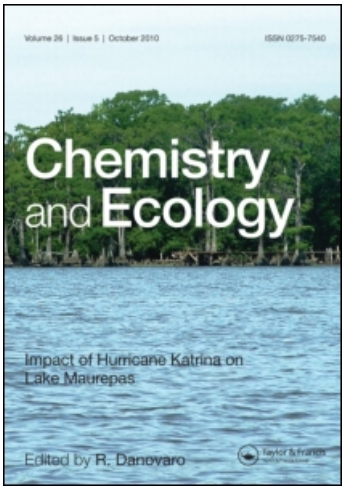

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CONCURRENT CHEMICAL AND HISTOLOGICAL ANALYSES: ARE THEY COMPATIBLE?

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Bivalves are often used as sentinel organisms in monitoring programmes for trace organic contaminants. The animal's physiological state may be important in interpreting trends in contaminant body burden. Simultaneous evaluation of physiological state and organic contaminant concentration in bivalves typically involves removal of a lipid-rich cross-section of the body mass for histopathological and/or gonadal analysis.

In this study, the bias introduced by this technique in the final trace organic concentrations, e.g. of polynuclear aromatic hydrocarbons, chlorinated pesticides and polychlorinated biphenyls, are evaluated on five different size groups of oysters. As a test case, we evaluated the use of this method in the NOAA's Status & Trends Mussel Watch (NS&T) Programme. The average biases introduced by this technique in the final trace organic concentrations in Gulf of Mexico oysters have been increasing since 1986 as a consequence of a continuous decrease in the size of the individuals sampled.

KEY WORDS Bivalves, oysters, trace organics, trends, Gulf of Mexico

INTRODUCTION

Seasonal variations in organic contaminant concentrations in bivalves have been attributed to a number of different factors including the stage of the reproductive cycle, nutritional status and ambient temperature (Wormell, 1979; Neff and Anderson, 1981; Jovanovich and Marion, 1987). Several studies have indicated the importance of considering the bivalve's physiological state when measuring contaminant loads (Fossato and Canzonier, 1976; Boehm and Quinn, 1977; Mix and Schaffer, 1979; Lunsford and Blem, 1982; Widdows *et al.*, 1982; Jovanovich and Marion, 1987). Monitoring programmes that use bivalves as sentinel organisms typically try to assess some of these problems. In NOAA's National Status and Trends Mussel Watch (NS&T) Programme, for example, reproductive state, condition index and disease incidence in oyster samples from the Gulf of Mexico have been monitored since 1986 (e.g. Craig *et al.*, 1989; Wilson *et al.*, submitted).

One aspect of the problem involves the determination of the reproductive state, which typically requires a histological analysis in most bivalves (e.g. Morales-Alamo and Mann, 1989). In some cases, where the seasonal variability in contaminant concentrations in bivalves was followed in relation to their reproductive cycle, the chemical and biological analyses were performed on two different groups of individuals collected at the same site (e.g. Jovanovich and Marion, 1987). Since the reproductive state of bivalves may vary considerably among individuals at certain times of the year (e.g. Wilson *et al.*, submitted), adequate comparison requires a large sample size and the approach necessarily restricts statistical analysis.

An alternative approach is to take a cross-section of tissue from the same individual that is used for trace organic analyses. Bivalves where the gonadal material is in the mantle, such as mussels (Bullogh, 1970), present only a minor problem; but oysters, where the gonadal tissue surrounds the visceral mass (Morales-Alamo and Mann, 1989), require removal of a tissue cross-section that may be rich in trace organic contaminants. Any additional histopathological analysis would, of course, require removal of a larger tissue cross-section in either species.

The objective of this study was to evaluate the bias in the final organic contaminant concentrations introduced by the selective removal of a tissue cross-section for histopathological or gonadal analysis. Five groups of oysters, *Crassostrea virginica,* of different average sizes were dissected and the portions normally used for histopathological and trace organic analysis were separately analyzed for selected polynuclear aromatic and chlorinated hydrocarbons to evaluate this bias.

