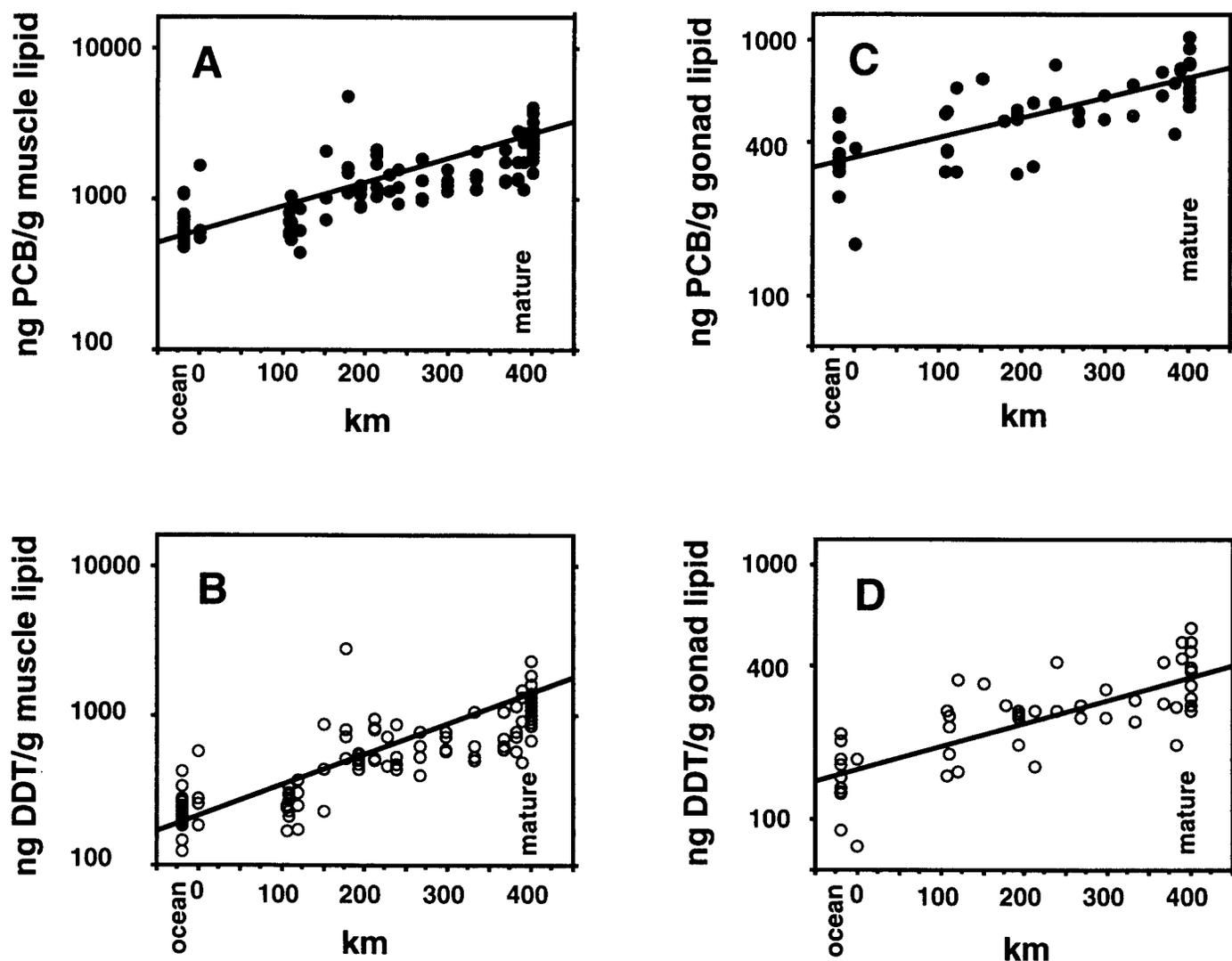


FIG. 2. Concentrations of PCB and DDT in muscle lipids (A and B, $n = 97$) and in female gonad lipids (C and D, $n = 53$) of sockeye salmon in relation to the length of upstream migration.

