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Trace Organic Contaminants (PAHS, PCBs, and Pesticides) in Oysters *Crassostrea virginica*, from the Caloosahatchee Estuary and Estero Bay, SW Florida.

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1. Introduction

Estuaries and coastal marine environments are some of the most anthropogenically-stressed environments in the world and are known to receive large amounts of hazardous organic chemicals in traces but deleterious quantities. Some of these chemicals include carcinogenic and toxic polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pesticides and herbicides. These chemicals can not only adversely benthic (Thompson et al. 1996, Capuzzo 1996, McDowell et al. 1999) and pelagic organisms but also accumulate to higher trophic levels, ultimately affecting humans.

The Caloosahatchee River and Estuary is located on the southwest coast of Florida (Fig. 1) and runs from Lake Okeechobee to the Franklin Lock and Dam (S-79) where it empties into the estuary at Shell Point. The estuary between the Franklin Lock and Dan and Shell Point is ~ 42 kilometers long. The Caloosahatchee River is the major source of freshwater to the estuary.

Historically, the Caloosahatchee River was a meandering riverine system with numerous oxbows, flowing from its headwaters at the marshlands of Lake Flirt and Lake Hicpochee, west of Lake Okeechobee, to the Gulf of Mexico. Alterations to this system have begun in the late 1800's, with Hamilton Disston's dredging and channelization project, which included a connection to Lake Okeechobee and construction of an extensive canal network associated with agricultural development in the watershed (Barnes 1995). The river has been permanently connected to Lake Okeechobee and about 20% of the water entering the estuary now comes from the Lake, mainly as regulatory releases to maintain the Lake at a prescribed water level (SFWMD 2009). The river has also been straightened, deepened and three water control structures (S-77, S-78, and S-79) have been added. The last structure S-79, was completed in 1966 to act, in part, as a salinity barrier. The channelization and canal building process (C-43) has changed the timing, quantity, quality and direction of runoff within the watershed and led to abnormal salinity fluctuations. The operation of three water

