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# Relationship of Parasites and Pathologies to Contaminant Body Burden in Sentinel Bivalves: NOAA Status and Trends ‘Mussel Watch’ Program

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## Abstract

The 1995-1998 database from NOAA’s National Status and Trends ‘Mussel Watch’ Program was used to compare the distributional patterns of parasites and pathologies with contaminant body burdens. Principal components analysis (PCA) resolved five groups of contaminants in both mussels and oysters: one dominated by PAHs, one dominated by pesticides, and three dominated by metals. Metals produced a much more complex picture of spatial trends in body burden than did either the pesticides or PAHs. Contrasted to the relative simplicity of the

contaminant groupings, PCA exposed a suite of parasite/pathology groups with few similarities between the sentinel bivalve taxa. Thus, the relationship between parasites/pathologies and contaminants differs significantly between taxa despite the similarity in contaminant pattern. Moreover, the combined effects of many contaminants and parasites may be important, leading to complex biological-contaminant interactions with synergies both of biological and chemical origin.

Overall, correlations between parasites/pathologies and contaminants were more frequent with metals, frequent with pesticides, and less frequent with PAHs in mussels. In oysters, correlations with pesticides and metals were about equally frequent, but correlations with PAHs were still rare. In mytilids, correlations with metals predominated. Negative and positive correlations with metals occurred with about the same frequency in both taxa. The majority of correlations with pesticides were negative in oysters; not so for mytilids. Of the many significant correlations involving parasites, few involved single-celled eukaryotes or prokaryotes. The vast majority involved multi-cellular eukaryotes and nearly all of them either cestodes, trematode sporocysts, or trematode metacercariae. The few correlations for single-celled parasites all involved proliferating protozoa or protozoa reaching high body burdens through transmission. The tendency for the larger or more numerous parasites to be involved suggests that unequal sequestration of contaminants between host and parasite tissue is a potential mediator. An alternative is that contaminants differentially affect parasites and their hosts by varying host susceptibility or parasite survival.

### **Introduction**

Bivalve mollusks, such as mussels or oysters, are often used as sentinel organisms in contaminant monitoring programs (Phillips, 1977; Farrington et al., 1983; Green et al., 1989; Oliver and Fisher, 1999; Peterson, 2001). Bivalves are preferentially chosen as sentinel organisms for their ability to concentrate both metals and organic contaminants, their immobility, their limited ability to metabolize accumulated contaminants, their relatively wide distribution among habitats including those relatively heavily polluted, their abundance, their persistence, and their ease of collection, all of which make them good long-term integrators of the surrounding environment (Phillips, 1977; O'Connor, 2002).

The National Status and Trends ‘Mussel Watch’ Program has been monitoring contaminants of environmental concern since 1986 using bivalves as sentinel organisms (O’Connor, 2002). As Mussel Watch was designed to document the geographic distribution and temporal trends of contamination on a national scale, most sites were placed away from known point sources of contamination, poorly flushed industrial waterways, and pilings or metal buoys (O’Connor and Beliaeff, 1995; O’Connor, 1996). Sampling sites of an earlier Mussel Watch program (Goldberg et al., 1978, 1983) were, when possible, incorporated into the current Mussel Watch program (O’Connor, 1994).

Bivalves are presently collected every other year at a network of over 250 estuarine, lakeshore, and coastal locations along the U.S. Great Lakes, East, West, and Gulf coasts, with approximately half being visited during each annual sampling. Bivalve samples are analyzed for a variety of contaminants, including polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyl (PCB) congeners, and major and trace elements. The Mussel Watch program also monitors the health of bivalve populations through the measurement of a variety of biological indices, including the prevalence and infection intensity of parasites, diseases, and tissue pathologies. The database thus obtained provides an opportunity to examine the relationships between parasites and pathologies on the one hand and contaminants on the other over a large geographic scale. The question of the relationship of contaminant body burden to parasites and pathologies is a complex one. Extrapolating from laboratory and field experimentation comparing parasites/pathologies with contaminant body burden to inferences at geographic scales has been difficult, due to the multiplicity of contaminants and parasites present in most environments and the limited geographic scale of most present-day databases inclusive of both variables.

Certain contaminants may increase parasite body burden favoring the propagation of parasites by excluding their natural predators, by reducing the resistance of their hosts, or by providing improved living conditions for their intermediate hosts (Möller, 1987). Contaminants may also interfere with parasite transmission or proliferation within hosts and thus reduce parasite burden (Lafferty and Kuris, 1999). The interaction of parasite and pathology with contaminant body burden is known

primarily from controlled experiments. Barszcz et al. (1978) showed that host oysters exposed to crude oil had an increase in prevalence and intensity of infection by parasites, for example. Experimental studies by Pascoe and Cram (1977) and Boyce and Yamada (1977) showed that parasitized host fishes were more sensitive to Cd and Zn, respectively. Ciliate epibionts increased host mortality in copepods exposed to PAHs (Puckett and Carman, 2002). In contrast, Khan and Kiceniuk (1983) found reduced infections of endoparasites in host fish exposed to crude oil. Whether the influence of the contaminant is directly on the parasite or mediated through the host is often unclear. The immune systems of host animals can be affected by contaminants (Sindermann, 1979; Rohde 1993), lowering the resistance of a host to a variety of parasites and increasing susceptibility to parasitic invasion and disease (Barszcz et al., 1978; Galli et al., 2001). Thulin (1989) pointed out that ectoparasites may be particularly sensitive to contaminants because they are in intimate contact with the external environment. Such parasites may be useful indicator organisms for early detection of adverse environmental effects (Thulin, 1989; MacKenzie et al., 1995; Galli et al., 2001).

The cause and effect explanation is most often the inference from observed correlations between parasite body burden and contaminant body burden. More recently, however, research has begun documenting an alternative explanation; the differential in body burden between parasites and their hosts may accrue from differential affinities for contaminant accumulation. Zimmerman et al. (1999), Sures et al. (1999), and Heinonen et al. (2001) provide salient examples. Although this potential is not yet included in models of contaminant body burden (e.g., Fraysse et al., 2002; Lee et al., 2002; Yamamoto et al., 2003), this realization leaves the obvious quandary for sentinel bivalves of whether parasite body burden by their tissue contribution influences the measured value for the host organism as a whole. Parasites may contribute a significant fraction of host biomass (e.g., Powell et al., 1999); thus, differential affinities could be important. This possibility offers one explanation for the frequent finding that contaminant body burden is non-normally distributed among individuals at a site, such that a few animals have unusually high contaminant body burdens (Boyden and Phillips, 1981; Daskalakis, 1996; Baldwin and Maher, 1997). Parasites tend to be skewed similarly in their distribution among hosts (Munger et al., 1989; Jones et al., 1991).

The interaction between tissue pathology and contaminant body burden has also received some attention. Lowe et al. (1971) demonstrated that oysters exposed chronically to the pesticide DDT showed edema, lesions, and necrosis of the digestive tubule epithelium. Farley (1976) suggested the possible induction of epizootic neoplasia in bivalve molluscs by carcinogenic compounds, such as pesticides (aldrin and dieldrin). Couch et al. (1979) found that oysters exposed to the carcinogen benzo(*a*)pyrene showed inflammation of the connective tissue and proliferation of a cell resembling a sarcomatoid neoplasm. Wintermyer and Cooper (2003) and Svärth and Johannesson (2002) provide additional examples. The distribution of pathologies in the field, however, can rarely be ascribed unequivocally to individual contaminants (Sindermann, 1983; Johnson et al., 1992; Landsberg, 1996). The establishment of parasitism and disease in aquatic populations is usually not due to a simple cause-and-effect relationship, but is dependent upon a complex interaction between organism and environment.

Thus, the use of parasites or pathologies as indicators of pollution stress has rarely been unequivocal (Möller, 1987). One important constraint is the rarity of surveys that include both contaminant and health assessments. Yevich and Barszcz (1983) correlated stations with high parasitism with stations with high PCB concentrations in host bivalves collected from natural coastal and estuarine waters in the first Mussel Watch Program. Wilson et al. (1992) and Kim et al. (2001) examined the relationship of contaminant body burden to health indices at Gulf of Mexico Mussel Watch sites. Johnson et al. (1992) and Schmidt et al. (2003) obtained promising results relating fish pathologies to contaminant burdens along the northeast coast of the U.S. and in the North Sea, respectively. The Mussel Watch Program is a unique multi-year study and the first of its kind that covers a large geographic area, multiple parasite species, and multiple contaminants. Here, we used the Mussel Watch database for 1995-1998 to examine the relationships between parasitism, pathology, and contaminant body burdens on regional scales with emphasis on identifying parasite assemblages and contaminant suites that resolve as significant relationships within the complex medley of potential contaminant/parasite/pathology combinations. Such relationships can provide the basis for mapping the results of individual cause and effect experiments to integrated population level processes observed in the field.

## Materials and Methods

### ***Species Sampled and Sample Collection***

The bivalve samples were collected annually from a network of sites established along the United States coastline. Bivalves were dredged or hand-picked in intertidal to shallow subtidal areas. Sampling targeted the largest animals available at each site. Each sampled site subsequently was assigned to one of 126 bays [see Lauenstein et al. (1997) for further details of site location]. A ‘bay’, as termed herein, represents all the sites in a single estuary, estuarine reach of a large estuary, or group of neighboring sites on an open coastline. Details on the allocation of sites to bays can be found in the Appendix of Kim and Powell (2006).

The introduced zebra mussel, *Dreissena polymorpha*, and quagga mussel, *D. bugensis*, were sampled at sites in the Great Lakes (Bays 1-10) and Hudson River (Bay 11). Zebra mussels were first discovered in the Great Lakes in 1988 (Hebert et al., 1989). Quagga mussels were found in 1991 (May and Marsden, 1992). Both likely were introduced from Europe in ship ballast water (Hebert et al., 1989). Both species spread rapidly throughout the Great Lakes system and then into the major river basins of the central and eastern U.S. (Griffiths et al., 1991; Strayer, 1991; Mills et al., 1996). Recently, the quagga mussel has displaced the zebra mussel in many areas (Stoeckmann, 2003). Rosenberg and Ludyanskiy (1994) discussed the taxonomy. Mussel Watch sites include all Great Lakes except Lake Superior (Lauenstein et al., 1997).