MATERIALS AND METHODS

Oysters were collected from Galveston Bay, Texas, near the Houston Ship Channel in December 1988. This area is one of the 71 sites that was sampled during the NS&T Programme in the Gulf of Mexico (Sericano *et al.,* 1990). The site, Galveston Bay Ship Channel (GBSC), is located at the mouth of Goose Creek in Tabbs Bay. Immediately after collection, the oysters were transported to the laboratory and sorted into five different size groups. A cross-section of the body of the oysters was separated by first making a transverse cut where the palps and gills meet. A second parallel cut was made about 5 mm from the first cut toward the centre of the organism. This cross-section contained portions of gonad, stomach, intestine, digestive diverticula and connective tissue as well as mantle and gill. In standard practice, 3 to 5 mm sections are cut for histological analysis; consequently, the 5 mm cross-section would represent a maximum estimate of any bias incurred. The cross-section and remaining body tissues from oysters within each size group were pooled into two separated samples and analyzed for PAHs, chlorinated pesticides and PCBs. The methods used to measure the analyte concentrations were fully described elsewhere (e.g. Sericano *et al.,* 1990).

RESULTS AND DISCUSSION

Average lengths of the five different groups of oysters used in this study ranged from 6.1 to 9.5 cm (Table 1). Also shown are the mean percent contribution on a dry weight basis of the cross-section and remaining body tissues to the total body mass, and the percentage of extractable lipids corresponding to each of these fractions.

In general, the concentrations of PAHs, pesticides and PCBs measured in oysters are similar in each of the five size groups when the same subsamples, cross-section (A) or remaining body tissues (B), are compared (Table 2). In contrast, the trace organic concentrations of the two subsamples differ substantially in all five size classes. The cross-section (A) is the portion that normally would have been used for histological analysis. Since aromatic and chlorinated hydrocarbons are hydrophobic, they tend to be associated with lipid-rich tissues. This could in part explain the higher concentrations measured in the cross-section tissues which contain between 35 to 50% more extractable lipid than the remaining body tissues (Table 1).

Table 1 Average shell length and percent contribution of the cross-section and remaining body tissues to the total body weight corresponding to the five groups of oysters analyzed. Lipid percentages for each fraction are also indicated.

Oyster Size	*n*	*Shell Length (cm)*	*Cross-Section Tissues*		*Remaining Body Tissues*	
			Dry Weight (%)	*Lipids (%)*	*Dry Weight (%)*	*Lipids (%)*
I	8	9.5±0.8	15.6±2.7	14.2	84.4±2.7	9.6
II	8	9.3±0.9	17.0±1.9	13.3	83.0±1.9	9.0
III	8	8.5±1.4	18.5±3.0	12.7	81.5±3.0	8.9
IV	14	6.7±0.9	22.2±3.4	14.9	77.8±3.4	10.2
V	14	6.1±0.6	22.5±2.7	14.5	77.5±2.7	10.9

The removal of the tissue cross-sections from the sample analyzed for trace organic compounds will introduce a bias towards lower total concentrations in the sample. The magnitude of this bias will largely depend on the sizes of the oysters sampled. In this study, the tissue cross-sections accounted for about 15% of the tissue dry weight in a 9 cm oyster, but for nearly 23% in a 6 cm oyster (Table 1). Accordingly, in large oysters, a proportionally smaller fraction is used for biological assays whereas, in smaller oysters, a 5 mm cross-section represents the removal of a comparatively large fraction of the total body mass. In the extreme, the cross-section of a very small specimen may include all of the tissues, from where the palps and gills meet, to the adductor muscle which removes most of the lipid-rich internal organs.