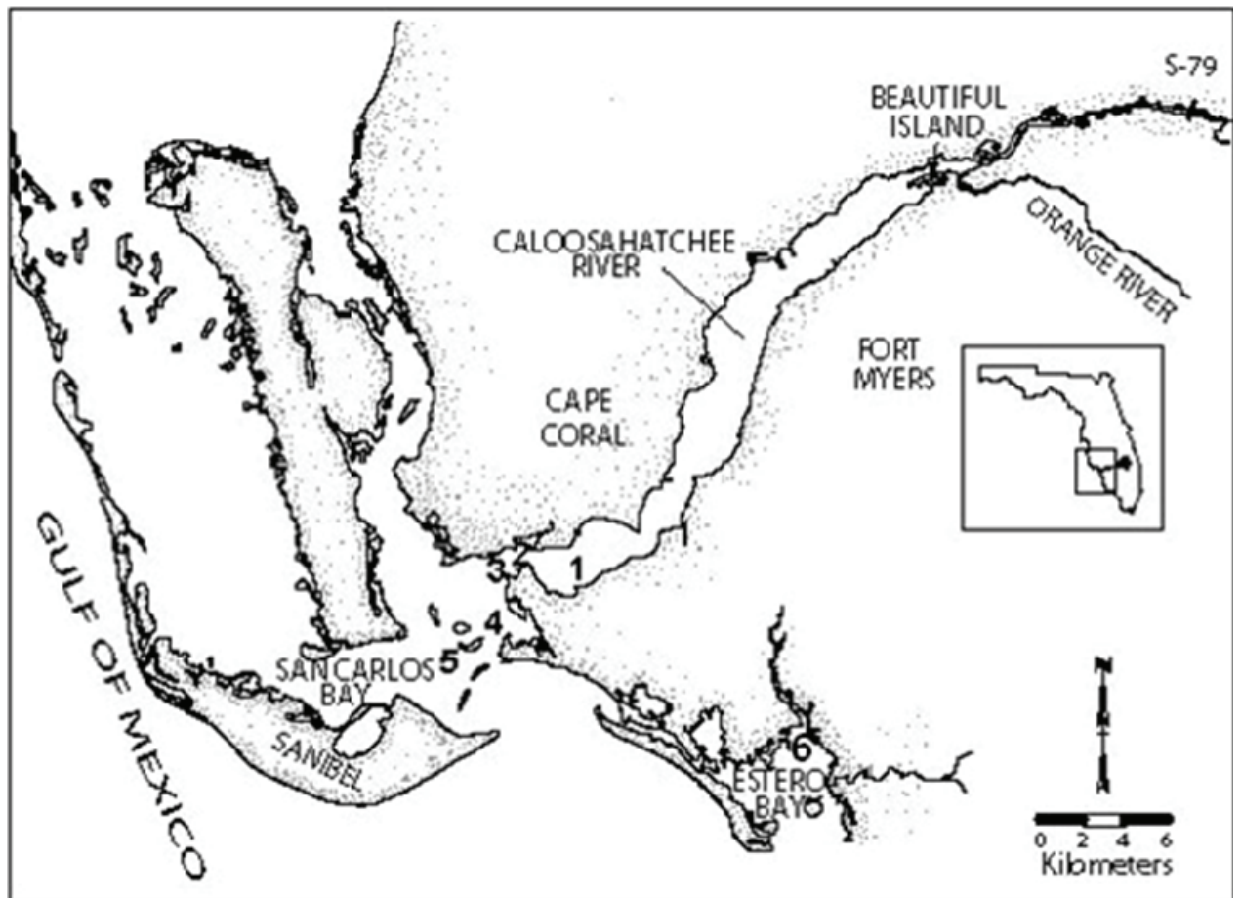


Fig. 1. Sampling Locations in the Caloosahatchee Estuary (1-5) and Estero Bay (6), Southwest Florida.

control structures allowing large periodic regulatory releases from Lake Okeechobee has reduced the tidally influenced portion of the estuary. The combination of over-draining and the addition of S-79 as a salinity barrier results in a truncated and highly variable salinity gradient in the estuary. During periods of low freshwater discharge typically during the dry season, salt water regularly intrudes all the way to the structure, often exceeding 10 ppt. By contrast, high freshwater discharge can cause salinity to drop below 5 ppt at the mouth. The transition between the two states can be rapid, sometimes requiring less than a week. The fluctuations observed at the head and mouth of the estuary exceeds the salinity tolerances of oligohaline and marine species. The result is that freshwater delivery to the estuary has been altered and shows characteristics consistent with a watershed that has lost storage capacity. Stormwater is not retained but runs off quickly and at higher peak flows. Because there is no retention base flows are low or non-existent during the dry season (Volety 2008, Volety et al. 2009).

In SW Florida, in addition to lake releases from Okeechobee, runoff from agricultural lands, golf courses, marinas, and housing subdivisions is directed into sensitive estuarine environments such the Caloosahatchee estuary. These water flow alterations have resulted in the input of organic and inorganic pollutants and altered salinity regimes, stressing the organisms inhabiting these ecosystems.

Oysters *Crassostrea virginica* are ubiquitous features in the estuaries along the eastern seaboard of the United States and the Gulf of Mexico including SW Florida and the

Caloosahatchee estuary (Volety et al. 2009). Given the enormous filtration activity (Newell 1988), and low detoxification capability of oysters, oysters are used for contaminant monitoring (O'Connor and Lauenstein 2006). The purpose of this study was to determine temporal and spatial variation in baseline concentrations of PAHs, PCBs, and selected pesticides in oyster tissue in Caloosahatchee Estuary and a reference site, Estero Bay.

2. Methods

2.1 Field collections

Oysters were collected by hand from the Caloosahatchee Estuary and Estero Bay in Southwest Florida (Fig. 1). Most of the sampling locations are intertidal to shallow-subtidal in nature and are subject to tidal flushing. Samples were scrubbed clean of fouling organisms and were kept frozen (-20 °C) until further processing as noted below.

2.2 Analytical protocol

Just prior to contaminant analyses, oysters were thawed at room temperature for ~ 2-4 h after which time they were shucked and all soft tissue removed for determination of water content and trace organic contaminants. Approximately 15 oysters were pooled and homogenized per composite sample. Composite wet oyster tissue from each station was placed in 100 mL pre-cleaned (450 °C for 4h) and tared glass jars and then dried at 60 °C. The drying process was continued for at least one week or longer until dry weights stabilized. Sample water content was determined using the difference in weight before and after drying.

Desiccated oyster tissue was homogenized in a stainless steel blender. In between samples, the blades were wiped down pre-cleaned glass wool soaked with GC-grade methanol. Dried homogenized oyster tissue samples were then placed in pre-weighed Pyrex test tubes. A surrogate standard consisting of deuterated PAHs and PCBs was added, followed by 30 mL of a dichloromethane:methanol (2:1, v:v) cocktail. Samples were extracted twice using ultrasonication for 30 minutes each time. Each extract was centrifuged and transferred to a rotary evaporator flask. Extracts were concentrated to 0.25 mL, exchanged to 100% hexane, and then purified using silica gel. The purification process consisted of passing the extract through a column containing 10 g of pre-cleaned (Soxhlet extraction with DCM for 24 h) deactivated silica (mesh size 100-200), topped with 0.5 cm precombusted (4 h at 450 °C) Na₂SO₄ for further purification. As an additional precautionary step against tissue lipids confounding gas chromatographic analysis, Florisil chromatography was also conducted. The purified extract was then reduced by rotoevaporation followed by nitrogen blow down to 1 mL. An internal standard containing deuterated select PAHs and pesticides was added, and the extract further reduced to 100 µL under N₂ for analysis and quantification using gas chromatography-mass spectrometer.