using the persistent pollutants in sources (salmon or atmospheric fallout) as the X-variate and the pollutants in receivers (the grayling in each of the two lakes) as the Y-variate. A high regression coefficient ($r^2 = 0.56$, $p < 0.0001$) was obtained for the pollutant composition in salmon and grayling in the salmon spawning lake. In the salmon-free lake, the regression coefficient was much lower ($r^2 = 0.18$, $p < 0.0001$). No relationship between the pollutants found in atmospheric deposition and those in the grayling was found for either lake ($r^2 < 0.01$, $p > 0.05$).

DISCUSSION

Fish with a high lipid content, such as salmonids, accumulate greater amounts of organic pollutants than lean fish (Geyer et al., 1985; Vuorinen, 1985; LeBlanc, 1995). As organic pollutants are distributed to different organs via the blood (Borlakoglu et al., 1990), they partition to different

tissues according to tissue lipid content and lipid composition, accumulating in triacylglycerol-rich tissues the most (Schneider, 1982; Kawai et al., 1988; Hellou et al., 1993; Bernhoft et al., 1994). Salmon have a high content of lipids in muscle tissue.

Toxic effects are correlated with both pollutant concentration and the load of pollutants in the body (Sijm et al., 1993). Accordingly, the higher wet weight concentrations of pollutants in fat fish species may lead to the false conclusion that these species are more vulnerable to organic pollutants than lean fish are. In fact, the opposite is the case: pollutants are relatively harmless as long as they are contained in triacylglycerol deposits. Several studies have concluded that the toxic effects of pollutants are less acute in those organisms that have a particularly high lipid content (Findlay and DeFreitas, 1971; Lassiter and Hallam, 1990; Geyer et al., 1990, 1993a, b). Thus, since the triacylglycerols serve mainly as an energy reserve, these deposits of neutral lipids withdraw pollutants from biologically more active structures, such as

