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ACCUMULATION OF METALS AND ORGANOCHLORINES IN TISSUES OF THE OYSTER *CRASSOSTREA ANGULATA* FROM THE SADO ESTUARY, PORTUGAL

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ABSTRACT

Sixty-eight composite samples of selected tissues (mantle, gills, adductor muscle and other soft parts) of *Crassostrea angulata* oysters collected in the upper Sado Estuary from May 1985 to May 1987 were analyzed for Fe, Mn, Zn, Cu, Cd, Pb, Ni, Cr, Co, PCB and total DDT. Principal component analysis demonstrated that Cd, Ni, Zn, Cu and Mn were more closely associated with mantle and gills, and their accumulation seemed to be highly influenced by oyster metabolic alterations. In contrast, partitioning of Pb and Cr in *C. angulata* tissues was largely related to environmental changes. As both biological and environmental factors tend to have major influences at the same period on the accumulation of PCB and DDT, the major factor defining their temporal partition in oysters from this upper estuary could not be clearly discerned. PCB and DDT were principally accumulated in the mantle and visceral mass.

INTRODUCTION

The ability of oysters to accumulate high concentrations of metals and organochlorines in soft tissues has been extensively documented (Establier and Gutierrez, 1970; Romeril, 1971; Establier, 1972; Lowe et al., 1972; Boyden and Romeril, 1974; Establier and Pascual, 1974; Pascual and Establier, 1975; Watling and Watling, 1976; Phillips, 1979, 1980; Eisler, 1981; Farrington, 1983).

Major factors known to control temporal fluctuations of metals and organochlorine concentrations in bivalves are: size (Boyden, 1977), weight (Cain and Luoma, 1986), lipid and metabolic changes associated with the reproductive cycle (Phillips, 1980), source input, the temporal variation of which may lead to aperiodic fluctuations (Boyden and Romeril, 1974; Farrington, 1983; Luoma and Phillips, 1988) and environmental changes, among which salinity variations may affect metal availability (Phelps et al., 1985).

The oyster *Crassostrea angulata* is native to Portugal and its occurrence is mainly restricted to intertidal areas of the upper and middle Sado Estuary. High concentrations of Cd, Zn and Cu occur in whole soft parts of *C. angulata* from the Sado Estuary (Assis and Nunes, 1972; Vale and Cortesão, 1988). Relatively high accumulation of PCB and total DDT also occur in this oyster species in the Sado Estuary (Barros, 1979). Both metal and organochlorine concentrations in whole soft parts have shown a well-defined seasonal fluctua-

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tion (Vale and Cortesão, 1988; Castro et al., 1988). Such high levels and their variations have been mainly attributed to both source inputs and biological factors. The River Sado exhibits a pronounced dry season/wet season division, which results in a considerable winter salinity decrease in the upper estuary, from ~ 25 to < 5‰ (Ferreira et al., 1989). The River Sado drains an active pyrite mining area, resulting in an important and variable fluvial input of several metals to the estuary (Vale and Cortesão, 1989). Other potential sources of pollutants, namely PCB, are located in the lower estuary, which is surrounded by a large industrial area.

Thus, metals and organochlorines in *C. angulata* in this estuarine system may be affected by a number of interacting chemical, biological, hydrological and anthropogenic factors. In order to understand the relative importance of the factors which, under natural conditions, may play major roles in the accumulation of metals and organochlorines, selected tissues of *C. angulata* were collected in different seasons and analyzed for Fe, Mn, Zn, Cu, Cd, Co, Ni, Pb, Cr, PCB and DDT. Principal component analysis was used to interpret the results.

SAMPLING METHODS

From May 1985 to May 1987, 17 oyster samples (6 cm average length) were collected from four natural oyster banks (Stations 1, 2, 3 and 4) of the upper Sado Estuary (Fig. 1). Approximately 25 individuals (coefficient of variation of length < 20%) were collected at each station and composite samples of dissected tissues, i.e. mantle, gills, adductor muscle and remaining soft parts (visceral mass) were homogenized, freeze-dried and analyzed. Oysters were not subjected to depuration of gut contents, to avoid metal and organochlorine redistribution by the dissected organs (Martincic et al., 1986).