Mytilid mussel taxa were collected from the Northeast (Bays 12-36) and West (Bays 94-125) coasts, including Alaska. According to Hilbish et al. (2000), mussels collected on the East coast were *Mytilus edulis sensu stricto*, as *M. edulis* is the predominant species from central Maine south (Rawson et al., 2001) to Cape Hatteras (Wells and Gray, 1960). On the West coast, three mussel taxa were collected, *M. californianus* and two species referable to the *M. edulis* complex, *M. galloprovincialis* and *M. trossulus*. The taxonomic status of species in the *M. edulis* complex is uncertain (His et al., 2000). *Mytilus californianus* was collected at 31 sites mostly located at jetties, points or capes among the 58 total West coast sites excluding Alaska (viz., 27 sites out of 36 in California, two sites among six in

Oregon and two sites among 16 in Washington). *Mytilus trossulus* was collected at the vast majority of the more northern stations from Oregon to Alaska. *Mytilus galloprovincialis* was introduced into the eastern Pacific in the 1880s and now occurs from central California to Baja California, with some populations probably farther north (McDonald and Koehn, 1988; Koehn, 1991; Seed, 1992), including a population in Puget Sound established at least by 1988 (Wonham, 2004). Thus, *M. galloprovincialis* was collected at some California sites and may have been present in some Puget Sound collections. In addition, some central and northern California sites yielded mussels that probably were hybrids of *M. galloprovincialis* and *M. trossulus* (Hilbish et al., 2000).

Four oyster taxa were sampled, *Crassostrea virginica*, *C. rhizophorae*, *C. gigas*, and *Dendostrea sandvichensis*. *Crassostrea virginica* was sampled from coastal and estuarine areas of the Mid-Atlantic and southeast coasts (Bays 37-61) and the Gulf of Mexico (Bays 63-93). *Crassostrea rhizophorae* was collected in Puerto Rico (Bay 62), and *C. gigas* and *D. sandvichensis* were collected in Hawaii (Bay 126). Considerable disagreement exists as to the taxonomic status of *C. virginica* and *C. rhizophorae* (Newball and Carriker, 1983; Ladrón de Guevara et al., 1996; LaPègue et al., 2002), whereas *C. gigas* and *D. sandvichensis* are clearly distinct from the other two. In total, however, *C. virginica* was collected at 55 of 57 bays where oysters were collected.

To simplify discussion when referring to groups of these taxa, the following inclusive terms will be used: dreissenid in reference to the combination of *D. bugensis* and *D. polymorpha*; oyster jointly for *C. virginica*, *C. rhizophorae*, *C. gigas*, and *D. sandvichensis*; mytilid for *M. edulis* on the East coast and the combination of *M. edulis*, *M. californianus*, *M. galloprovincialis*, and *M. trossulus* on the West coast.

Except in the Great Lakes, the sampling sites were visited annually during winter months with each site occupied within 30 days of an annual target date (O'Connor, 1994). The sampling was done in winter to minimize the influence of reproduction on contaminant body burden as spawning may influence contaminant body burden (Jovanovich and Marion, 1987; Ellis et al., 1993; Baldwin and Mayer, 1997). In the Great Lakes, dreissenid mussels were collected in late August through

September. Access to sampling sites in this area is difficult in winter as the Great Lakes are frequently frozen over during this period. As a consequence of sampling program design, analysis of the Mussel Watch dataset may be biased for those parasites that exhibit a strong seasonal cycle of infection intensity (e.g., Wallet and Lambert, 1986; Burreson and Ragone Calvo, 1996; Ford et al., 1999).

### ***Parasite/Pathology Quantification***

Tissue preparation followed the original NOAA Status and Trends protocols (Ellis et al., 1998a), as delineated by Kim and Powell (2006). Tissue samples were embedded in paraffin after dehydration and clearing, sectioned at 5  $\mu\text{m}$ , and the sections stained in a pentachrome series (Ellis et al., 1998a).

Major tissue types examined included gill, mantle, gonad and gonoducts, digestive gland tubules, stomach/digestive gland, and connective tissue. For oysters, *Perkinsus marinus* was assayed by the more precise thioglycollate method (Powell and Ellis, 1998). All other parasites and pathologies were scored microscopically for intensity based on either a quantitative or semiquantitative scale. Quantitative scores were used for parasites, pathologies, and selected morphological conditions, including prokaryotic inclusions (rickettsia, chlamydia, etc.), ciliates, gregarines, nematodes, cestodes and metacercariae of trematodes, that could be tallied individually, following procedures described by Ellis et al. (1998b) and Kim et al. (2006). Ciliates were quantified by tissue type (viz., gill and digestive tract), as were the gregarines (viz., body, gill, and mantle). Each nematode cross-section observed was counted, although a single individual may be responsible for a number of tissue cross-sections. Certain tissue pathologies and tissue components were also quantified by direct counts, including cases of hemocytic infiltration that were scored separately as focal and diffuse (Kim and Powell, 2004), granulocytomas (Lowe and Moore, 1979), and ceroid bodies or pigment cells (Mackin, 1951; Stein and Mackin, 1955).

Some parasites and morphological conditions, such as ramifying trematode sporocysts and the disease-producing protozoans, *P. marinus* and *Haplosporidium nelsoni*, were assigned to semiquantitative scales depending on the intensity or extensiveness of the affected area. Definitions of scale values can be found in Ellis et

al. (1998b) and Kim et al. (2006). A semiquantitative 0-to-4-point scale was used for ramifying trematode sporocysts of the families Fellodistomidae and Bucephalidae due to the extensiveness of infections and the difficulty in obtaining quantitative counts of the large branching sporocysts. Intensity of *P. marinus* infection was assigned using the semiquantitative 0-to-5-point scale of Mackin as modified by Craig et al. (1989). *Haplosporidium nelsoni* infection intensity was scored on a 0-to-4-point scale of Kim et al. (2006) adapted from Ford and Figueras (1988). For each specimen examined, the presence of neoplasms and unusual digestive tubules was recorded. Abnormal gonadal development, characterized by unusual development at the base of the follicles and by presence of foreign cells and cellular debris in the follicles, was given a semiquantitative 0-to-4-point score related to the spatial coverage of the condition (Kim et al., 2006). For digestive gland atrophy, a condition known to be caused by a variety of stressors most likely related to poor nutrition (Palmer, 1979; Winstead, 1995; Kim and Powell, 2004), the average degree of thinning of the digestive tubule walls was assigned a numerical rating on a 0-to-4-point scale (Kim et al., 2006).

### ***Contaminant Analysis***

The contaminant analyses were carried out on pooled samples of twenty bivalves from each site using methods developed for the National Status and Trends Program (Lauenstein and Cantillo, 1993). Only data which passed rigorous QA/QC tests using blanks, spikes, and reference materials were used. In addition, each analytical laboratory successfully completed yearly QA/QC programs involving analysis of blind samples supplied by the National Institute of Standards and Technology and the National Research Council of Canada (Cantillo, 1992). Detailed descriptions of analytical methods for contaminant analyses can be found in Lauenstein and Cantillo (1998).

Oysters were sampled from the Housatonic River (Bay 37) and Port Jefferson (Bay 38), New York, in only one of the four years (1998). Consequently, data from these collections were deleted from analysis.

### ***Statistical Analysis***

Data for 24 contaminant categories were used for data analysis: 11 trace metals

(Ag, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn); the five PAHs normally present in highest concentration (naphthalene, phenanthrene, fluoranthene, pyrene and chrysene) and the sum of 24 PAH compounds (total PAHs), as defined by Jackson et al. (1998); the sum of concentrations of DDT and its metabolites, DDE and DDD (total DDTs); four chlorinated pesticides other than DDT (aldrin, dieldrin, lindane and mirex); the sum of concentrations of all chlordanes (total chlordanes) [see Jackson et al. (1998) for constituents] and the sum of concentrations of 18 individual PCB congeners (total PCBs) [see Jackson et al. (1998) for constituents]. Bay mean values, determined using all measurements from all sites within each designated bay, were obtained geometrically for contaminants. Mean values of individual contaminants for each bay are given in Figures 1-8.

Data for 17 parasite/pathology groups were used for data analysis: rare parasites, prokaryotic inclusions, *Haplosporidium nelsoni*, *Perkinsus marinus*, gill ciliates, gut ciliates, body, mantle, and gill gregarines, nematodes, trematode sporocysts, trematode metacercariae, cestodes, major pathologies, tissue pathologies, ceroid bodies, and digestive gland atrophy. The sum of the counts of the rare parasites was obtained by excluding the common taxa (e.g. gregarines, ciliates, nematodes, cestodes and prokaryotic inclusions). The category of total prokaryotes included prokaryotic inclusions of chlamydia-like organisms and rickettsia-like organisms. Pathologies were grouped into two categories, major and tissue. Major pathologies included neoplasms, unusual digestive tubules, and gonadal abnormalities. Tissue pathologies included focal and diffuse hemocytic infiltration and granulocytomas as described in Villalba et al. (1997).

Parasite assemblages vary significantly between mussels, oysters, and dreissenids. In addition, mussels and oysters are characterized by inherently different contaminant affinities, at least for a number of metals (Rainbow, 1997; O'Connor, 1998; Valette-Silver et al., 1999; O'Connor, 2002). For these reasons, no between-taxon comparisons are presented.

The largest bivalves were chosen due to the larger tissue volume required for multiple chemical analyses and the increased likelihood of equilibrium between contaminant exposure and contaminant body burden (e.g., Lee et al., 1972; Roesijadi et al., 1984; Hinch and Green, 1989; Palmer et al., 1993; Abbe and Sanders, 1990).

These bivalves, being older, can also be presumed to have an increased incidence of some parasites and pathologies (e.g., Anderson and Gordon, 1982; Anderson and Crombie, 1984; Aguirre-Macedo and Kennedy, 1999; Curtis et al., 2000; Wilson-Ormond et al., 2000). Animal size and age vary between habitats and with latitude, as does the relationship of size with age (e.g., Hofmann et al., 1994; Weinberg, 1999). Some parasites have extended life spans; others do not (Powell et al., 1999; Curtis et al., 2000; Poulin and Morand, 2000), but the life spans of most bivalve parasites are very poorly known. The degree to which variation in the relationship of host size and age, as well as parasite transmission rate and life span, among Mussel Watch sites affects parasite and contaminant body burden cannot be evaluated; however, the focus on the largest animals can be expected to enhance the likelihood of identifying a relationship between parasite and contaminant.