Sample extracts were analyzed on a Shimadzu QP5050A gas chromatograph-mass selective detector (GC/MS). A J&W DB-35MS capillary column (30 m length, 0.25 mm diameter, 0.25 µm film thickness) was used and compounds were quantified using selective ion monitoring. Method parameters for PAH and pesticide analysis were as follows: 70 °C initial hold time of 1 min; 70-150 °C at 20 °C min⁻¹; 150-280 °C at 4 °C min⁻¹, hold for 15 min; 280-295 °C at 5 °C min⁻¹, hold for 2 min; source temperature 150 °C. The carrier gas was helium set to flow at 1.0 mL min⁻¹ with a velocity of 39 cm s⁻¹. The PAHs analyzed in this manner included

naphthalene, azulene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, biphenyl, acenaphthene, fluorene, phenanthrene, anthracene, 1-methylfluorene, 2-methylphenanthrene, 2-methylanthracene, 1-methylanthracene, 1-methylphenanthrene, 9-methylanthracene, fluoranthene, pyrene, 3,6-dimethylphenanthrene, 9,10-dimethylanthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene, coronene. The sum of these compounds are referred to as total PAHs. The organochloride compounds analyzed in this manner include DDT and metabolites, chlordane and isomers, hexachlorocyclohexanes, and heptachlor. Organophosphates targeted in this study include: methylparathion, fenthion, parathion, and azinophos (methyl,ethyl). Triazine herbicides, carbamates, and pyrethroids were also quantified. Minimum detectable quantities were established via quantification of relative response factor dilutions of known concentrations, with minimum acceptable signal to noise ratio of 10. Instrument detection limits for the GC/MS ranged from 0.002 ng to 0.056 ng per 1 μ L injection.

3. Results and discussion

3.1 Trace organic contaminant concentrations in oyster tissue – compound class totals

In general, the concentrations of PAHs, PCBs, organochlorides, and organophosphates found in oyster tissue in this study are similar in magnitude to concentrations found in oyster tissue globally (KUMAR et al., 2008; LAUENSTEIN et al., 2002a). Detectable concentrations of total PAHs (tPAHs) in oysters ranged from 2 to 800 ng grams dry weight⁻¹ throughout both Caloosahatchee Estuary as well as in Estero Bay (Table 1), with generally higher levels in Caloosahatchee Estuary. There did not seem to be any obvious seasonal trend in tPAH concentrations at both sites (Figure 2). Samples which were designated as “not quantifiable” (NQ) contained excessive lipid interferences in the chromatography which precluded confirmation of PAH compound identity. In general, the range of tPAH concentrations in oyster tissue from both sites in this study are lower than that found in Gulf of Mexico oysters (WADE et al., 1988) but similar in concentration to that found in coastal waters of North and South Carolina (LAUENSTEIN et al., 2002b). Many of the PAHs detected at both sites are known to be carcinogens (e.g. benzo[a]pyrene). In general, polychlorinated biphenyl (PCBs) concentrations were only detected in a oyster tissue from a few months during the sampling period and moreover, they were detected slightly more frequently at the reference site, Estero Bay (Table 1). Their limited detection precludes us from making specific statements about their spatial or temporal trends at each location.

The organochlorides analyzed in the oysters in this study are listed in Table 2. Interestingly, organochloride compound concentrations which were detected at mg L⁻¹ concentrations in some cases, were higher in oysters from the reference site, Estero Bay, in December of 2007 and in March, June, September and November of 2008. These levels of organochlorides are higher than those found in oysters isolated from a marsh/estuarine environment in coastal Georgia, USA (Kumar et al., 2008). Concentration ranges of organophosphate pesticides varied broadly between ~5 to 15,000 ng gdw⁻¹ (Table 2). While there were no discernable trends across both sites (Figure 5), organophosphate levels were higher in Estero Bay, the reference site than in Caloosahatchee Estuary. Both organochlorides and organophosphate compounds have relatively high vapor pressures (SCHWARZENBACH et al., 2003). Their

polycyclic aromatic hydrocarbons (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	213.04	20.52	100.04	22.68
Jan-08	144.57	1.44	43.48	10.68
February - 08	143.92	0.00	5.58	2.51
March-08	8.04	4.96	78.79	59.03
April-08	556.66	47.56	11.37	1.55
May-08	0.00	0.00	61.49	20.26
June-08	57.11	46.22	181.21	0.00
July-08	2.79	0.00	0.00	0.00
August-08	293.67	94.41	1.76	0.00
September-08	497.58	313.36	154.34	101.08
October-08	760.91	746.92	82.33	10.76
November-08	151.86	146.75	33.75	0.00