Concentrations of Fe, Mn, Zn, Cu, Cd, Pb, Ni, Cr and Co were determined by either atomic absorption spectrophotometry or electrothermal atomic absorption using Perkin Elmer 400 and 4000 spectrophotometers and a P-E HGA 500 carbon furnace. Approximately 200 mg of dry material was digested in a Teflon bomb with nitric acid/hydrogen peroxide. The accuracy of the determination procedure was assessed by the analysis of a standard reference material (NBS Bovine Liver). Blanks and the concurrent analysis of the standard reference material were used to detect possible contamination during analysis. For organochlorine compound (PCB and total *p,p'*-DDT) determinations, freeze-dried samples were extracted with n-hexane for 6 h in a Soxhlet apparatus and the extract purified according to Faubert-Maunder et al. (1964) methodology. Alkaline hydrolysis of the extracts was performed to allow the quantification of PCB, which was carried out using Aroclor 1260 as standard. Organochlorine residues were determined using a gas-chromatograph (Perkin Elmer F-17) equipped with a ^{63}Ni electron capture detector and packed columns under the operational conditions described elsewhere (Vale et al., 1985). Both metal and organochlorine results are expressed on a dry weight basis.

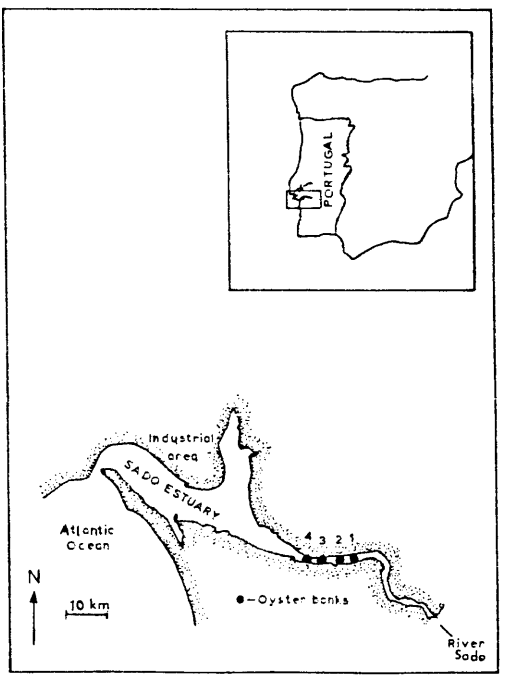

Fig. 1. Upper Sado Estuary showing the locations of the four sampling stations (●).

Principal component analysis was used to describe the variability of the results (Arfi et al., 1983). In this statistical method, each sample corresponds to a point in multi-dimensional space and the distance between points is proportional to the chemical composition dissimilarities of the corresponding samples. Multivariate vectors are viewed through two-dimensional space (Estrada, 1975).

RESULTS

The ranges of metal and organochlorine concentrations in the soft tissues of the oyster *C. angulata* collected from four oyster banks on the upper Sado Estuary are shown in Table 1. Of the analyzed metals, zinc was most abundant, reaching concentrations $> 30\,mg\ g^{-1}$ in some tissues. Copper was the second most abundant metal in oyster tissues and its concentration attained $3\,mg\,g^{-1}$.

TABLE 1

Range of metals (Fe, Mn, Zn, Cu, Cd, Pb, Ni, Cr and Co), PCB and Σ DDT concentrations in soft parts (mantle, gills, adductor muscle and remaining soft tissues) of *C. angulata*; results expressed on a dry weight basis

Tissue	Fe ($\mu g\,g^{-1}$)	Mn ($\mu g\,g^{-1}$)	Zn ($mg\,g^{-1}$)	Cu ($mg\,g^{-1}$)	Cd ($\mu g\,g^{-1}$)	Pb ($\mu g\,g^{-1}$)	Ni ($\mu g\,g^{-1}$)	Cr ($\mu g\,g^{-1}$)	Co ($\mu g\,g^{-1}$)	PCBs ($ng\,g^{-1}$)	∑DDT ($ng\,g^{-1}$)
Mantle	345–1186	34–79	9.5–35.0	1.39–2.65	4.6–40.0	0.8–4.5	0.8–3.6	1.0–3.3	0.4–2.5	81–1119	10–80
Gills	308–690	35–169	13.5–37.4	1.79–3.11	6.1–29.3	0.4–2.1	1.1–6.0	0.3–2.3	0.4–2.4	33–526	6–97
Remaining soft tissues	170–652	15–56	8.3–27.0	1.02–1.89	4.6–25.9	0.2–1.8	0.2–3.3	0.2–3.7	0.4–1.5	52–1642	11–92
Muscle	57–345	5–26	4.0–13.5	0.15–0.76	4.6–15.2	< 0.1–6.1	0.4–2.2	0.2–3.3	0.1–1.1	44–253	4–16