Analysis of parasite/pathology occurrence began with the computation of prevalence and infection intensity. Prevalence, the fraction of individuals with the parasite or pathology, was calculated as:

$$\text{prevalence} = \frac{\text{number animals affected}}{\text{number animals analyzed}}.$$

Infection intensity, the average number of occurrences of a parasite or pathology in the affected individuals only, was calculated as:

$$\text{infection intensity} = \frac{\sum_{i=1}^n \text{number of occurrences of parasite or pathology}}{\text{number affected individuals}}.$$

For conditions rated using semiquantitative scales, the scale rating replaced the number of occurrences in this calculation. If a parasite or condition did not occur in a bay, infection intensity was undefined for that bay. A value of zero was not assigned.

Correlations were common among parasites for both variables (Kim and Powell, 2006) and occurred commonly as well among the contaminants (see also Ringwood et al., 1999; Green et al., 1983, 1993). Accordingly, to evaluate the potential relationships between parasites/pathologies and contaminant body burden in sentinel bivalves, principal components analysis (PCA) was used to reduce the many correlated variables in each dataset down to a few orthogonal variables for further analysis. Accomplishing this requires data points for each variable in each of the 126 bays. Infection intensity, unfortunately, is not defined for some variables in

some bays because prevalence was zero. A full set of values exists for prevalence, but this variable does not provide any information about infection intensity. To resolve this dilemma, we calculated weighted prevalence or mean abundance, a measure often used in the bivalve literature (e.g., Ford, 1988; Kim and Powell, 2004), that combines prevalence and infection intensity:

$$\text{weighted prevalence} = \text{prevalence} \times \text{infection intensity}.$$

Bay average prevalences and infection intensities are reported in Kim and Powell (in press).

Separate PCAs were conducted for each species using the weighted prevalence of 17 parasite/pathology categories and 24 contaminant categories. Each variable was standardized to  $\bar{x} = 0$  and  $s^2 = 1$  prior to using them in the PCA. Factor scores for factors having eigenvalues above 1.0 obtained from the weighted prevalence and contaminant body burden datasets were compared by ANOVA. The suite of individual variables with factor loads exceeding 0.5 for each significant contaminant factor  $\times$  weighted prevalence factor in ANOVA were then compared in *a posteriori* tests to resolve potential reasons for the significant relationships between factor scores.

The *a posteriori* comparisons were conducted in two ways. First, we tested the significance of correlations between variables with factor loading scores  $\geq 0.5$  for factors identified as significantly related by ANOVA using Spearman's Rank correlation tests. We confirmed that such correlations did not occur by chance using binomial tests (Conover, 1980). Second, double-zero pairs could not be removed from the PCA because all variables were simultaneously analyzed. These double-zero pairs are ambiguous. In some cases, double zeros occur simply because the bay was beyond the range of both parasites. In other cases, they occur if no affected animals were found for either condition in an area where each could occur. For this analysis, double-zero pairs introduced a possible bias because some relationships elucidated by PCA and *a posteriori* tests might exist due to the coincidence of low or zero values. Relationships of substance should be characterized by the co-occurrence of two high values more frequently, for positive correlations, or less frequently, for negative correlations, than expected by chance. We defined a 'high' value as a value exceeding the 75<sup>th</sup> percentile of all values for a variable. So, the

results of significant Spearman's Rank tests were examined further by tallying the number of observations for each biological and contaminant variable greater than the 75<sup>th</sup> percentile value of all observations of that variable. Then, the occurrence rate for cases where the two variables were simultaneously high was compared to that expected by chance using a binomial test.

## Results

Parasites and pathologies were rarely observed in dreissenid mussels, likely due to their recent introduction into the U.S. (Kim and Powell, in press, 2006). Oysters from Puerto Rico and Hawaii were different species and these sites were not readily assigned to geographic regions. As a consequence, dreissenids were not further analyzed and oyster analyses were limited to the East and Gulf coasts where *C. virginica* was sampled.

### ***Principal Components Analysis***

PCAs were run separately for mytilids and oysters. Factor axes for parasites/pathologies were determined by substantively different groups of parasites/pathologies in mytilids and oysters (Table 1). Similarities are remarkably few, and this is in keeping with the divergent geographic trends among the parasitic fauna, observed in these two taxa (Kim and Powell, in press). In mytilids, Factor 1 (P1) contained the three gregarines (Table 1). Kim and Powell (in press) suggested that mantle and body gregarines might be the same species, but the evidence supports a separation between these two and most gregarines occurring in the gill [see also Sprague and Orr (1952, 1955)]. The gregarines did not fall on the same factor axis in oysters. Factor 2 (P2) in mytilids was determined by the pathologies and trematode sporocysts. The rare parasites and prokaryotic inclusions determined Factor 3 (P3). Trematode metacercariae, digestive gland atrophy, and ceroid bodies determined Factor 4 (P4). Factor 5 (P5) was determined by the ciliates. Again, in oysters, as for the gregarines, the ciliates did not fall on the same axis as they did for the mytilids. For oysters, P1 was determined by gill gregarines and *P. marinus*. Prokaryotic inclusions and major pathologies determined P2. The remaining gregarines determined P3. P4 combined the tissue pathologies, ceroid bodies, and cestodes. Trematode sporocysts and gut ciliates fell on P5. Kim and

Powell (2006, in press) discuss the geographic relationships of these parasites and pathologies in more detail.

The contaminant PCAs were much more consistent between the two taxa (Table 1). Each contained a PAH-dominated factor (C1), a pesticide-dominated factor (C3), and three metal-dominated factors. Ag and Zn fell on the same PCA axis in both PCAs. Mirex and Pb fell on the same PCA axis in both PCAs. Cu and Hg did likewise, as did Zn, Ni, and Cd. Total chlordanes, for both species, contributed significant influence to both the PAH-dominated and the pesticide-dominated axis. Finally, the PAH-dominated axis was defined by 6 contaminants common to both taxa and the pesticide-dominated axis was defined by 4 contaminants common to both taxa (Table 1). For the mytilids, Cu, Hg and Pb combined with two pesticides on Factor axis 2 (C2). Cd, Cr, Fe, Ni, and Zn loaded on Factor axis 4 (C4). Ag, As and Zn defined Factor axis 5 (C5). In oysters, Ag, Cd, Cu, Ni and Zn loaded on C2; Cr, Cu and Hg on C4; and Fe, Pb, and Se, with mirex, on C5. Common to C1 in both PCAs were phenanthrene, fluoranthene, pyrene, chrysene, total chlordanes, and total PAHs. Total PCBs contributed to this axis in oysters. Common to C3 were total DDTs, aldrin, dieldrin, and total chlordanes. Lindane and total PCBs contributed to this axis in oysters. Presley et al. (2004) also observed correlations between Cu and Zn in oysters from Gulf of Mexico Mussel Watch sites, in agreement with their co-occurrence on Factor axis 2. The tendency for the metals to be distributed independently of the organics also conforms to earlier analyses of Mussel Watch data (Wilson et al., 1992; Kim et al., 2001). Interestingly, one common contaminant, naphthalene, did not fall on any of the five primary PCA axes in either taxon suggesting an inherent difference in the distribution of this contaminant in comparison to the others.

Significant relationships between contaminants and parasites/pathologies were examined by ANOVAs comparing PCA factors. A total of 25 tests, 5 parasite/pathology factors  $\times$  5 contaminant factors, were performed for each taxon. Six of these tests were significant for mytilids (Table 2), considerably more than anticipated by chance at the least significant significance level obtained (Table 2) (tested probability  $p$ , significance level  $P$ ;  $p = 0.015$ ,  $P < 0.0001$ ). Five were significant for oysters. Once again, this number is considerably more than anticipated

by chance ( $p = 0.029$ ,  $P < 0.0001$ ). We also differentiated the oysters on the East and Gulf coasts and the mytilids on the East and West coasts. For the oysters, at this geographic scale, 5 tests were significant in the Gulf and 2 on the East coast. The number of significant comparisons for Gulf oysters exceeded that expected by chance ( $p = 0.026$ ,  $P < 0.0001$ ). The number on the East coast did not. Thus, significant results overall were principally a function of the Gulf of Mexico portion of the oyster dataset. For the mytilids, 3 comparisons were significant on the East coast. This exceeded the number anticipated by chance ( $p = 0.033$ ,  $P < 0.01$ ). The frequency on the West coast, however, did not exceed the frequency expected by chance.

The PAH-dominated factor, C1, generated significant comparisons with parasite/pathology factors in both oysters and mussels. For oysters, the significant comparison was with P4 (tissue pathologies, cestodes, ceroid bodies). Interestingly, for mussels, the significant factor, P3, contained a very different parasite complement, rare parasites and prokaryotic inclusions. Thus, PAHs were associated with a number of pathologies and tissue components, ceroid bodies, thought to have detoxification abilities (Zarogian et al., 1989, 1993) in oysters, but not in mussels. The pesticide-dominated factor (C3) had significant comparisons also with P4 in oysters, but with no mytilid parasite/pathology factors.

Among the metals, a mytilid factor containing gill gregarines (P1) was significantly related to a factor containing the metals Ag and Zn (C5). Cu and Hg fell on the same axis on both mytilid and oyster PCAs. In mytilids, C2 that included Cu, Hg, Pb, and mirex demonstrated significant comparisons with P1 (gregarines) and P2 (pathologies and trematode sporocysts). The factor containing Cu and Hg in oysters (C4), however, showed no significant comparisons, but C5 that contained Pb and mirex (plus Se, Fe) showed significance with P1, also a gregarine containing factor, and P2, a pathology containing factor (with prokaryotic inclusions). Ni, Zn, and Cd fell on the same PCA axis in both mytilid and oyster PCAs. For oysters, this axis (C2) was significantly related to P4 (tissue pathologies, ceroid bodies, and cestodes). For mytilids, C4 was significantly related to P2 (pathologies) and P4 (trematode metacercariae, ceroid bodies and digestive gland atrophy).