polychlorinated bihenyls (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	0.00	0.00	1.50	0.61
Jan-08	0.69	0.00	0.00	0.00
February - 08	1.03	0.00	0.00	0.00
March-08	0.83	0.00	1.34	0.00
April-08	0.00	0.00	0.47	0.00
May-08	0.00	0.00	0.81	0.00
June-08	0.00	0.00	0.79	0.00
July-08	0.00	0.00	0.00	0.00
August-08	0.73	0.12	0.00	0.00
September-08	0.00	0.00	21.41	0.00
October-08	0.00	0.00	0.81	0.00
November-08	3.40	0.00	0.00	0.00

Table 1. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls in oyster tissue.

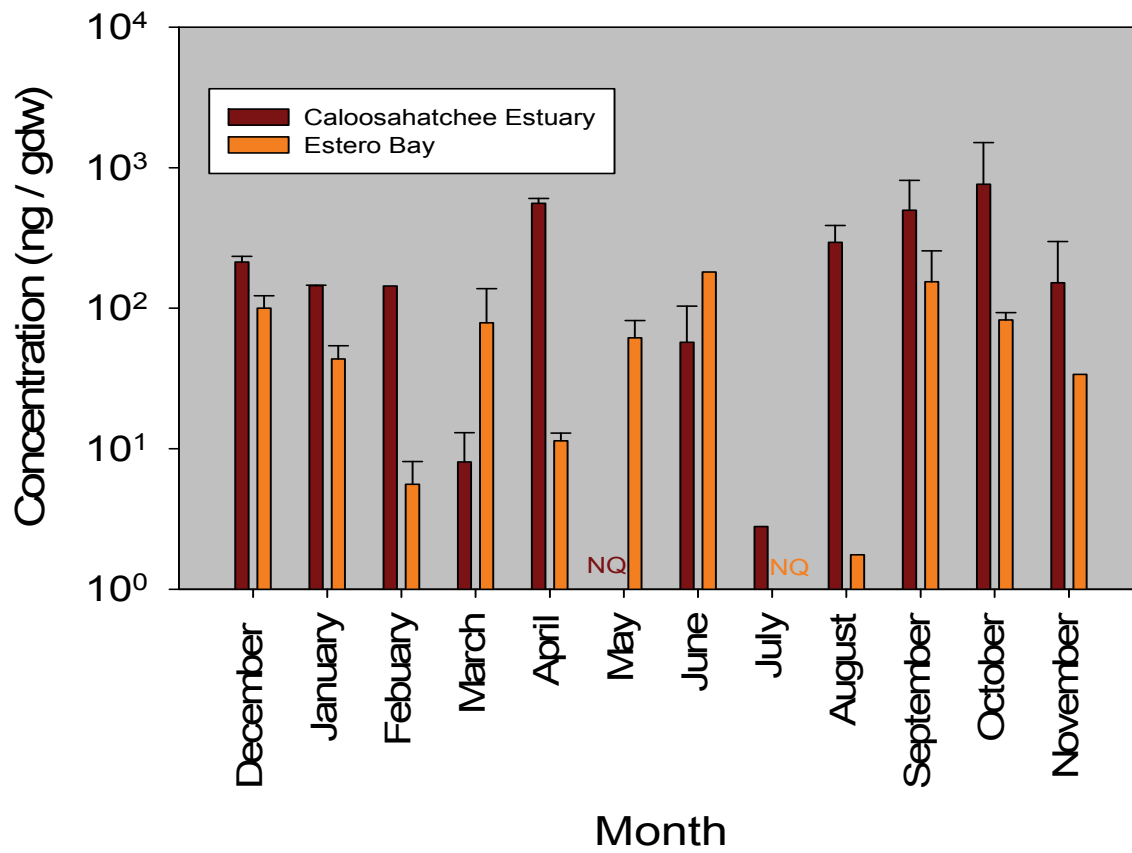


Fig. 2. Total polycyclic aromatic hydrocarbons (PAH) concentrations in Caloosahatchee Estuary and Estero Bay oyster tissue.

presence at detectable levels in both the Caloosahatchee Estuary and Estero Bay may ultimately have resulted from volatilization from agricultural soils from proximal or distal regions (THOMANN and CONNOLLY, 1984). This would account for similar levels of occurrence at both sites. Typically, oysters accumulate trace organic contaminants from sediments in the seabed in areas where they reside. In that context, sedimentary resuspension may have resulted in periodic exposure of the trace organic contaminants in this study. Sediment resuspension events have been demonstrated to result in greater bioaccumulation of metals by oysters in the Gulf of Mexico (JOHNSON et al., 2009). Given that all the sampling collection areas are intertidal and shallow-subtidal, oysters from these areas are exposed to sediment re-suspension through wind- and tidal-driven forces as well as boat wakes.