Iron and manganese concentrations ranged up to 1186 and 169 $\mu g\,g^{-1}$, respectively. Cadmium concentration in oyster tissues reached 40 $\mu g\,g^{-1}$. Concentrations of Pb, Ni, Cr and Co remained $\lesssim 6\,\mu g\,g^{-1}$. Residues of total DDT and PCB were < 100 and $< 1700\,ng\,g^{-1}$, respectively.

Principal component analysis (PCA) was applied to the results for each dissected tissue separately, and to those for all data sets combined. The variance explained by the first two components (E1 and E2) for each tissue (mantle, gills, adductor muscle, remaining soft tissues and all tissues combined) is presented in Table 2. For analyzed tissues, except adductor muscle, the first two components explain > 60% of the total variance, therefore only these two axes will be considered. The projections of the paired individual and global tissues data, in planes defined by the two principal axes, are shown in Figs 2 and 3. In these figures, lines are drawn to include points determined from several surveys corresponding to similar distributions. Parameters most correlated ($p < 0.001$) were linked by segments.

Individual analyses of dissected tissues

The results of PCA analysis for each dissected tissue are presented in Fig. 2. Based on best correlation coefficients ($p < 0.001$), aggregation of parameters into distinct groups and their preferential association with seasonal periods was obtained for mantle, gills, adductor muscle and remaining soft tissues.

Figure 2A displays the results obtained for mantle tissues in a plane defined by the two principal coordinates. Parameters may be collected into three groups: one containing Zn, Cu and Cd; a second Cr, Pb, Co, Mn and Fe; and a third PCB and DDT. Metals of Group I are closely associated with samples collected in May and June 1985, March 1986 and May 1987, while organochlorines and elements of the second group are associated with January, March and April 1987 samples.

Results of PCA of the data for gills are plotted in Fig. 2B. Axis E1 shows the presence of two distinct groups, one formed by Cd, Ni, Zn, Mn, Cu and Co and the other by Pb, Cr, PCB and DDT. Unlike those observed for mantle tissues, a better defined association of Mn with Zn and of Co with Cu is seen. Though Pb and Cr in gill samples remain correlated, PCB, DDT and Fe display a poor

TABLE 2

Percent variance explained by the first two components (E1 and E2) and accumulated (E1 + E2) for each tissue

Analyzed tissues	E1	E2	E1 + E2
Mantle	37	30	67
Gills	39	24	63
Adductor muscle	37	17	54
Remaining soft tissues	35	26	61
All tissues	44	22	66