The concentrations of PAHs, chlorinated pesticides and PCBs can be corrected for the contribution to the total body burden of the cross-section removed for histology. The differences between the uncorrected concentrations, which represent the values that would normally be reported, and corrected concentrations for each of the oyster groups are shown in Figure 1. As expected, the biases in the individual concentrations of trace organic compounds increase as the oyster sizes decrease. Average biases are 6.1±2.0, 8.8±2.4, 10.5±2.7, 14.0±2.6, and 14.3±2.4% for PAHs, 10.5±2.1, 11.7±0.7, 13.3±1.0, 13.9±0.8 and 16.8±1.5 for pesticides, and 6.3±0.9, 8.9±1.7, 10.9±1.2, 13.3±1.4 and 13.9±0.9 for PCBs in oyster groups I to V, respectively.

As an example for this study, we consider the NS&T programme in the Gulf of Mexico. In this programme, oysters are used as sentinel organisms to monitor the current status and long-term trends of selected organic and inorganic environmental contaminants along the Atlantic, Pacific and Gulf coasts of the United States. In 1986, the overall average oyster size collected for the Gulf of Mexico portion of the NOAA's S&T Programme was 8.5±1.4 cm (Brooks *et al.*, 1987) (Figure 2). During the following sampling years there was a continuous decrease in the sizes of the oysters that were sampled. In 1987, the average oyster size for the Gulf of Mexico was 7.6±1.8 cm (Brooks *et al.*, 1988); in 1988, the average size was 7.2±1.4 cm (Brooks *et al.*, 1989); and in 1989, it was 7.0±1.3 cm (Brooks *et al.*, 1990).

Wilson *et al.* (submitted) discuss the possible reasons for this decline in the sizes of the sampled oysters and concluded that the trend toward smaller sizes was probably a manifestation of decreased population health. For our purposes, this downward trend could introduce a bias in the trace organic values.

The bias imposed by the continuous decrease in oyster sizes with the successive sampling years in the observed PAH concentrations in the Gulf of Mexico can be estimated from the regression lines in Figure 1. Assuming that the cross-section was 5 mm in each case, the average percent biases in PAH concentrations that were