Triazine herbicides and carbamates were detected periodically in oyster tissue throughout the sampling period at both sites. Concentration ranges varied broadly from the low ng g^{-1} levels to mg g^{-1} levels per dry weight of oyster tissue. Both systems are located proximal to upland areas and/or receive water from agricultural and urban areas that lie within the watershed, where triazine and carbamate compounds may be applied as herbicides and pesticides. Triazines and carbamates remain some of the mostly widely used classes of trace organic pesticides in the continental US. Thus, their detection in these samples is not surprising, given some of the regional applications noted above.

organochlorides (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	32.71	8.18	136.75	36.93
Jan-08	593.84	12.89	37.12	27.07
February - 08	3.73	0.28	2.54	0.21
March-08	54.20	13.16	95.38	5.53
April-08	28.56	6.97	3.76	0.12
May-08	0.00	0.00	29.30	5.60
June-08	88.23	47.87	9346.45	15.65
July-08	10.29	0.00	0.00	0.00
August-08	74.49	59.77	40.88	0.00
September-08	26.44	7.60	1033.27	55.13
October-08	41.27	27.70	24.10	8.89
November-08	5.43	0.46	486.25	0.00

organophosphates (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	25.03	22.74	179.54	122.26
Jan-08	0.00	0.00	5.86	4.21
February - 08	0.51	0.00	1.14	0.00
March-08	303.49	294.65	1344.75	8.29
April-08	38.84	26.34	36.80	0.00
May-08	0.00	0.00	5.88	6.12
June-08	318.45	2.38	10873.40	0.00
July-08	0.00	0.00	0.00	0.00
August-08	2994.87	4697.27	215.17	0.00
September-08	15.52	8.92	15507.29	0.00
October-08	9.98	6.12	15.61	11.07
November-08	25.24	24.48	0.00	0.00

Table 2. Organochlorides and organophosphates in oyster tissue.

triazines (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	2.44	0.00	0.56	0.00
Jan-08	0.00	0.00	2.73	0.29
February - 08	2.37	0.00	24.70	15.64
March-08	37.55	2.19	2.30	1.22
April-08	291.42	264.53	1.36	0.00
May-08	0.00	0.00	1.33	0.00
June-08	11.65	2.61	421.15	0.00
July-08	0.00	0.00	0.00	0.00
August-08	5.76	0.00	0.00	0.00
September-08	77.87	75.58	0.00	0.00
October-08	5.49	0.00	0.54	0.00
November-08	0.00	0.00	0.00	0.00

carbamates (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	11.63	10.52	63.06	0.00
Jan-08	749.80	0.00	0.82	0.00
February - 08	0.00	0.00	2.53	0.83
March-08	0.00	0.00	15.24	0.00
April-08	0.23	0.00	0.00	0.00
May-08	0.00	0.00	0.98	0.00
June-08	3.17	0.00	35.18	0.00
July-08	0.00	0.00	0.00	0.00
August-08	13.93	0.00	0.00	0.00
September-08	0.00	0.00	0.00	0.00
October-08	3.24	0.00	6.03	0.00
November-08	13.21	0.00	2586.04	0.00

Table 3. Triazine herbicides and carbamates in oyster tissue.