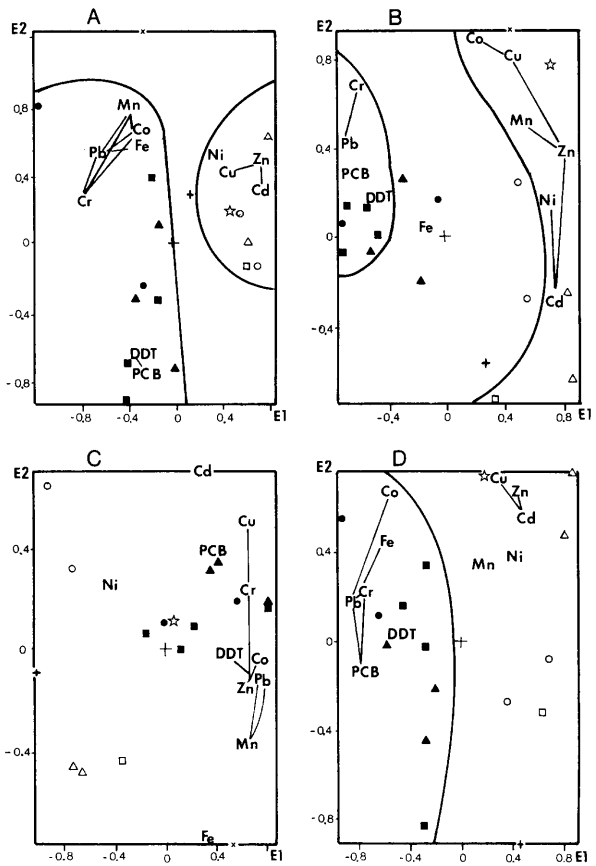

Fig. 2. Distribution of variables in the space delimited by principal components E1 and E2. (A) mantle, (B) gills, (C) adductor muscle, (D) remaining soft tissues. Samples were collected in May (□), June (△) and October (+) 1985; March (○) and September (x) 1986; and January (●), March (■), April (▲) and May (*) 1987.

correlation to each other as well as to Pb and Cr. The first group of metals plots close to points corresponding to samples collected in June 1985 and May 1987, while PCB and DDT are closely associated with samples from January, March and April 1987.

Results for adductor muscle are plotted in Fig. 2C. A group consisting of Mn, Pb, Co, Zn, DDT, Cr, and Cu can be discerned. In this tissue, Cr is best correlated ($p < 0.001$) with Zn and Cu, while Pb is correlated with Mn, Co and DDT. Poor correlations of PCB, Ni and Cd with other components were found. Samples collected in March and May 1986 and June 1985 are located in the negative region of E1, while components are located in the positive region. Chromium, PCB and Cu are more closely associated with samples of 1987.

Results for the remaining soft tissues are plotted in Fig. 2D. Parameters may be separated into two groups: one including Cd, Zn and Cu, and another formed by Pb, Cr, PCB, Fe, Co and DDT. Poorer correlations of DDT with PCB ($p < 0.05$) and of Mn and Ni to the first group of metals were obtained. Cadmium, Zn and Cu showed a closer association with samples collected in June 1985, while parameters of the second group remain more closely associated with samples collected in January, March and April 1987.

Analysis of global results

Principal component analysis was applied to all metal and organochlorine concentrations from all tissues, stands and seasons.

Figure 3A shows segments linking parameters most correlated ($p < 0.001$). Two major groups may again be defined: one containing Cd, Ni, Zn, Cu and Mn, with correlations between all of them, and another embracing PCB, Cr, DDT and Pb for which fewer cross-correlations were obtained. Iron and Co are located in an intermediate position with correlations with several elements of both groups.

Figure 3B presents parameters and samples with the identification of the analyzed oyster tissue. Along E1, a sequential distribution of the tissues, adductor muscle, visceral mass, gills and mantle, can be clearly discerned. As most metals are located in the positive region of E1, an increasing association of metals from adductor muscle to mantle and gills can be seen. Though PCB, DDT and Cr are included inside the mantle area, its location close to the remaining soft tissues suggests an indistinct association of those parameters with both tissues.

In Fig. 3C, the same points, but with identification of the sampling periods, are shown. Samples collected in winter and spring 1987 are located in the positive region of E2 and, in contrast, samples collected in spring and early summer of 1985/86 and May 1987 are located in the negative region of E2. So, as parameters are distributed along E2, samples collected in later sampling periods (May/June 1985, March 1986 and May 1987) are more closely associated with the first group of metals (Cd, Zn, Ni, Cu and Mn), while samples obtained in earlier sampling periods (January/March/April 1987) show a preferential association with the second group (Pb, Cr, PCB and DDT).