### ***A Posteriori Spearman's Correlations***

*A posteriori* tests were confined to cases where significant ANOVA comparisons occurred more frequently than expected by chance. For the mytilids, six significant relationships existed between contaminant and parasite/pathology PCA factors out of a possible 25 (Table 2), an incidence that far exceeds the incidence anticipated by chance. From these, 43 significant contaminant-parasite/pathology *a posteriori* correlations were obtained out of a possible 81. Among the 43 pairs with significant relationships, 30 achieved significant levels at  $\alpha \leq 0.01$  (Table 3). Thirty of 81 far exceeds the incidence expected by chance ( $p = 0.01$ ,  $P < 0.0001$ ). Forty-three of 81 reached significance at  $\alpha \leq 0.05$ , a frequency also exceeding chance ( $P < 0.0001$ ). Of these 43, 26 were negative, a frequency not exceeding chance. Thus, negative and positive correlations were about equally likely.

The majority of significant correlations in the mytilids involved metals, 33 of 43 (Table 3). Correlation tests with metals involved 70% of the 81 possible tests. Both significance tests with metals and with organics occurred more frequently than expected by chance (metals: 33 of 57,  $p = 0.05$ ,  $P < 0.0001$ ; organics: 10 of 24,  $p = 0.05$ ,  $P < 0.0001$ ). Taking into consideration the differential in the number of tests, 57 versus 24, significant differences with metals occurred significantly more frequently than with organics ( $P < 0.0001$ ). Finally, positive correlations occurred in 7 of 10 significant tests for the organics, a frequency no different than chance. For the metals, the ratio was 10 of 33, a frequency much lower than expected by chance ( $P < 0.02$ ); hence significant correlations with metals were significantly more likely to be negative. A substantial number of the significant correlations for mytilids involved the gregarines; however, gregarines were absent from East-coast mytilids and this absence might have reinforced an East coast-West coast difference in metal body burden yielding the observed significant *a posteriori* tests. Removing the suspect gregarines does not materially change conclusions deduced from this battery of tests, with one exception. The frequency of positive versus negative correlations no longer differs from that expected by chance.

Excluding the suspect gregarines, the majority of the correlations with metals, whether positive or negative, involved major pathologies, tissue pathologies (mostly granulocytomas), and trematode sporocysts and metacercariae. Single-celled para-

sites offered no significant correlations with metals in mytilids. Among organic contaminants, mirex was positively associated with tissue pathologies. Fluoranthene, pyrene, and total PAHs showed weak positive relationships with rare parasites. Total PCBs were positively correlated with major/tissue pathologies and trematode sporocysts. Excluding the gregarines, all correlations with organics were positive, higher weighted prevalence being associated with higher contaminant body burden. Among the metals, Cd, Cr, Fe, Ni, and Zn exclusively demonstrated significant negative correlations, whereas Cu, Hg, and Pb were exclusively involved in significant positive correlations. Such an outcome would not be expected by chance.

Limiting the analysis to East-coast *M. edulis* produced significant relationships in ANOVA more frequently than expected by chance. From these, nine significant contaminant-parasite/pathology pairs were identified out of 33 possible, a frequency that is greater than expected by chance ( $p = 0.05$ ,  $P < 0.0001$ ). About one-third were positive correlations. With one exception, all involved digestive gland atrophy, ceroid bodies, and trematode metacercariae (Table 4). All of the latter two were negative. All for digestive gland atrophy were positive. Such an outcome would not be expected by chance. The single significant ANOVA for West-coast mytilids may have occurred by chance; nevertheless, we include the *a posteriori* tests in Table 4 because of their unusual nature; all involve PAHs and major pathologies, and all are positive.

The ANOVAs for oysters yielded five significant relationships between contaminant and parasite/pathology factors (Table 5), an incidence not anticipated by chance. *A posteriori* Spearman's Rank correlations identified 19 significant individual contaminant-parasite/pathology pairs out of 70 possible. Ten achieved significance levels exceeding  $\alpha = 0.01$  (Table 5). Ten of 70 possible far exceeds the incidence expected by chance ( $p = 0.01$ ,  $P < 0.0001$ ), as does 19 of 70 at  $p = 0.05$ . Of these, 8 were positive, a frequency possibly obtained by chance. Negative and positive correlations were equally likely.

Among the 19 correlations in oysters, nine significant comparisons occurred with the metals, and ten with organic contaminants (Table 5). Correlation tests with metals involved 38.6% of the 70 possible tests. Both significance tests with metals and with organics occurred more frequently than expected by chance (metals:

9 of 27,  $p = 0.05$ ,  $P < 0.0001$ ; organics: 10 of 43,  $p = 0.05$ ,  $P < 0.0001$ ). Taking into consideration the differential in the number of tests, 27 versus 43, significant differences with metals occurred less frequently than with organics ( $P < 0.05$ ). Finally positive correlations occurred in 3 of 10 significant tests for the organics, a frequency no different than chance. For the metals, the ratio was 5 of 9, a frequency also no different than expected by chance.

All negative correlations in oysters were with metals and pesticides or PCBs (Table 5). The vast majority of these significant results involved cestodes and tissue pathologies. All positive correlations were with metals and PAHs. Interestingly, these covered a range of parasite and pathology types. Tissue pathologies were more frequently significant with metals than with pesticides, whether the correlation was positive or negative.

Overall ANOVAs were driven principally by the Gulf oyster dataset. Not surprisingly, restricting *a posteriori* tests to the Gulf dataset yielded essentially the same results, with one important exception. Body and mantle gregarines were negatively correlated with a variety of pesticides (Table 6).

### ***Re-examination of Spearman's Rank Correlations***

Correlations obtained from the Spearman's Rank correlation tests may occur because of frequent co-occurrences of low parasite/pathology-low contaminant values. The most important correlations should be those produced by a coincidence of high parasite/pathology and high contaminant values exceeding (positive) or falling below (negative) the number expected by chance. We re-examined each significant correlation focusing on the values that exceeded the 75<sup>th</sup> percentile value of all observations.

Restricting significant correlations to those meeting this more stringent criterion decreased the number of significant results for the mytilids from 43 to 22 (Table 3). The overall distribution of significant results was not much changed, however. Some correlations with gregarines were lost, but some remained. The frequency of significant results with major pathologies and tissue pathologies was much reduced, but these two biological variables continued to manifest important relationships with contaminants. No pesticide or PAH relationship survived this

analysis, however, with one exception, mirex. Only metals and PCBs remained. Separate analyses for East- and West-coast mytilids also remained qualitatively the same, although the more stringent criterion retained only 57% of the original Spearman's Rank correlations.

The more stringent 75<sup>th</sup> percentile requirement retained only five of the 19 significant Spearman's Rank correlations for oysters. These involved metals and pesticides exclusively; no PAH correlation was retained. These dominantly involved cestodes; no relationship with tissue pathologies or ceroid bodies was retained. Restricting the analysis to the Gulf of Mexico oysters reduced the 20 significant Spearman's Rank correlations to three, again involving exclusively pesticides and metals; no PAH correlation was retained.

## Discussion

### ***Approach***

McDowell et al. (1999) argued that establishing a causal relation between parasites/pathologies observed in bivalve populations and a specific contaminant is inherently difficult, as different types of contaminants simultaneously occur as mixtures, as organisms are exposed to these complex environmental mixtures, not individual contaminants, and as synergistic or antagonistic effects of several classes of contaminants may occur. We add that this same complication exists with parasites and pathologies: most animals are parasitized by multiple taxa (Kim and Powell, 2006); some taxa promote certain pathologies (Kim and Powell, 2004; Kim and Powell, in press); and the infection process is inherently and substantially influenced by processes external to prospective hosts (e.g., Mollison and Levin, 1995; Ford and Tripp, 1996). Bearing these points in mind, we resolve possible relationships between biological and contaminant variables in this study by first utilizing PCA to identify relationships between multiple contaminants and multiple parasites/pathologies (Table 2); we then investigate these relationships in more detail. Because the co-occurrence of low values of parasite/pathology and contaminant might occur for many reasons, a stepwise progression was used to remove from consideration parasite/pathology-contaminant pairs that might originate strictly from frequent co-occurrences of low parasite/pathology-low contaminant pairs. The final

stringent culling of statistical results focused solely on the frequency of co-occurrence of high values of parasite/pathology and contaminant (Tables 3-6).

Positive associations surviving the final cull are cases in which a high contaminant and a high parasite/pathology value co-occurred more frequently than expected by chance. A diversity of possibilities exists to explain these findings: contaminant stresses may reduce host resistance thus increasing susceptibility to parasitism; some parasites may preferentially accumulate certain contaminants in their tissues to a greater extent than their hosts; environmental influences may simultaneously increase contaminant availability and the chance of infection; some parasites may increase host contaminant body burden by modulating accumulation rate; and/or some parasites, through their influence on reproduction or physiology, may decrease depuration or detoxification rate. Negative relationships surviving the final cull are cases in which a high contaminant value and a high parasite/pathology value co-occurred less frequently than expected by chance. A diversity of possibilities exists to explain these findings as well: some contaminants may negatively impact the parasite directly; environmental influences may increase contaminant availability and simultaneously reduce the chance of infection; and/or lower tissue affinity in the parasite may lower apparent host body burden at high infection intensity.

One caveat applies to the comparisons that include data from multiple coasts. Parasites/pathologies vary between coasts; the most obvious case being the absence of gregarines in mytilids on the East coast (Kim and Powell, in press). Some relationships might simply be due to the coincidence of changes in parasite availability and transmission process and the availability of contaminants in the watershed. As one cannot, in most cases, discount a more causative interaction, we include these larger-scale analyses. However, emphasis is placed on within-coast comparisons.

Finally, we note that large-scale climate processes acting through variations in temperature and rainfall (salinity) may play an important role in establishing many of the observed parasite/pathology distributional patterns over broad geographic regions (Kim and Powell, in press). Because the process of contaminant accumulation is normally temperature-dependent (Reid et al., 1997; Kennedy and Walsh, 1997) and because contaminant source loadings and local concentrations are

affected by rainfall and freshwater inflow (Wilson et al., 1992; Kim et al., 2001), trends in the physiology of contaminant accumulation could be similar to trends in the occurrence of parasitism (e.g., Wenzloff et al., 1979) without any inherent interaction between the two. Accordingly, we focus on distinctive patterns that, by their uniqueness, seem unlikely to be explained in so simple a fashion.