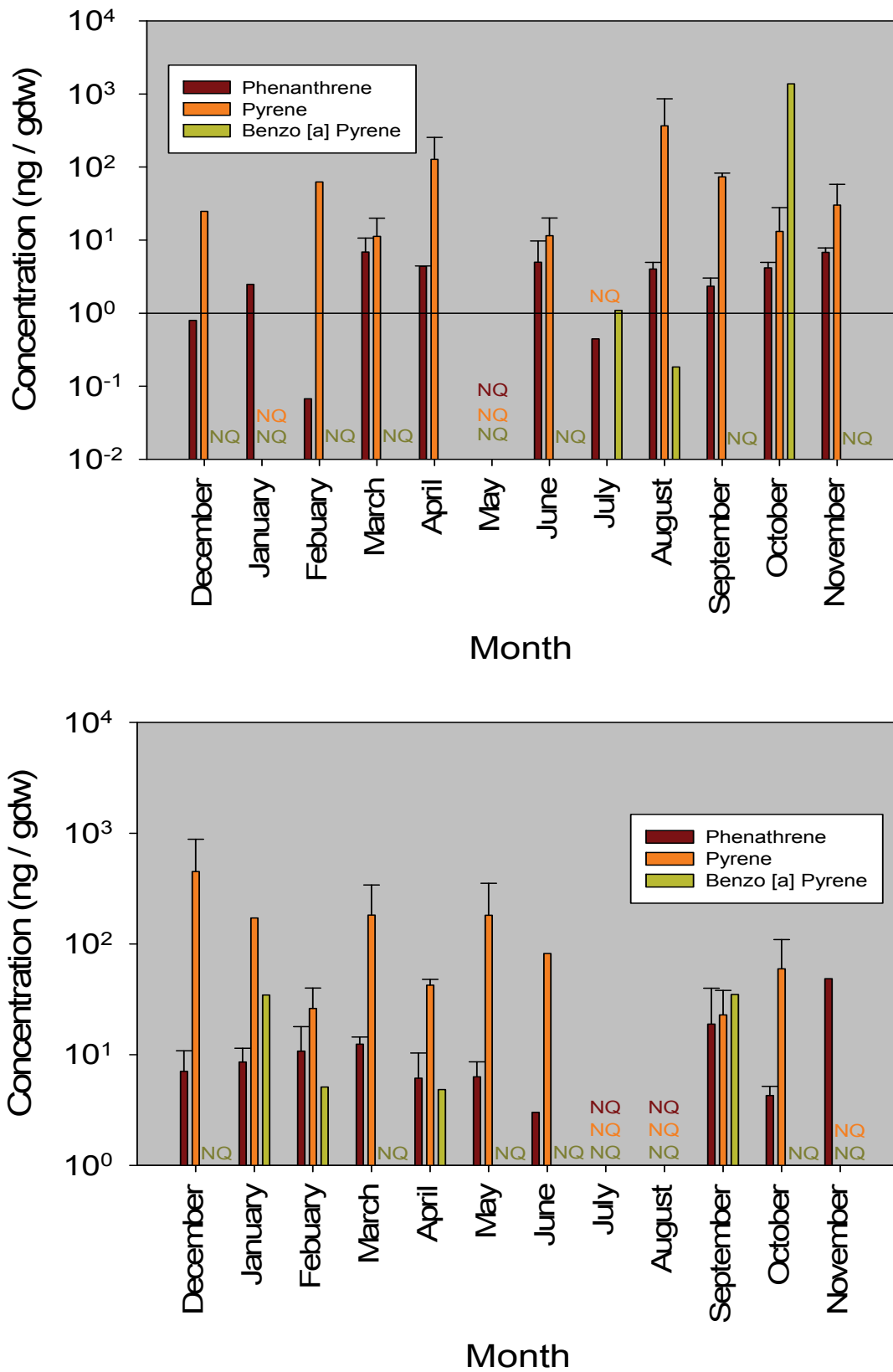


Fig. 3. Selected PAHs in oyster tissue isolated from Caloosahatchee Estuary (top panel) and Estero bay (bottom panel).

pyrethroids (ng g ⁻¹ dry wt)				
Month	Caloosahatchee		Estero	
	average	Std. Dev.	average	Std. Dev.
Dec-07	0.00	0.00	0.00	0.00
Jan-08	0.00	0.00	0.00	0.00
February - 08	0.00	0.00	0.00	0.00
March-08	2.21	0.00	2.21	0.00
April-08	0.00	0.00	0.41	0.00
May-08	0.00	0.00	0.21	0.00
June-08	0.00	0.00	0.00	0.00
July-08	0.00	0.00	0.00	0.00
August-08	0.00	0.00	0.00	0.00
September-08	0.00	0.00	0.00	0.00
October-08	1.51	0.00	0.33	0.00
November-08	0.00	0.00	174.17	0.00

Table 4. Pyrethroids in oyster tissue.