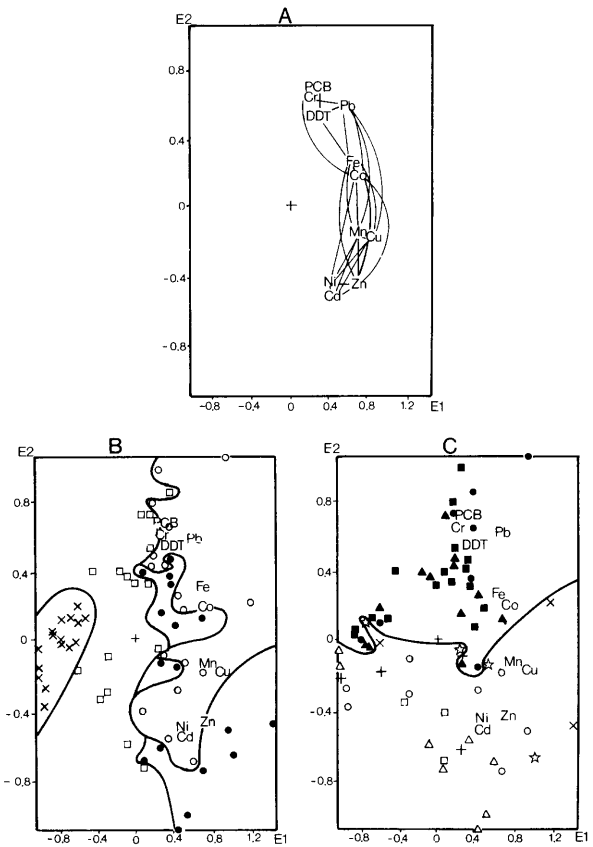

Fig. 3. Factorial plane E1–E2 of principal component analysis. Representation of all tissues and parameters. (A) Parameters best correlated. (B) Identification of the analyzed tissue, mantle (○), gills (●), adductor muscle (×) and remaining soft tissues (□). (C) Evidence for temporal separation of the samples. Samples were collected in May (□), June (△) and October (+) 1985; March (○) and September (×) 1986; and January (●), March (■), April (▲) and May (*) 1987.

DISCUSSION

The sampling schedule followed in this study enabled us to obtain complex information concerning metal and organochlorine concentrations in tissues of oysters collected in a highly dynamic upper estuarine zone in different seasons.

Principal component analysis applied to the results provided a multivariate concept of all the information and large-scale variabilities. That is: (i) the relative bioaccumulation capacity of the mantle, gills, remaining soft tissues and adductor muscle; (ii) the separation of parameters into two distinct bioaccumulation behaviour groups, one embracing Zn, Cu, Cd and Ni, and the other Pb, Cr, PCB and DDT, for all tissues except adductor muscle.

Results obtained in this study indicate that metals are highly associated with the mantle and gills and poorly associated with the adductor muscle. An intermediate association was found for the remaining soft tissues. Such accumulation heterogenity has been observed in other oyster species (Brooks and Rumsby, 1967; Cunningham and Tripp, 1975; Zaroogian, 1980) and the ability of mantle and gills to concentrate heavy metals has been attributed to their mucus and large surface area (Korringa, 1952; Romeril, 1971; Martincic et al., 1986). For organochlorine compounds a slightly different pattern was obtained, as both mantle and remaining soft tissues contained high concentrations of residues. Due to the high lipid solubility of organochlorines (Marchand et al., 1976), the gonads and digestive gland are often important storage sites for these compounds (Butler, 1966; Gutierrez-Galindo et al., 1984). The accumulation of organochlorines in the mantle and remaining soft tissues of *C. angulata* may also be explained by lipid content, since both tissues showed approximately the same lipid seasonal variations (ranging from 2.8 to 11.2% and from 3.3 to 17.5%, respectively).

Clear separation of samples collected in winter from those collected in later periods (Figs 2 and 3C) indicates a distinct seasonal variation in accumulation. However, samples collected in March 1986 and March 1987 were plotted in two distinctly different areas. This suggests that important inter-annual variations (hydrological, reproductive cycle) may also occur. Examples of such time-scale fluctuations have been found elsewhere, for example San Francisco Bay (Luoma et al., 1985).

Seasonal variations of Zn, Cu and Cd in oyster whole soft tissues were found to be greatly influenced by body weight changes associated with the sexual cycle (Boyden and Phillips, 1981). Frazier (1975) and Martincic et al. (1984) suggested that Zn, Cu and Cd in oysters are closely related to gonadal development and spawning. In *C. angulata* from the Sado Estuary, gametogenesis/spawning and concurrent body weight changes are known to take place usually in the spring/early summer period (Vale and Cortesão, 1988). Large amounts of Mn are known to occur during maximum shell growth (Frazier, 1975) and high Mn values were found in ovary and gills (Korringa, 1952; Brooks and Rumsby, 1967; Pascual and Establier, 1974). Galtsoff (1964) and Frazier (1975, 1976) also found higher contents of manganese, zinc, copper and cadmium in oysters during the warm summer months when shell formation is greatest. In our study, the closer association of elements of Group I (Zn, Cu, Cd, Ni and Mn) with spring/early summer samples is in agreement with those findings and suggest that the concentration of these elements in *C. angulata* tissues seems to be influenced by the reproductive cycle of the oyster.