### ***Trends in Correlations: The Contaminant Perspective***

Different contaminants seldom occur in isolation in the environment but are present as mixtures with other organic and inorganic contaminants (Moore et al., 1984; Hagopian-Schlekat et al., 2001). Murphy (1980) suggested that identified biological responses to chemical stress are unlikely to have been caused by a single contaminant (see also Ringwood et al., 1999). Synergies are commonly reported from controlled multi-contaminant experiments (Hagopian-Schlekat et al., 2001; Chu and Chow, 2002). PCA analyses of 1995-1998 Mussel Watch data resolved five groups of contaminants (Table 1). In both mussels and oysters, these five included one dominated by PAHs, one dominated by pesticides, and three dominated by metals. Most of the common contaminants fell on one or the five primary PCA axes. The most significant exception was naphthalene that, among the five most common PAHs, did not load on any of the primary contaminant PCA axes. Metals produced a much more complex picture of spatial trends in body burden than did either the pesticides or PAHs. Furthermore, certain similarities existed in the metal groupings between the two taxa. Ag and Zn fell on the same PCA axis in both PCAs. Mirex and Pb fell on the same PCA axis in both PCAs. Cu and Hg did likewise, as did Zn, Ni, and Cd.

Contrasted to the relative simplicity of contaminant trends was the diversity exposed by the PCAs for parasites and pathologies. Very few similarities between the sentinel bivalve taxa existed. This was due in part to the different taxa parasitizing these bivalves. Cestodes and nematodes were present only in oysters. Trematode metacercariae were vastly more abundant in mussels, as were major pathologies. Further examples abound. Thus, the relationship between parasites/pathologies and contaminants can be expected to be different between taxa despite the similarity in contaminant pattern.

Despite the complexity of the biology, significant contaminant versus parasite/pathology differences occurred much more frequently than expected by chance when the two suites of PCA factors were compared. Nevertheless, when the contributors to the PCA factors were compared individually, not all individual variable pairs in each significant factor showed correlations in *a posteriori* Spearman's Rank tests (Tables 3-6), even though the overall comparison between factor scores reached high levels of significance (often  $P \leq 0.001$ ). In some cases, none of them did! Thus, the analysis suggests that combined effects of many contaminants and parasites may be important, leading to complex biological-contaminant interactions with synergies both of biological and chemical origin.

Overall, correlations between parasites/pathologies and contaminants were more frequent with metals, frequent with pesticides, and less frequent with PAHs in mussels. In oysters, correlations with pesticides and metals were about equally frequent, but correlations with PAHs were still rare. Most associations may be due to inherently different contaminant loads in species and coasts, but even at the lowest spatial scale (i.e. East-coast *M. edulis*, Gulf-coast oysters), the metals (and pesticides in oysters) produced most of the significant correlations. Inasmuch as metals contribute a large portion of the analytes examined in this study, this outcome might be anticipated. However, the frequency accrues not from this source, but from the diversity of metal groups in the PCA, of which three were identified in each taxon, and the common occurrence of significant Spearman's Rank correlations with individual metals contributing to these metal-dominated PCA factors. Negative and positive correlations occurred with about the same frequency, both in oysters and in mytilids, once the suspect gregarines were excluded for the mytilids. For metals, important positive associations were recognized between Pb, Hg, and Cu and trematode sporocysts in mytilids. Negative correlations occurred between a number of metals and trematode metacercariae, as well as both types of pathologies in mytilids. For the oysters, positive correlations involved principally Pb, with Cd and Ni contributing most of the negative ones. The positive relationship between major pathologies and Pb, and the negative relationship between tissue pathologies and both Cd and Ni occurred in both mytilid and oyster datasets

Correlations with pesticides and PCBs, though less common than with the metals in mussels, were relatively frequent in both taxa and more common in oysters than in mytilids. The majority of associations related to pesticides were negative ones. In mytilids, positive correlations were limited to the PCBs. None existed in the oysters. Negative correlations occurred with mirex for the mytilids and with a variety of pesticides and the PCBs in oysters. A negative correlation between cestodes from East-coast oysters with total hexachlorocyclohexane (HCH), a synthetic pesticide that does not occur naturally in the environment, was identified by Kim et al. (1998). In this study, all potential relationships of cestodes in oysters with manufactured organic chemicals (i.e. total DDT, aldrin, dieldrin, lindane, and total PCBs) were negative (Tables 5 and 6), suggesting that locations with higher infection intensity of cestodes tended to co-occur with lower body burden of these contaminants.

PAHs rarely showed significant relationships with parasites/pathologies in this study. Powell et al. (1999) reported similarly that parasites observed in petroleum seep mussels were nevertheless rarely significantly associated with PAH body burdens.

Lipophilic organic contaminants (e.g., PAHs) are easily depurated by spawning as they are enriched in gametic tissue (Jovanovich and Marion, 1987; Ellis et al., 1993). Ellis et al. (1993) noted that pesticides are concentrated in eggs but not in sperm, so that depuration through spawning would influence only a portion of the bivalve population. Metals are less influenced by spawning (e.g., Cunningham and Tripp, 1975; Greig et al., 1975) than organics, and thus metals tend to stay in the tissues. This may be the reason why the majority of associations identified in this study came from metals, as they are present in higher concentrations over longer periods, and so can more strongly influence the biology of both host and parasite. Pesticides fell in between as anticipated by their apparently more limited depuration through spawning (Ellis et al., 1993). PAHs, most easily depurated through spawning, were rarely characterized by significant correlations.

The inverse ranking of spawning as a depuration mechanism and the frequency of significant correlation are gripping and argue for a much closer look at this key linkage in explaining spatial trends in monitoring studies. The same ranking would

promote a greater and longer-term interaction between parasites/pathologies and metals than PAHs, as is observed. The infrequency of significant effects between Se and parasites/pathologies is potentially noteworthy in this regard as this metal may be lost through spawning (Baldwin and Maher, 1997). However, one cannot exclude the differential in effects levels that tend to be much lower for pesticides than PAHs (Long et al., 1995) as contributing to the intermediary nature of the pesticides. PAHs may simply be less toxic and therefore less influential in affecting the occurrence of parasites/pathologies.

### ***Trends in Correlations: The Biological Perspective***

Of the 23 significant correlations involving parasites, after discounting the gregarine relationships in mytilids and the rare parasite group that combines a variety of taxa, only 3 involve single-celled eukaryotes or prokaryotes (Tables 3 and 5). This is significantly fewer than expected by chance ( $p = 0.01, P < 0.0001$ ). The remaining involve multi-cellular eukaryotes and nearly all of them either cestodes, trematode sporocysts, or trematode metacercariae. Some of the frequently significant relationships observed with tissue pathologies, particularly the hemocytic infiltration in oysters, probably accrue also in this way, as these worms, with the exception of the trematode sporocysts, are often observed to locally increase hemocyte concentration in bivalves (Cheng, 1966; Cheng and Rifkin, 1968; Kim and Powell, 2004). The few correlations for single-celled parasites all involve proliferating protozoa (*P. marinus*) or protozoa reaching high body burdens through transmission (the gregarines). With two exceptions, the parasites involved in significant correlations with contaminants are not known to do great damage to the host, the exception being *P. marinus* and the trematode sporocysts. The non-random distribution of significant correlations and the tendency for the larger or more numerous parasites to be involved argue that unequal sequestration of contaminants between host and parasite tissue is a potential mediator. Negative and positive correlations occur with about equal frequency, suggesting that, if present, the principal differential may be either a lower or higher body burden in parasitic tissue. This expectation differs from that expected from Sures et al. (1999).

One interesting tendency was for correlations with pathologies to be principally with the mytilids. The few in oysters always co-occurred with cestodes and are

likely derived from hemocytic infiltration near cestode cysts. With the mytilids, pathologies, major and tissue (mostly granulocytomas in this case), were common and correlations widespread among the metal, pesticide, and PAH contaminants.

*A posteriori* correlations did not occur more frequently than expected by chance in East-coast oysters and West-coast mussels. The ready explanation of multiple taxa on the West coast compromising the analysis is belied by the case of oysters on the East coast. These two coastline stretches are unique in having the widest temperature range. Each contains significant provincial boundaries (Hall, 1964; Valentine, 1966). The influence of the large temperature range on parasite distributional pattern potentially minimizes the possibility that correlations with contaminants can be identified. Shorter coastline stretches bounded by provincial boundaries might be investigated advantageously.

#### ***Noteworthy Specific Relationships***

Couch (1985) found that industrialized sites had a tendency to show a higher frequency of oysters parasitized or diseased, suggesting that pollution stress can be a major contributing factor in the occurrence of disease in animals from degraded habitats. Villalba et al. (1997) noticed a higher parasite load in mussels from areas of higher levels of pollution in Spain. Other studies have suggested possible relationships between certain parasites or disease conditions in marine bivalves and pollution by identifying chemical contaminants as causative factors for detrimental biological effects. Kraak and Davids (1991) found that organisms weakened by parasitism contained higher levels of some metals, for example. From all of these studies, one might anticipate that positive correlations between parasite body burden and contaminant body burden would be the norm. Nevertheless, in this study, positive correlations were no more common than negative ones. A strong tendency existed for the correlations to be of equivalent trend in both mytilid and oyster taxa. Thus, correlations with Cd and Ni were negative in both taxa. Positive correlations occurred in both taxa for Pb.

Exposure to contaminants can increase the bivalve host's susceptibility to disease and thus enhance disease infections (Chu and Hale, 1994; Chu, 1996; McDowell et al., 1999). This study identified two positive associations with

*P. marinus*, for Pb and Se. No correlations were negative. Guth et al. (1977) showed that snails parasitized by trematodes were more susceptible to Zn than unparasitized snails. In contrast, Zn entered into a number of significant correlations with trematodes in this study; all were negative. From a previous Mussel Watch Program by the EPA, Yevich and Barszcz (1983) reported correlations between locations with high parasitism and locations high in PCBs. Kim et al. (1998) also reported that trematode sporocysts in *M. edulis* were positively correlated with body burdens of total PCBs. Evidence to support these observations from this study includes the fact that total PCB levels in mytilids were positively associated with infections by trematode sporocysts.

Barszcz et al. (1978) reported that oysters exposed to crude oils had an increased intensity of infection, showing that contaminant stress plays a role in increased parasitism. Neff et al. (1987) also noted a relationship between parasitism and oil exposure. In this study, relationships with PAHs were rare; the only noteworthy exception was the relationships elucidated in West-coast mussels between a variety of PAHs and pathologies. Barszcz et al. (1978) also noted that bivalves with reduced resistance to infection due to higher contaminant body burdens are more likely to become hosts to opportunistic parasites and that contaminant exposure may help parasites already present in hosts to proliferate. Laird (1961) forwarded a similar hypothesis. Our analysis of rare parasites, anticipated to follow this trend, yielded few significant correlations, though all were positive.