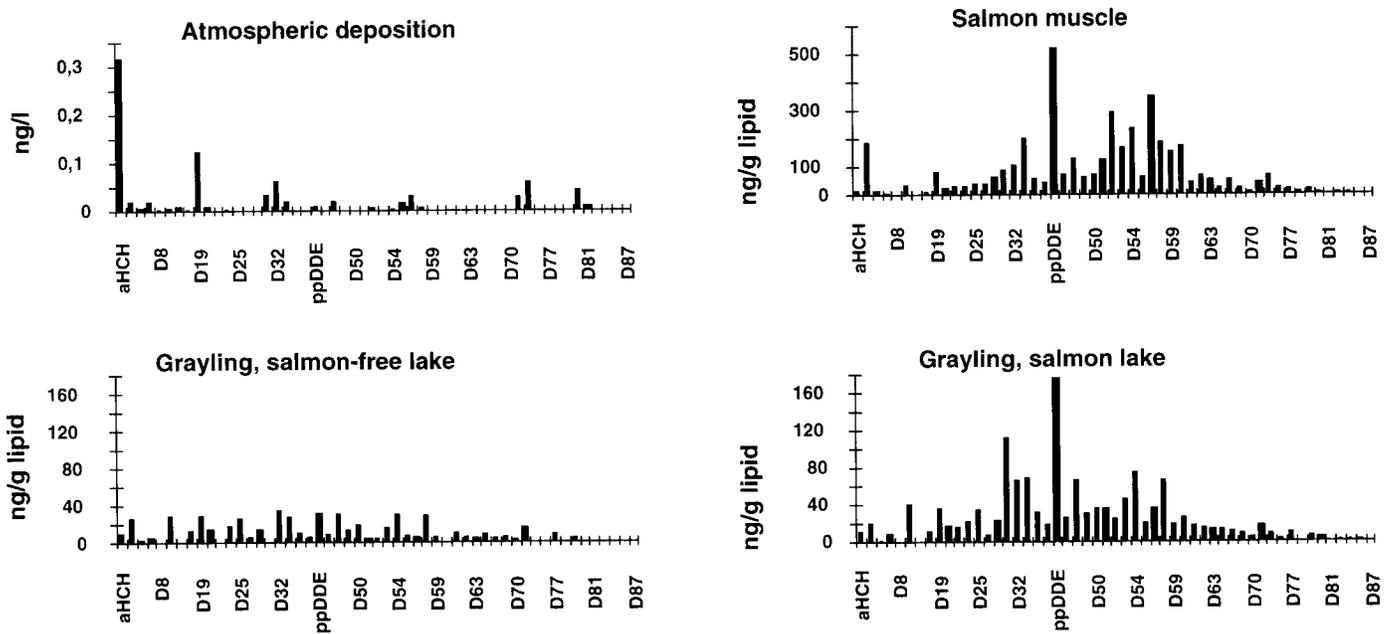


FIG. 3. The distribution and concentration of persistent organic pollutants in the muscle of mature sockeye salmon ($n = 20$), in arctic grayling caught in the salmon spawning lake ($n = 23$) and in the nearby salmon-free lake ($n = 23$), and in atmospheric deposition from the catchment areas of the lakes ($n = 20$). The PCB domains (Schulz et al., 1989) and other persistent pollutants are shown on the x-axis in the following order: α -hexachlorohexane, hexachlorobenzene, pentachloro-anisol, γ -hexachlorohexane, D8, D13, D14, D15, D19, D20, D21, D23, D25, D27, D30, D31, D32, D38, D39, D42, p,p-DDE, D44, D46, D47, D50, p,p-DDD, o,p-DDT, D53, D54, D55, p,p-DDT, D58, D59, D60, D61, D62, D63, D66, D67, D68, D70, D72, D73, D74, D77, D78, D79, D80, D81, D82, D84, D86, D87.