Other processes have to be considered for the other elements. Wong et al.

(1978) suggest that Pb (a Group II element) in oysters appears not to be absorbed through any apparent physiological demand process, but randomly incorporated and subject to the amount of bioavailable lead. Association of Pb with samples obtained in winter/early spring may thus result from the retention of particles forming the turbidity zone which became enriched in Pb (increase from 40 to 80 $\mu g\, g^{-1}$) during the rainy season (C. Vale, unpublished data). The accumulation of Cr in *C. virginica* is known to occur more rapidly by direct absorption (Preston, 1971), and in marine molluscs its concentration increases at low salinity (Zaroogian and Johnson, 1983; Eisler, 1986). The close association of Cr with samples collected in winter (a period in which salinity decreases from $\sim$25 to 5%) suggests also the importance of environmental factors on Cr accumulation by *C. angulata* in the upper Sado Estuary. Particles, which might have been undigested by the oysters, also displayed an increase in Cr in winter (from 300 to 500 $\mu g\, g^{-1}$). Elements of Group I and II showed contrasting temporal accumulation patterns in mantle, gills and remaining soft tissues. This overall pattern is in agreement with the findings for such metals referred to in the literature.

More than 90% of the annual riverine input of metals takes place during run-off and changes in metal distributions have been recorded in the upper estuary (Vale and Cortesão, 1989; C. Vale, unpublished data). In spite of the influence of hydrological conditions on metal cycling in the Sado upper estuary, fluctuations of Zn, Cu, Cd and Ni accumulation in oyster tissues seem to be mainly related to biological processes, and for Pb and Cr fluctuations appear to result principally from environmental changes.

A less clear-cut temporal pattern was found for Fe and Co, since the same seasonal association in the mantle, gills and remaining soft tissues was not evident. Windom and Smith (1972) found for *C. virginica* a difference between the uptake of Fe and other metals such as Cu and Zn and attributed the difference to the geochemical characteristics of the metals. This differential uptake of iron was also observed for most of the analyzed tissues of *C. angulata*. Boyden and Phillips (1981) observed for *C. gigas* that fluctuations in Co concentrations in the whole soft body were mainly determined by alterations in tissue weight, which is in agreement with the closer association of Co with the Group I elements in the remaining soft tissues of *C. angulata*.

Higher concentrations of PCB and DDT were found in samples collected in winter/early spring 1987 and, except for the adductor muscle, these compounds were only correlated with elements of the second group. However, such temporal association could not be interpreted as resulting only from environmental changes. Indeed, the lipid content of Sado oysters increases in late winter (Castro et al., 1988) and such biological changes could explain this temporal pattern. Principal component analysis applied to the data, in which organochlorines were expressed on a lipid weight basis, produced results similar to the tissue partition pattern obtained on a dry weight basis. This indicates that factors other than lipid content may influence the accumulation of organochlorines. Therefore, both biological and environmental changes may

explain the organochlorine accumulation pattern of oysters obtained for this upper estuarine area. The continued PCB–DDT association with winter samples, when expressed on a lipid basis, suggests the importance of run-off on the transport of these compounds to this area. Indeed, run-off causes a large increase of DDT and PCB in the upper estuary, although other factors such as size of the suspended particles also play an important role (Ferreira et al., 1989). Increase of organochlorine residues in bivalves due to enhanced levels in the environment resulting from run-off or accidental spills has also been observed (Barros, 1979; Phillips, 1980).

The grouping of parameters suggests, therefore, two different bioaccumulation behaviour patterns for mantle, gills and remaining soft tissues. Accumulation of Zn, Cu, Cd, Ni and Mn (Group I) by *C. angulata* in the Sado Estuary seems to be largely dependent on the different stages of the oyster reproductive cycle. Alternatively, Pb and Cr (Group II) incorporation in these oyster tissues suggests a more prominent role for changes in environmental conditions, such as salinity and metals' availability. The relative importance of such factors on the accumulation of PCB and DDT could not be discerned, as higher concentrations of residues in winter/early spring may be due to both an increase in tissue lipids and to run-off.

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