Lowe and Moore (1979) showed that areas of high pollution have a high incidence of granulocytomas characterized by an inflammatory cellular condition in mytilid mussels from England and Wales. Confirmatory data come from Villalba et al. (1997), Svärddh (1999), and Svärddh and Johannesson (2002). In this study, tissue pathologies, principally granulocytomas, in mytilids were more common and abundant in East-coast *M. edulis*, especially from Massachusetts to Delaware, than in West-coast mytilids. Contaminant concentrations are also high in these animals, on the average (O'Connor and Ehler, 1991; O'Connor, 2002). With the exception of Cd, highly significant correlations between tissue pathologies and contaminants were positive in mytilids. This included metals (Pb, Hg) and PCBs.

Ceroid bodies or brown cells are thought to have detoxification abilities (Zaroogian et al., 1989, 1993). Cd, Ni, and certain PAHs are implicated (Zaroogian et al., 1993). Interestingly, correlations with ceroid bodies were always positive, with one exception in mytilids. These positive correlations occurred solely in oysters and involved Zn and certain PAHs. Digestive gland atrophy has been shown to be responsive to environmental stress in mytilids and other bivalves (Widdows et al., 1982; Axiak et al., 1988; Hinton et al., 1992; Da Ros et al., 1998), particularly metals. In this study, all significant correlations were observed in mytilids and all were positive. Their absence in oysters is possibly explained by the relationship of this tissue condition and nutritional status in oysters (Winstead, 1995; but see Gold-Bouchot et al., 1995).

### Conclusions

Estuarine and coastal marine waters receive many kinds of organic and inorganic contaminants that potentially have adverse effects on biota living in these habitats and possibly on humans who consume them. Sessile bivalve mollusks can be used effectively as bioindicators to monitor the status and trends of coastal water quality. Whether parasites/pathologies can be used as biomarkers is still unclear, but some observations are noteworthy. The distribution of parasites/pathologies within taxa and along coasts is sufficiently complex that a universal relationship with contaminants is an unachievable goal. Relationships may be present at smaller spatial scales, however, and within taxon.

Several studies have suggested parasites as potential biomarkers of pollution. Some focus on particular parasite types (Thulin, 1989); others on broader indices of parasite species richness and diversity (Schmidt et al., 2003). In this study, the gregarines, the most common parasites in oysters exclusive of the disease-causing *P. marinus*, were responsible for a large fraction of the relationships with contaminants found in Gulf-coast oysters. These were exclusively inversely associated with pesticides, suggesting an underlying relationship. Cestodes, another common parasite, were exclusively negatively associated with metals and pesticides. The possibility that some parasites such as the gregarines and cestodes in oysters might be useful biomarkers is indicated by this study. That the common parasites are most important is particularly critical to establish biomarkers of general useful-

ness. The trend for common parasites to display correlations with contaminants is also present in mussels, where trematode sporocysts and metacercariae contributed most significant correlations, and nearly exclusively with metals. In contrast to the promise shown by the metals, and by pesticides in oysters, PAH biomarkers are unlikely to be found among the parasites. Correlations with PAHs were remarkably rare. The ease of depuration of PAHs through spawning offers a likely explanation.

Interactions between different types of contaminants present as mixtures in estuarine and coastal waters must be common. Not all individual parasite/pathology-contaminant pairs in each significant PCA factor pair showed significant correlations, although the overall comparison between PCA factors loaded by multiple biological and contaminant variables reached high levels of significance. Thus, this study re-enforces the contention that combined effects of many contaminants and parasites may be important, and emphasizes the complexity of parasite-contaminant interactions, considering the other biological and environmental variables involved. For this reason, parasites are unlikely to be responsive to single contaminants, but they are likely to respond to classes of contaminants and the number of such classes is relatively few; five in the case of mytilids and oysters in this study. Thus, these results also support continued evaluation of parasite/contaminant interactions.

As parasitism can influence the sensitivity of host bivalves to contaminant exposure and affect the spawning of animals as a means of contaminant loss, and as parasites may respond directly to contaminants and influence host body burden directly, the study of parasites and pathologies should be included in biomonitoring and toxicity studies as is now the case in the Mussel Watch Program. The supposition that increased parasitism and increased contaminant body burden should co-occur was not uniformly supported, however. A surprising number of significant correlations between contaminants and parasites/pathologies, about half of all correlations, were negative, suggesting that some contaminants may negatively affect parasites more than their hosts. The importance of parasites in community diversity is attracting increasing attention (Hawkins, 1990; Sousa, 1991; Thomas et al., 1997; Rohde, 2002). As parasites are potentially more sensitive than hosts to environmental disturbance, the possibility that contaminants may reduce the abundance of some parasites is deserving of attention. More multidisciplinary

studies on the interactive biological influences of environmental stressors (e.g., multiple contaminants) on marine organisms, both hosts and parasites, in estuaries and coastal waters are required to further elucidate these relationships.

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**Legends for Figures**

- Figure 1. Bay-mean body burdens of Ag, As and Cd. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.
- Figure 2. Bay-mean body burdens of Cr, Cu and Fe. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.
- Figure 3. Bay-mean body burdens of Hg, Ni and Pb. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.
- Figure 4. Bay-mean body burdens of Se, Zn and naphthalene. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.
- Figure 5. Bay-mean body burdens of phenanthrene, fluoranthene and pyrene. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.
- Figure 6. Bay-mean body burdens of chrysene, total PAHs and total DDTs. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid

sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.

Figure 7. Bay-mean body burdens of aldrin, dieldrin and lindane. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.

Figure 8. Bay-mean body burdens of mirex, total chlordanes and total PCBs. Vertical bars mark sectional boundaries. Great Lakes/Hudson River dreissenid sites, Bays 1-11; East-coast mytilid sites, Bays 12-36; East-coast oyster sites, Bays 37-61; Puerto Rico, Bay 62; Gulf-coast oyster sites, Bays 63-93; West-coast mytilid sites, Bays 94-125; Hawaii, Bay 126. Data for Bays 37 and 38 not included.

**Table 1.** Factor load analysis for the four PCAs. PCAs were conducted separately for mytilids and oysters. For each taxon, a parasite/pathology PCA and a contaminant PCA were run. Variables assigned had factor loads  $\geq 0.5$  on the indicated factor axis.

### Mytilid PCA

#### Parasite/Pathology

P1	body gregarine	gill gregarine	mantle gregarine	
P2	major pathology	tissue pathology	trematode sporocyst	
P3	rare parasite	prokaryotic inclusion		
P4	trematode metacercaria	ceroid body	digestive gland atrophy	
P5	gill ciliate	gut ciliate		

#### Contaminant

C1	phenanthrene	fluoranthene	pyrene	chrysene	total PAH	total chlordanes
C2	Cu	Hg	Pb	mirex	total PCB	
C3	total DDT	aldrin	dieldrin	total chlordanes		
C4	Cd	Cr	Fe	Ni	Zn	
C5	Ag	As	Zn			

### Oyster PCA

#### Parasite/Pathology

P1	gill gregarine	<i>Perkinsus marinus</i>				
P2	prokaryotic inclusion	major pathology				
P3	body gregarine	mantle gregarine				
P4	tissue pathology	cestode	ceroid body			
P5	trematode sporocyst	gut ciliate				

#### Contaminant

C1	phenanthrene	fluoranthene	pyrene	chrysene	total PAH	total PCB	total chlordanes
C2	Ag	Cd	Cu	Ni	Zn		
C3	total DDT	aldrin	dieldrin	lindane	total chlordanes	total PCB	
C4	Cr	Cu	Hg				
C5	Fe	Pb	Se	mirex			

**Table 2.** Results of ANOVAs comparing contaminant (C) factors with parasite/pathology (P) factors derived from PCA analysis of mytilids and oysters. Factor loads are defined in Table 1. Only comparisons significant at  $\alpha \leq 0.05$  are reported.

Taxon	Parasite/ Pathology	Contaminant	Significance	
	Factor	Factor	Level	
<b>All Mytilids</b>	P1	C2	0.027	
	P1	C5	0.015	
	P2	C2	0.0001	
	P2	C4	0.0015	
	P3	C1	0.0046	
	P4	C4	0.0046	
	<i>East Coast only</i>	P3	C1	0.0056
P3		C5	0.0049	
P4		C4	0.033	
<i>West Coast only</i>	P2	C1	0.024	
<b>All Oysters</b>	P1	C5	0.0018	
	P2	C5	0.007	
	P4	C1	0.0029	
	P4	C2	0.0041	
	P4	C3	0.0015	
	<i>East Coast only</i>	P3	C1	0.028
		P3	C5	0.017
	<i>Gulf Coast only</i>	P2	C5	0.0097
		P3	C3	0.026
		P4	C1	0.0001
		P4	C2	0.001
		P4	C3	0.023

**Table 3.** Significance levels for significant ( $\alpha = 0.05$ ) Spearman's Rank correlation tests on parasite/pathology-contaminant pairs found significant in ANOVA, for all mytilids. Parentheses indicate negative correlations. Factor pairs are defined in Table 1. Significance levels are provided in Table 2. Results of subsequent binomial tests to determine if the correlation is based solely on the co-occurrence of low values are given in the righthand column by a non-significant result.