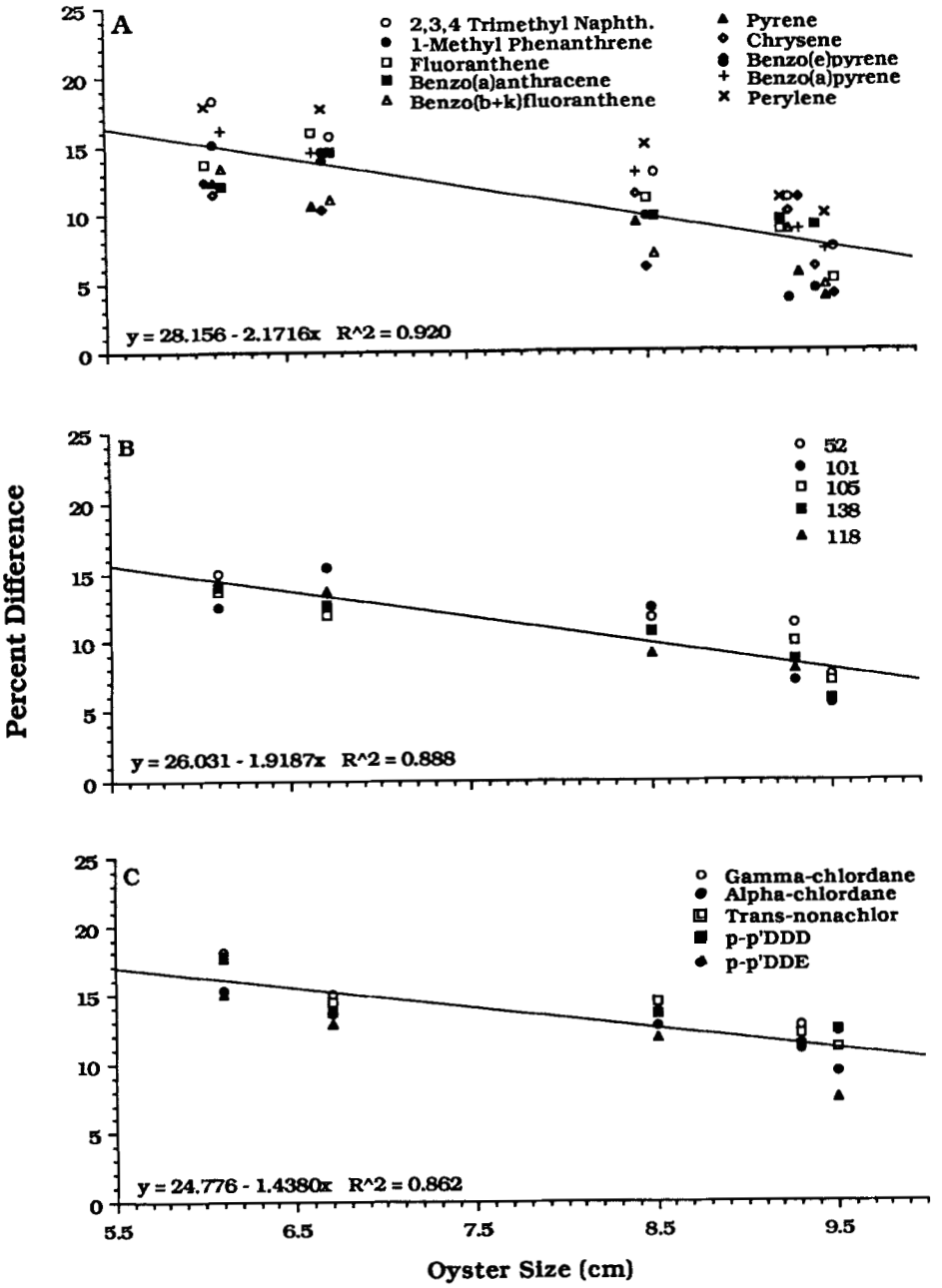

Figure 1 Percent differences between corrected and remaining body concentrations of polynuclear aromatic hydrocarbons (A), selected PCB congeners (B) and chlorinated pesticides (C) versus oyster size.

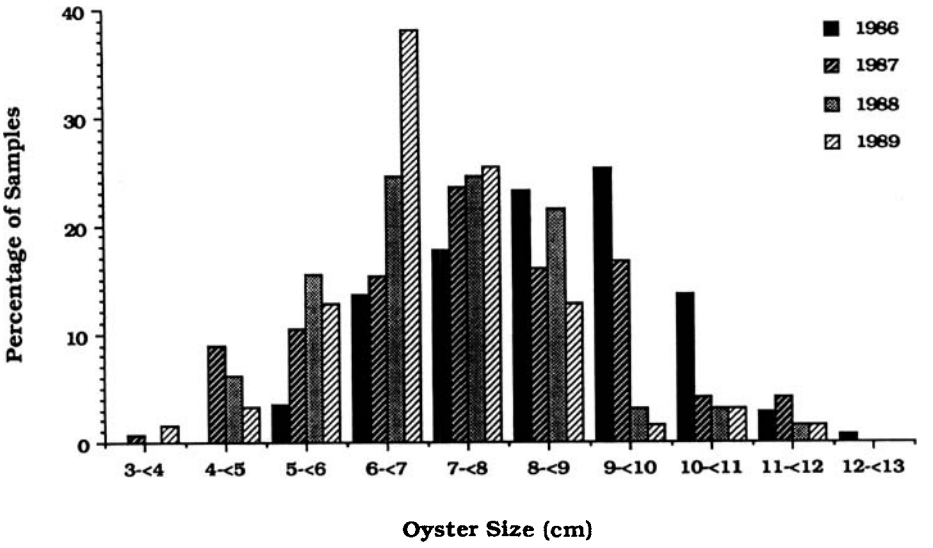

Figure 2 Size distributions of oysters sampled in the Gulf of Mexico during the NOAA's S&T Program between 1986 and 1989.

reported for 1986, 1987, 1988 and 1989 can be estimated as 9.7, 11.7, 12.5 and 13.0%, respectively. Similarly, average percent biases for chlorinated pesticides and PCBs can be estimated as 12.6, 13.8, 14.4, and 14.7% and 9.7, 11.4, 12.2 and 12.6%, respectively. However, under the protocol used for the NS&T programme, a cross-section of tissue is removed from only 10 of the 20 oysters collected per sampling station. Thus, the estimated bias for each group of analytes would be about half of these values.