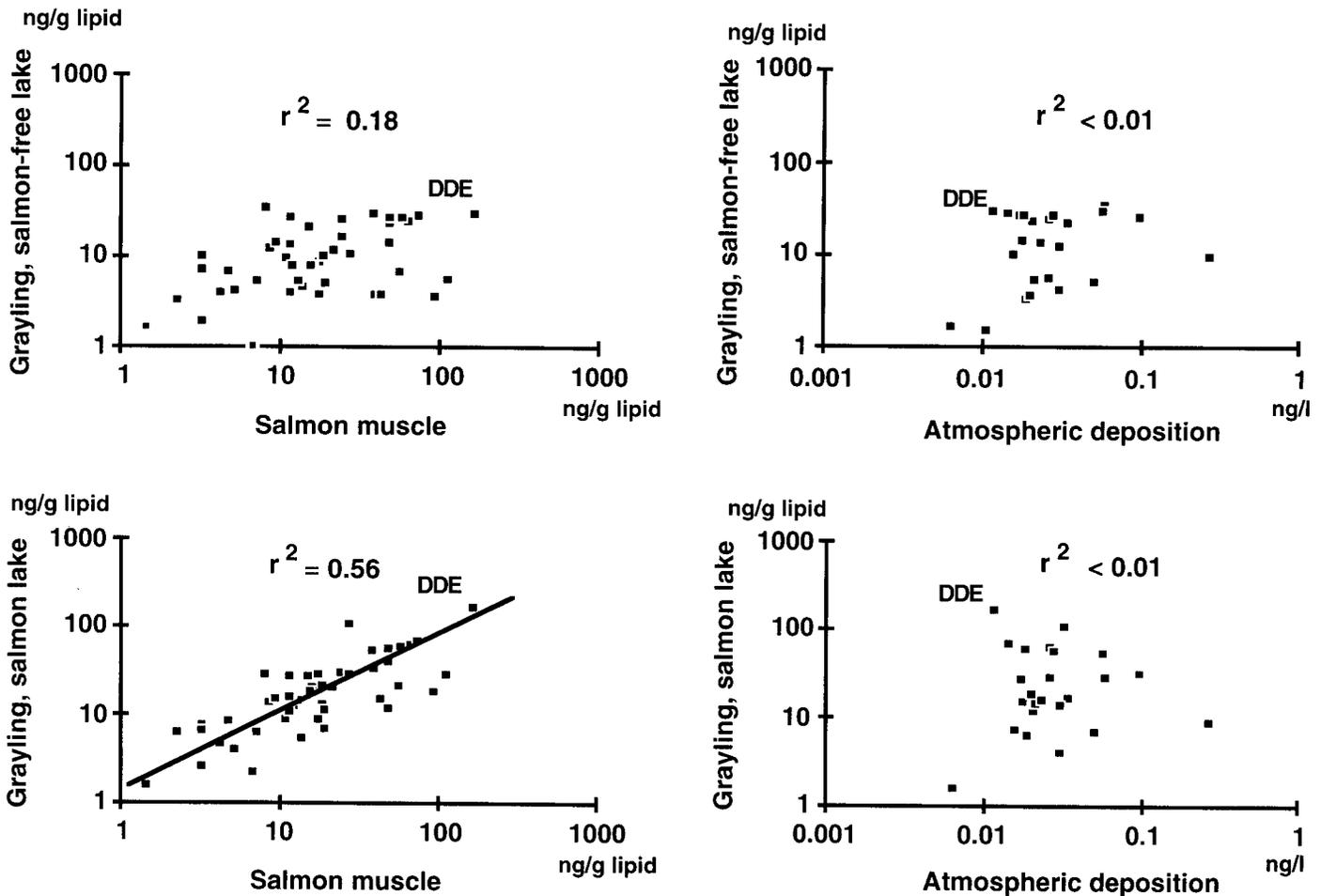


FIG. 4. Regression analysis comparing pollutant distribution in atmospheric and salmon muscle sources to pollutant distribution in grayling from the two lakes, using the concentration of each individual pollutant in source and receiver as an x/y pair. Each square represents an individual pollutant. A complete match between the composition in the source (x-axis) and that in the potential receiver (y-axis) would result in a regression coefficient (r^2) of 1. Total dissimilarity, in contrast, would give a regression coefficient of zero. DDE is indicated in the different plots as a reference point.

cell membranes, which are thus affected less than they would otherwise be. Nevertheless, the triacylglycerols can sooner or later become depleted, as during starvation, hibernation, or—as in this study—migration.

The initial result of a depletion of the triacylglycerol deposits is an increase in lipid pollutant concentration due to the decrease in the solving volume (Findlay and DeFreitas, 1971; Addison, 1982; Henriksen et al., 1996). This higher concentration results in turn in a redistribution via the blood to other tissues, such as nerve tissue, in which pollutant concentrations may increase and give rise to toxic effects (Ecobichon and Saschenbrecker, 1969). During river ascent, the salmon use their muscle lipid deposits for energy and gonad development. In the females, the organic pollutants redistribute, accumulating in the lipid-rich gonads. Our results indicate that the lipid depletion during river ascent is not coupled to a simultaneous elimination of the organic pollutants; most of the pollutants remain in the body. Further, the pollutant concentration in lipids in the gonads increases as a response to the overall lipid depletion in the salmon. The gonads receive pollutants transferred from muscle tissue, which implies that the salmon roe, an important food source for grayling in the spawning lake (Skopets and Prokopyev, 1990), contain pollutants similar to those in the salmon carcasses.