Factor Pair	Contaminant	Parasite/Pathology	Spearman's	Binomial
			Significance	Significance
			Level	Level
<b>C2-P1</b>	Cu	body gregarine	(0.0049)	
	Cu	gill gregarine	(0.0019)	0.05
	Cu	mantle gregarine	(0.0016)	
	Hg	body gregarine	(0.0001)	0.02
	Hg	gill gregarine	(0.0001)	
	Hg	mantle gregarine	(0.0001)	0.02
	Pb	body gregarine	(0.019)	
	Pb	gill gregarine	(0.0068)	0.02
	mirex	body gregarine	(0.0004)	
	mirex	gill gregarine	(0.0002)	
	mirex	mantle gregarine	(0.0053)	
	<b>C5-P1</b>	Zn	body gregarine	0.016
Zn		gill gregarine	0.0001	0.01

Table 3. continued

Factor Pair	Contaminant	Parasite/Pathology	Spearman's	Binomial
			Significance Level	Significance Level
<b>C2-P2</b>	Cu	trematode sporocyst	0.0011	0.025
	Hg	major pathology	0.036	
	Hg	tissue pathology	0.0004	0.05
	Hg	trematode sporocyst	0.0001	0.005
	Pb	major pathology	0.003	
	Pb	tissue pathology	0.0001	0.0005
	Pb	trematode sporocyst	0.0001	0.005
	mirex	tissue pathology	0.02	0.005
	total PCB	major pathology	0.0001	0.005
	total PCB	tissue pathology	0.0001	0.005
	total PCB	trematode sporocyst	0.0002	0.01
<b>C4-P2</b>	Cd	major pathology	(0.0001)	
	Cd	tissue pathology	(0.0001)	0.005
	Cd	trematode sporocyst	(0.0001)	0.02
	Cr	major pathology	(0.013)	
	Cr	tissue pathology	(0.013)	
	Fe	tissue pathology	(0.014)	
	Ni	tissue pathology	(0.022)	
	Zn	major pathology	(0.0005)	
	Zn	tissue pathology	(0.044)	
<b>C1-P3</b>	fluoranthene	rare parasite	0.047	
	pyrene	rare parasite	0.025	
	total PAH	rare parasite	0.027	
<b>C4-P4</b>	Cd	trematode metacercaria	(0.0001)	0.005
	Cr	trematode metacercaria	(0.0004)	0.05
	Fe	trematode metacercaria	(0.0001)	
	Fe	ceroid body	(0.0001)	0.01
	Fe	digestive gland atrophy	0.035	
	Ni	trematode metacercaria	(0.0001)	0.005
	Zn	trematode metacercaria	(0.0013)	0.02

**Table 4.** Significance levels for significant ( $\alpha = 0.05$ ) Spearman's Rank correlation tests on parasite/pathology-contaminant pairs found significant in ANOVA, for mytilids on the East and West coast. Parentheses indicate negative correlations. Factor pairs are defined in Table 1. Significance levels are provided in Table 2. Results of subsequent binomial tests to determine if the correlation is based solely on the co-occurrence of low values are given in the righthand column by a non-significant result.

<b>East Coast</b>				
<u>Factor Pair</u>	<u>Contaminant</u>	<u>Parasite/Pathology</u>	Spearman's	Binomial
			Significance	Significance
			<u>Level</u>	<u>Level</u>
<b>C5-P3</b>	Ag	rare parasite	(0.0069)	
<b>C4-P4</b>	Cr	trematode metacercariae	(0.0008)	0.05
	Cr	digestive gland atrophy	0.0004	0.05
	Fe	trematode metacercariae	(0.0001)	0.05
	Fe	ceroid body	(0.0005)	
	Fe	digestive gland atrophy	0.0038	0.05
	Ni	trematode metacercaria	(0.0001)	0.05
	Ni	digestive gland atrophy	0.028	
	Zn	trematode metacercaria	(0.0063)	
<b>West Coast</b>				
<u>Factor Pair</u>	<u>Contaminant</u>	<u>Parasite/Pathology</u>	Spearman's	Binomial
			Significance	Significance
			<u>Level</u>	<u>Level</u>
<b>C1-P2</b>	phenanthrene	major pathology	0.0001	0.02
	fluoranthene	major pathology	0.0001	0.02
	pyrene	major pathology	0.0001	0.005
	chrysene	major pathology	0.0001	
	total PAH	major pathology	0.0001	

**Table 5.** Significance levels for significant ( $\alpha = 0.05$ ) Spearman's correlation tests on parasite/pathology-contaminant pairs found significant in ANOVA, for all oysters. Parentheses indicate negative correlations. Factor pairs are defined in Table 1. Significance levels are provided in Table 2. Results of subsequent binomial tests to determine if the correlation is based solely on the co-occurrence of low values are given in the righthand column by a non-significant result.

Factor Pair	Contaminant	Parasite/Pathology	Spearman's	Binomial
			Significance	Significance
			Level	Level
<b>C5-P1</b>	Pb	gill gregarine	0.02	
	Pb	<i>Perkinsus marinus</i>	0.0088	
	Se	<i>Perkinsus marinus</i>	0.033	0.01
<b>C5-P2</b>	Pb	major pathology	0.0024	0.005
<b>C1-P4</b>	fluoranthene	cestode	0.031	
	pyrene	cestode	0.024	
	pyrene	ceroid body	0.048	
	total PCB	cestode	(0.019)	
<b>C2-P4</b>	Cd	tissue pathology	(0.04)	
	Cd	cestode	(0.0001)	0.005
	Ni	tissue pathology	(0.034)	
	Ni	cestode	(0.0001)	
	Zn	ceroid body	0.0008	
<b>C3-P4</b>	total DDT	cestode	(0.0009)	0.05
	aldrin	tissue pathology	(0.0011)	
	aldrin	cestode	(0.0001)	
	dieldrin	cestode	(0.0037)	0.05
	lindane	cestode	(0.0005)	
	total PCB	cestode	(0.019)	

**Table 6.** Significance levels for significant ( $\alpha = 0.05$ ) Spearman's Rank correlation tests on parasite/pathology-contaminant pairs found significant in ANOVA, for Gulf oysters. Parentheses indicate negative correlations. Factor pairs are defined in Table 1. Significance levels are provided in Table 2. Results of subsequent binomial tests to determine if the correlation is based solely on the co-occurrence of low values are given in the righthand column by a non-significant result.

Factor Pair	Contaminant	Parasite/Pathology	Spearman's	Binomial
			Significance	Significance
			Level	Level
<b>C5-P2</b>	Pb	major pathology	0.0047	0.02
<b>C3-P3</b>	total DDT	body gregarine	(0.0001)	
	dieldrin	body gregarine	(0.0001)	0.05
	dieldrin	mantle gregarine	(0.01)	
	lindane	body gregarine	(0.0062)	
	lindane	mantle gregarine	(0.0091)	
	total chlordane	body gregarine	(0.0002)	
	total chlordane	mantle gregarine	(0.039)	
	total PCB	body gregarine	(0.0084)	
<b>C1-P4</b>	phenanthrene	ceroid body	0.042	
	pyrene	ceroid body	0.044	
	total PAH	ceroid body	0.044	
<b>C2-P4</b>	Cd	tissue pathology	(0.0062)	
	Cd	cestode	(0.0001)	0.05
	Ni	tissue pathology	(0.011)	
	Ni	cestode	(0.0005)	
	Zn	ceroid body	0.014	
<b>C3-P4</b>	aldrin	tissue pathology	(0.012)	
	aldrin	cestode	(0.0041)	
	lindane	cestode	(0.038)	