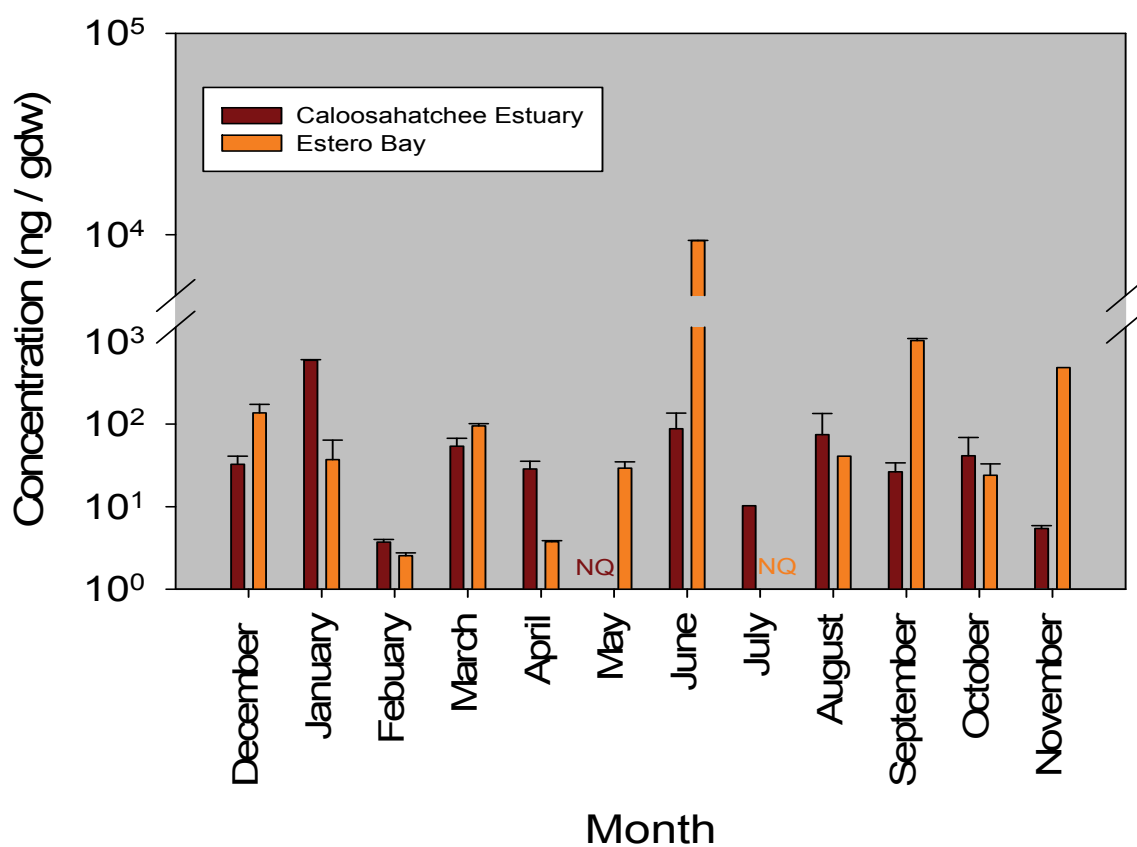


Fig. 4. Organochlorides in Caloosahatchee Estuary and Estero Bay oyster tissue.

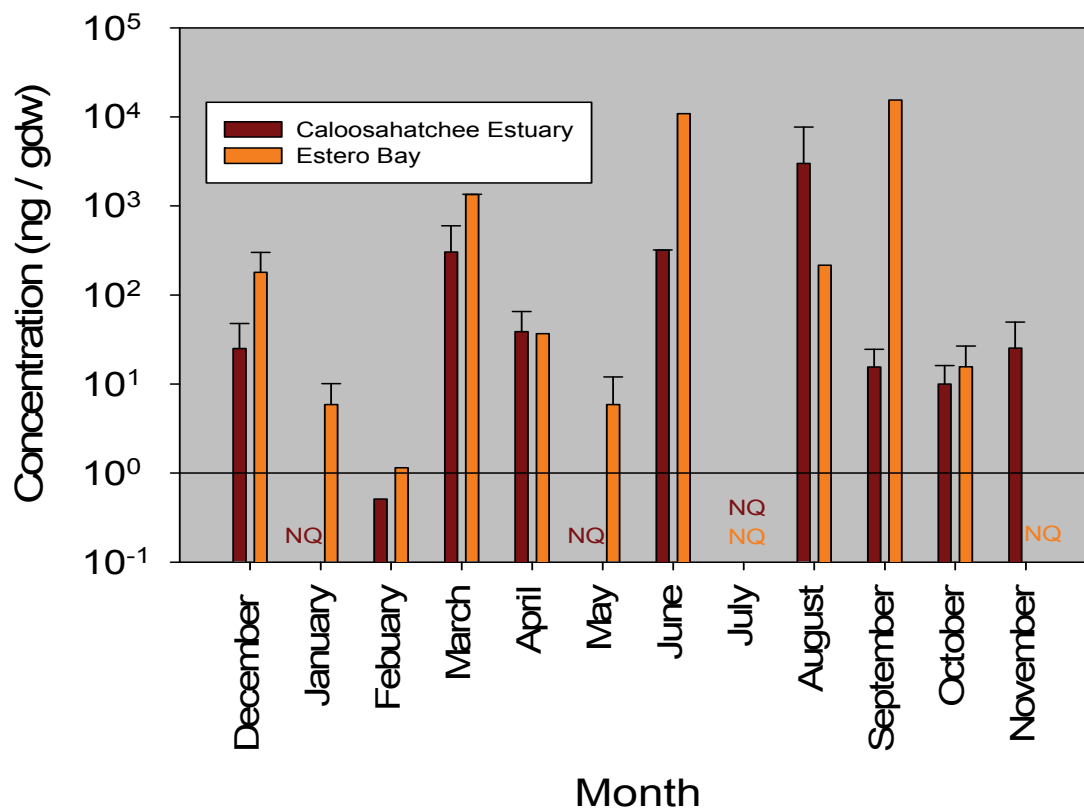


Fig. 5. Organophosphates in Caloosahatchee Estuary and Estero Bay oyster tissue.