The transfer of pollutants from muscle tissue to gonads prior to spawning may negatively affect roe hatching and fry survival. Recent studies suggest that both the decrease in spawning success of Atlantic salmon in the Baltic Sea and the decline in lake trout reproduction in the Great Lakes are related to vitamin B₁ (thiamine) deficiency, which in turn has a possible, though yet unproved, connection with persistent pollutants (Bengtsson et al., 1996; Fisher et al., 1996). Furthermore, the processes affecting reproduction are probably not restricted to fish, but are general for animals that migrate for long distances. Also, the transfer of pollutants to roe is a process similar to that of pollutant transfer to milk in lactating mammals (Gallenberg and Vodcicnik, 1987). However, the salmon in this study have concentrations of pollutants far below the levels that have caused concern with regard to human consumption or fish reproduction: pollutant levels are approximately 10 times lower than those found in salmon (*Salmo salar*) in the Baltic Sea (Larsson et al., 1996) and more than 20 times lower than those reported for salmonids in Lake Ontario (Oliver and Niimi, 1988).

The energy expenditures of migrating sockeye salmon in the Fraser River have been thoroughly described elsewhere (Idler and Clemens, 1959; Idler and Bitners, 1960), and our results on lipid depletion and gonad growth are in line with results of those investigations. The relatively high variation in salmon lipid content in the lower part of the river may be due to the fact that salmon sampled there belonged to different stocks with different spawning sites along the river, and thus were at varying stages of maturity.

Clear and statistically determinable similarities of the compositions of pollutants in salmon and grayling in the salmon lake support our hypothesis that the salmon have

strong influence on pollutant levels in their spawning lake. The fact that a weak relationship was obtained between the pollutant composition in the salmon and that in the grayling in the salmon-free lake can be seen as reflecting a selective process of bioaccumulation and transfer of pollutants in the food web. Pollutants appear to accumulate in proportion to their chemical and biological persistence and their lipophilicity, resulting in similarities in pollutant composition in species found at the same trophic level in the ecosystem, even if the pollutant sources differ (Oliver and Niimi, 1988). Our results show that the quality of the persistent pollutants differs between predators that inhabit aquatic environments in which there is biotransport and those inhabiting environments in which there is atmospheric transport alone.

The nature of the two organic pollutant “vectors,” atmospheric transport and biotransport, differs in two important respects in addition to that of the amounts of pollutants they introduce into the lake ecosystem. First, the pollutants in the salmon are more readily available for bioaccumulation. The migrating salmon, the salmon roe, and the carcasses are fed upon directly by such predators as bald eagles, bears, and grayling, allowing the pollutants to be transferred to biota in a direct and efficient way. In contrast, the atmospherically deposited pollutants are subjected to various abiotic processes, such as adsorption to suspended matter and sediments, prior to possible bioaccumulation.

Secondly, biotransport provides a long-range transport route for pollutants not chemically persistent or volatile enough to permit atmospheric transport. The significantly higher concentrations of chlorinated fatty acids found in lipids of the grayling in the salmon lake (compared to levels in the grayling of the salmon-free lake) clearly support this (Mu, 1996). Since chlorinated fatty acids are not volatile, they are easily degraded outside an organism by various oxidation processes such as UV-radiation. Even if some chlorinated fatty acids found in fish may have a natural origin, they are most probably a direct or indirect result of anthropogenic activities. Salmon are a very likely source of the chlorinated lipids found in grayling in the salmon spawning lake, as such lipids may be transferred within the food web via prey (Ewald et al., 1996), but do not have the chemical persistence that would allow them to be transported in the atmosphere. Similarly, it can be assumed that other, as yet unknown, organic pollutants that would otherwise be degraded by abiotic processes are brought into pristine areas by biotransport.

The geographic distribution of Pacific salmon extends from San Francisco Bay, in California, northward along the Canadian and Alaskan coasts to rivers draining into the Arctic Ocean, and southward down the Asian coastal areas of Russia, Japan, and Korea (Groot and Margolis, 1991). Most probably, all seven species of Pacific salmon are “biotransporters” of pollutants from the Pacific Ocean to their spawning sites in freshwater. The extent of this process is determined by pollutant exposure in their foraging areas, food choice, and the structure of the ecosystem at the

spawning sites. How the process of biotransport contributes to the inland ecosystems on a larger scale is yet to be determined.

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