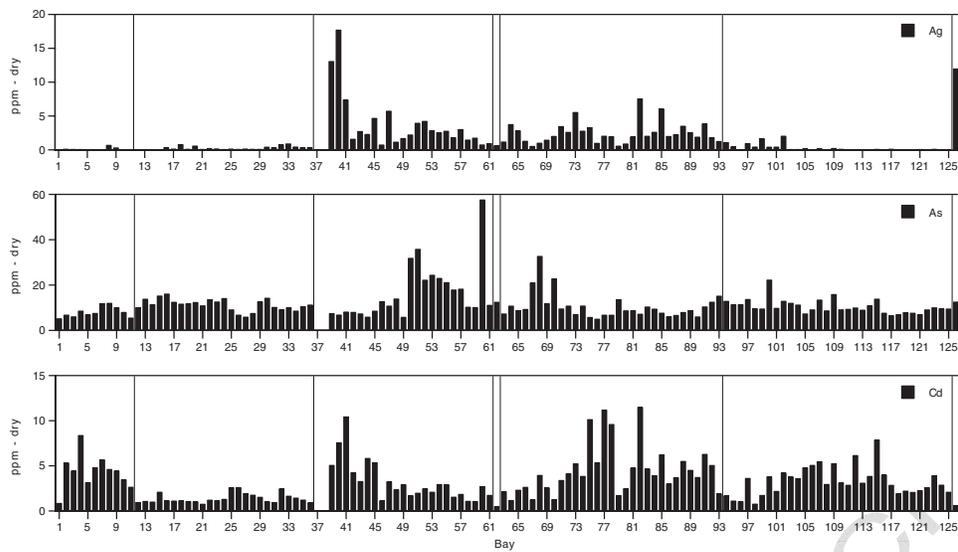


Fig.1

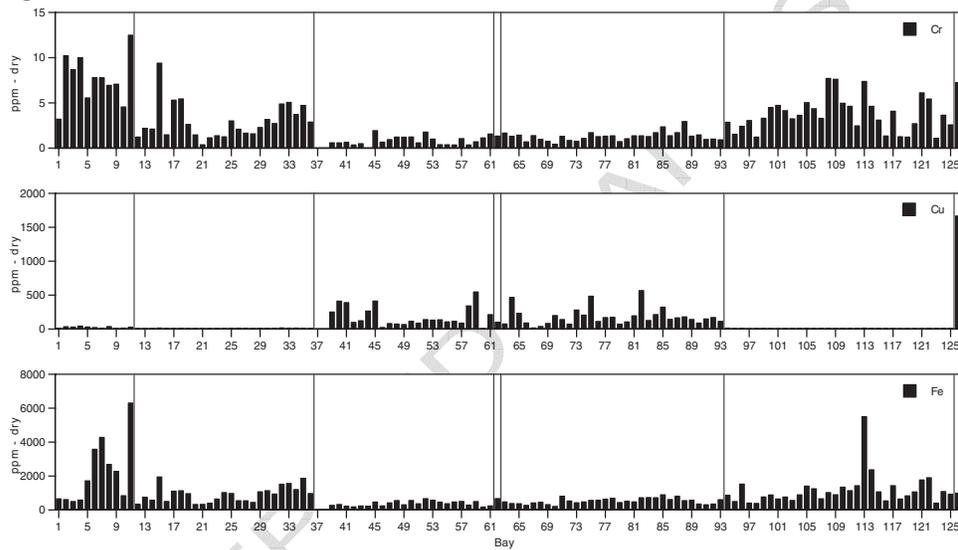


Fig.2

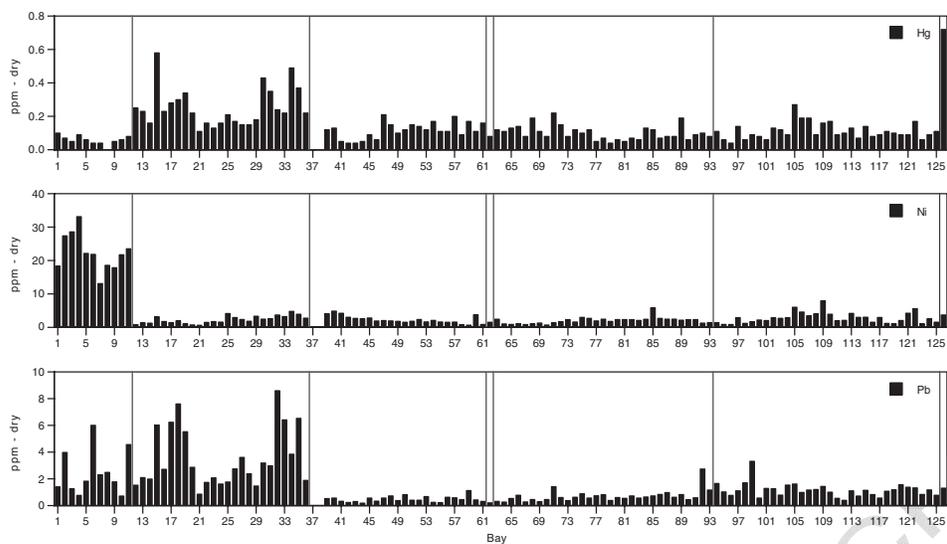


Fig.3

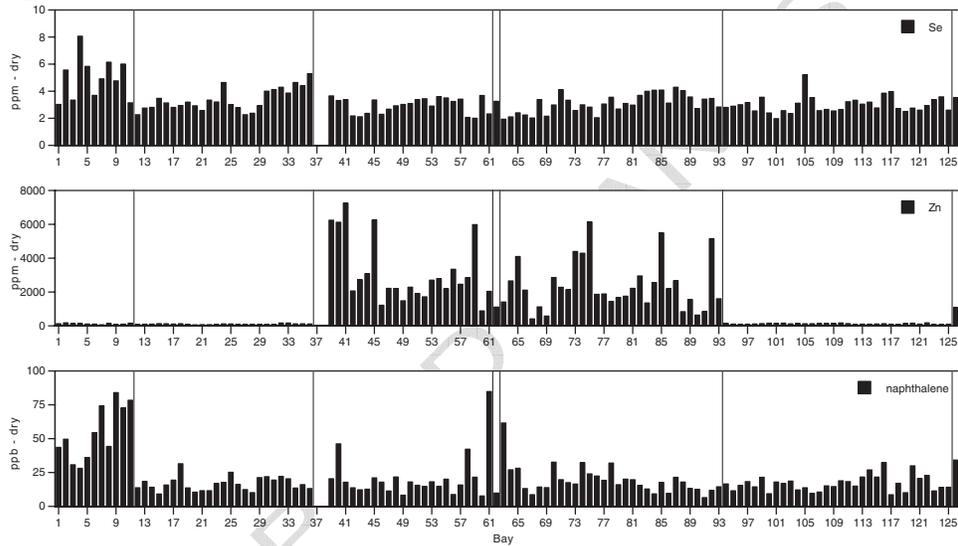


Fig.4

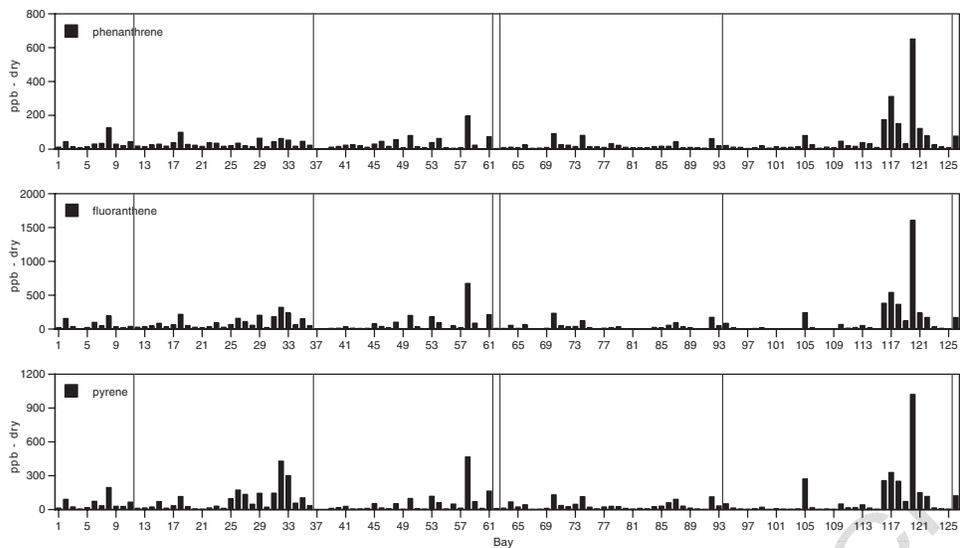


Fig.5

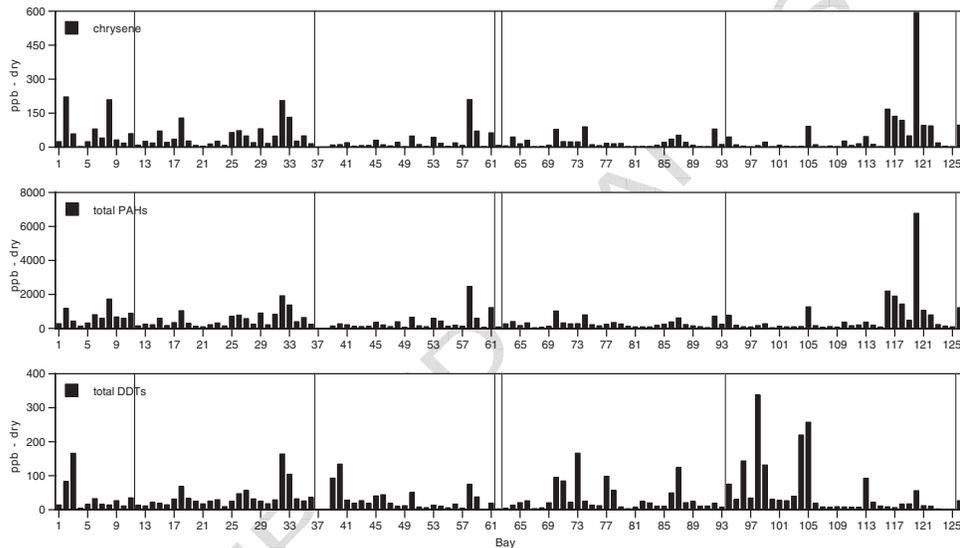


Fig.6

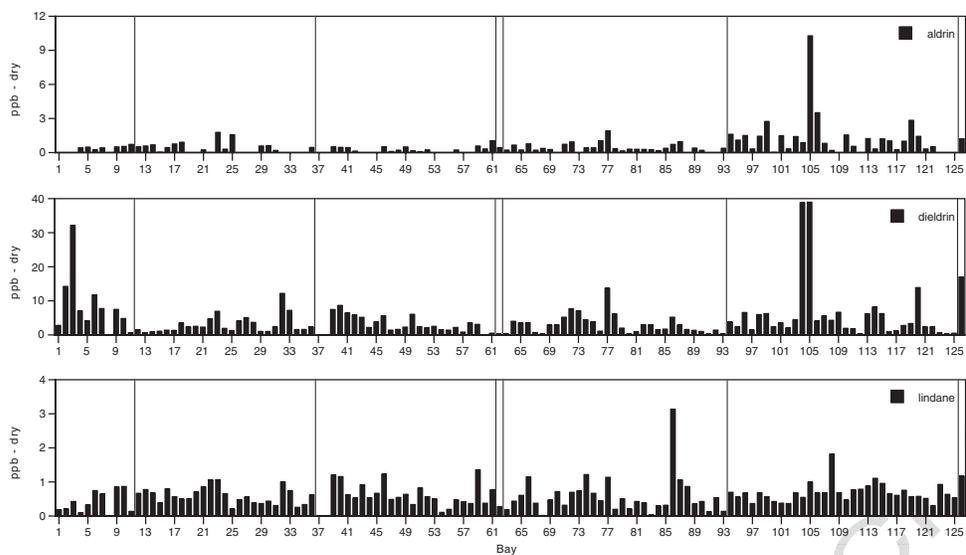


Fig.7

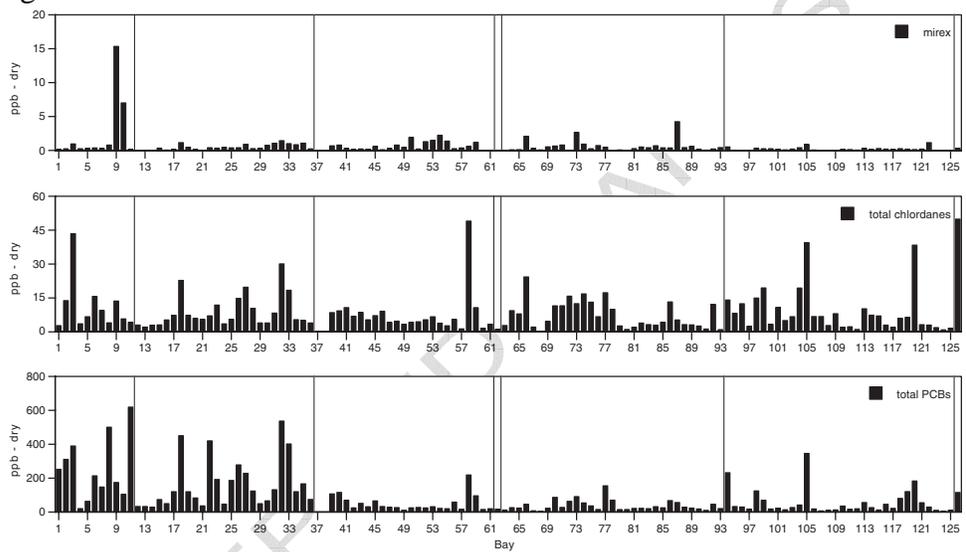


Fig.8