In order to avoid misleading interpretations of comparative spatial and temporal data, it is imperative to understand how the methodology affects the trace organic concentration measurements in bivalves. This understanding is of particular importance if tissue cross sections are removed for histological analysis and it is especially important at sites where considerable variability exists in the sizes of the individuals sampled over the years and in cases where smaller organisms must be used. The development of non-histologically based gonadal indices (e.g. Choi *et al.*, 1989; 1990) offers one way to avoid this problem.

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Table 2 Cross-section and remaining body PAH, pesticide and PCB concentrations, ng g^{-1}, measured in the five different groups of oysters. Average concentrations for each analyte in the subsamples and percent differences are also listed.

Analyte	*Oyster size*										*Average*		*Change*
	I		*II*		*III*		*IV*		*V*				
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	Δ%
PAHs													
2,3,4 Trimethyl naphthalene	95.2	64.6	106	64.5	98.4	57.2	101	59.6	124	68.2	105±11.3	62.8±4.38	67
1 Methyl phenanthrene	111	86.3	112	91.8	123	80.7	104	63.3	158	93.0	121±21.5	83.0±12.1	46
Fluoranthene	615	462	676	446	626	392	686	402	766	474	674±60.0	435±36.0	55
Pyrene	1300	1030	1430	1070	1470	970	1440	976	1750	1130	1480±165	1040±67.1	42
Benz (a) anthracene	210	132	229	147	204	132	214	131	219	142	215±9.47	137±7.26	57
Chrysene	392	281	439	277	426	264	443	273	487	321	437±34.2	283±22.1	54
Benzo (b+k) fluoranthene	220	170	221	147	232	169	254	172	299	186	245±33.0	169±14.0	45
Benzo (e) pyrene	253	201	282	172	267	200	298	204	352	226	290±38.3	201±19.2	44
Benzo (a) pyrene	86.4	58.6	84.6	55.8	100	58.3	98.3	59.8	107	62.1	95.3±9.51	58.9±2.30	62
Perylene	140	85.5	155	94.8	160	89.6	173	96.0	182	101	162±16.3	93.4±5.99	73
Chlorinated Pesticides													
Gamma-chlordane	20.0	11.2	21.1	12.1	21.3	12.2	23.1	13.8	23.7	13.2	21.8±1.52	12.5±1.01	74
Alpha-chlordane	18.9	11.8	21.4	13.0	21.9	12.9	23.8	14.7	23.4	13.9	21.9±1.94	13.3±1.10	65
Trans-nonachlor	17.2	10.0	18.7	10.9	19.2	10.8	20.0	12.1	20.8	11.6	19.2±1.36	11.1±0.80	73
p-p'DDE	42.2	28.5	48.1	28.6	43.5	26.5	50.2	31.7	47.8	28.6	46.4±3.37	28.8±1.86	61
p-p'DDD	45.2	25.2	48.0	28.8	49.1	28.3	51.6	31.9	53.8	30.2	49.5±3.31	28.9±2.49	71
PCBs													
52	71.3	48.1	82.5	49.7	75.8	46.6	81.9	52.3	79.0	47.6	78.1±4.64	48.9±2.23	60
101	102	75.3	109	77.1	127	76.3	132	78.1	122	78.1	118±12.5	77.0±1.20	53
105	26.6	18.4	32.6	20.7	32.2	20.4	34.1	22.2	33.6	20.9	31.8±3.02	20.5±1.37	55
118	74.0	54.2	82.6	56.3	82.9	55.6	93.3	57.7	92.2	55.6	85.0±7.94	55.9±1.27	52
138	52.5	38.5	64.6	42.8	67.5	42.8	66.0	42.1	68.0	42.1	63.7±6.41	41.7±1.80	53

A = Cross-section Tissues

B = Remaining body Tissues

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