3.2 Trace organic contaminant concentrations in oyster tissue – individual PAHs

In general, PAHs enter modern aquatic environments as a byproduct of combustion processes or are petroleum derived. While some PAHs are low enough in molecular weight that they remain in solution, in general, mid to high molecular weight PAHs (e.g. phenanthrene to indeno[1,2,3-cd]pyrene) associate with particulate or dissolved organic matter in the environment. Such particulate or dissolved organic matter upon association with sediments in aquatic systems can enter the benthic food webs. Although we did not quantify organic contaminants in sediment, we can use the size/molecular weight of various PAHs to help understand PAH fate and transport, and ultimately their sources in the environment. Low molecular weight PAHs are more water soluble relative to their high molecular weight counterparts (MACKAY et al., 1992). Thus, observations of low molecular weight PAH bioaccumulation may imply that the aqueous phase served as a relatively more efficient route of entry into an organism. In contrast, high molecular weight PAHs tend to be more particle reactive so their abundance in oyster tissue could be indicative of more-particle rich sources of trace organic contaminants (MACKAY et al., 1992). Alternatively, an absence of high molecular weight PAH accumulation may suggest that PAH availability to oysters was limited by desorption from particles. Indeed, PAHs tightly absorbed to combustion-derived particles such as soot can be minimally bioavailable (ACCARDI-DEY and GSCHWEND, 2003; LOHMANN et al., 2005).

Concentrations of phenanthrene, pyrene, and benzo[a]pyrene were examined closely in oyster tissue from both sites. In most cases, oyster tissue was higher in pyrene relative to

phenanthrene in both Caloosahatchee Estuary and in Estero Bay. This observation of a mid-molecular weight PAH being accumulated to a greater extent than a low molecular weight PAHs, has been observed in other contaminant accumulation studies with benthic organisms (Landrum, 1989). One explanation for this observation is that lower molecular weight PAHs such as phenanthrene may be accumulated and then metabolized and subsequently excreted by benthic organisms in contrast to larger PAHs which are more difficult to metabolize (Landrum, 1989). Alternatively, low molecular weight PAHs as well as other low molecular weight trace organic contaminants may simply not be in abundance as they have higher vapor pressures, volatilization rates, and microbial mineralization rates, resulting in faster disappearance from the aquatic ecosystem in which they reside (MACKAY et al., 1992). In contrast, high molecular weight and more particle reactive PAHs may have a higher residence time in the environment within which the oysters reside. Benzo[a]pyrene was only detectable in oyster tissue in 3 months out of the year 1 samples at Caloosahatchee Estuary and in four months out of year in samples from Estero Bay. We propose that the infrequency periods of detectable levels of benzo[a]pyrene compared to other PAHs can be explained by benzo[a]pyrene may be irreversibly be bound to soot-like residues obtained from combustion as noted above (LOHMAN and GSCHWEND, 2003).

In conclusion, we observe that middle molecular weight PAH's were present in the highest concentrations. This shows oysters are accumulating their contaminants from both dissolved and particulate-associated compounds. Highest concentrations of PAH's were observed in the Caloosahatchee Estuary. Varying levels of organochlorides were observed within the Caloosahatchee Estuary and Estero Bay. Slightly higher levels of organophosphates were found within the Estero Bay Aquatic Buffer Preserve. Continued monitoring is needed in both of these systems along with an approach designed to target the possible routes of entry of these trace organic contaminants into oysters.

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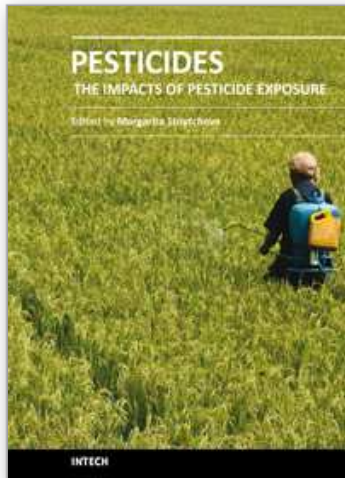
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Pesticides are supposed to complete their intended function without “any unreasonable risk to man or the environment”. Pesticides approval and registration are performed “taking into account the economic, social and environmental costs and benefits of the use of any pesticide”. The present book documents the various adverse impacts of pesticides usage: pollution, dietary intake and health effects such as birth defects, neurological disorders, cancer and hormone disruption. Risk assessment methods and the involvement of molecular modeling to the knowledge of pesticides are highlighted, too. The volume summarizes the expertise of leading specialists from all over the world.

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