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ENDOCRINE-DISRUPTING ALKYLPHENOLS ARE WIDESPREAD IN THE BLOOD OF LOBSTERS FROM SOUTHERN NEW ENGLAND AND ADJACENT OFFSHORE AREAS

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ABSTRACT Endocrine-disrupting pollutants in rivers and oceans represent a poorly understood but potentially serious threat to the integrity of aquatic and coastal ecosystems. We surveyed the hemolymph of lobsters from across southern New England and adjacent offshore areas for 3 endocrine-disrupting alkylphenols. We found all 3 compounds in hemolymph from every year and almost every region sampled. Prevalence of contamination varied significantly between regions, ranging from 45% of lobsters from southern Massachusetts to 17% of lobsters from central Long Island Sound. Mean contamination levels varied significantly as a function of region, year sampled, and collection trip, and were highest overall in lobsters from western Long Island Sound and lowest in lobsters from central Long Island Sound. Surprisingly, lobsters from offshore areas were not less contaminated than lobsters from inshore areas. Contamination levels also did not vary as a function of lobster size or shell disease signs. Contaminated lobsters held in the laboratory did not retain alkylphenols, suggesting that hemolymph contamination levels represent recent, rather than long-term, exposure. Our data set is the first, to our knowledge, to survey endocrine-disrupting contaminants in a population across such a broad temporal and spatial scale. We show that alkylphenol contamination is a persistent, widespread, but environmentally heterogeneous problem in lobster populations in southern New England and adjacent offshore areas. Our work raises serious questions about the prevalence and accumulation of these endocrine-disrupting pollutants in an important fishery species.

KEY WORDS: endocrine disruptor, alkylphenol, lobster, *Homarus americanus*, shell disease

INTRODUCTION

Alkylphenols are vertebrate and inve/rtebrate estrogenic endocrine disruptors (Biggers & Laufer 2004, Laufer et al. 2012a) widely used in the production of alkylphenol ethoxylates, which are used in industrial and household detergents, surfactants, paints, wetting agents, wood pulping, textile manufacture, plastic manufacture, petroleum recovery, and phenolic resins; as antioxidants, polymer stabilizers, and curing agents; and in many other products (Naylor et al. 1992, Naylor 1995, Ying et al. 2002, Soares et al. 2008). Worldwide annual alkylphenol production was estimated to be 500,000 t and increasing in 1997 (Ying 2006), and a large proportion of this material ends up in aquatic or marine environments via discharge from wastewater treatment plants or industry (Soares et al. 2008, David et al. 2009, Harman et al. 2011). Alkylphenol contamination has been reported widely in both freshwater (Soares et al. 2008) and marine (David et al. 2009) ecosystems, particularly in sediments and filter-feeding organisms (e.g., Hale et al. 2000, Munshi et al. 2009, Bouzas et al. 2011). Alkylphenols were also detected in the urine of 95% of humans tested in 2004 (Calafat et al. 2005).

Widespread environmental contamination by alkylphenols is increasingly seen as a serious public health concern because of their high potential to act as endocrine disruptors (Crain et al. 2007, vom Saal et al. 2007). Alkylphenol exposure has been tied to fertility problems, carcinogenic effects, feminization, and a host of other health problems (vom Saal et al. 2007, Soares et al. 2008, David et al. 2009, Meier et al. 2011). In this article, we explore exposure in the American lobster (*Homarus americanus,* Milne Edwards), a commercially and culturally important fishery in southern New England.

Laufer et al. (2004) previously isolated 4 alkylphenolic compounds from the hemolymph of lobsters from Long Island Sound (LIS). The compounds were identified as 2-t-butyl-4-(dimethylbenzyl)phenol (compound 1), 2,6-bis-(t-butyl)-4-(dimethylbenzyl)phenol (compound 2), 2,4-bis-(dimethylbenzyl) phenol (compound 3), and 2,4-bis-(dimethylbenzyl)-6-t-butylphenol (compound 4) (Biggers & Laufer 2004). All 4 of these compounds had juvenile hormone activity, inducing metamorphosis in larvae of the annelid *Capitella* (Biggers & Laufer 1996, Biggers & Laufer 1999, Biggers & Laufer 2004). Juvenile hormone and its crustacean analog, methyl farnesoate, regulate metamorphosis and molting in arthropods (Laufer et al. 1987, Riddiford 1994). All 4 compounds were also detected at high levels in marine sediments from a broad geographical area in the northwest Atlantic (reviewed in Biggers & Laufer (2004)).

Alkylphenols have the potential to affect lobsters negatively in several ways: through disruption of tanning and sclerotization during molting (e.g., Sacher 1971, Zomer & Lipke 1981, Sugumaran et al. 1992), and through endocrine disruption (e.g., Sumpter 1995, Hayes et al. 2006, Johnson et al. 2008, Ostrach et al. 2008, Planello et al. 2008, Ramakrishnan & Wayne 2008, Zhang et al. 2008, Meier et al. 2011). Laufer et al. (2012b) have demonstrated that alkylphenols are incorporated into lobster cuticles during molting, and that this is correlated with weaker cuticular structure, possibly because of interference with protein

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cross-linking during shell hardening. If weaker shells are more susceptible to microbial invasion, then this may provide a mechanistic link between these pollutants and shell disease (Smolowitz et al. 2005).

Endocrine-disrupting activity on lobsters has been demonstrated for compound 3 and the related alkylphenol bispheno A. Larval lobsters exposed to 5 ng/day or 10 ng/day of alkylphenols in their diets experienced abnormal metamorphosis, as well as reduced survival and delay of metamorphosis (Laufer et al. 2012a). Compound 2 has been shown to disrupt sclerotization and metamorphosis of mosquito larvae, even at relatively low (0.1 ppm) doses (Sacher 1971, Zomer & Lipke 1981, Semensi & Sugumaran 1986). Larval crabs exposed to compound 2 also experienced high mortality and behavioral effects on swimming speed and phototaxis (Forward & Costlow 1976).

The purpose of the current study was to assess alkylphenol levels in lobsters by measuring spatial and temporal patterns of contamination in the hemolymph of lobsters from southern New England and adjacent offshore waters. We compared prevalence and severity of contamination between geographical areas, over time, and between collection trips. We looked for correlations between alkylphenol levels and shell disease status, as well as carapace length. We also held lobsters in the laboratory to measure retention time of alkylphenols in hemolymph. To our knowledge, this is the first long-term and large-scale study of alkylphenol contamination in a marine population.

MATERIALS AND METHODS

Animals

We obtained 766 lobsters from commercial fishermen, the Connecticut Department of Environmental Protection, Millstone Environmental Laboratory, University of Rhode Island and Rhode Island Department of Environmental Management, and the Massachusetts Division of Marine Fisheries during 2002 to 2008. Lobsters were from 1 of 7 regions (Fig. 1): western LIS (LIS West); central LIS (LIS Central); eastern LIS (LIS East); Narragansett Bay, RI (RI); Buzzards Bay or Vineyard Sound, MA (MA South); Cape Cod Bay; or offshore between Munson Canyon and the Hague Line (Offshore). The number of sampling trips for each region for each year can be found in Figure 2.

On delivery, lobsters were bled and maintained in cooled running seawater (circa 18 ± 2°C) in A-frame fiberglass tanks at the Marine Biological Laboratory, Woods Hole, MA, or in fiberglass tanks filled with recirculating artificial seawater equipped with aeration and biofilters at the University of Connecticut. The animals were fed 3 times per week with fish or squid.

Hemolymph samples (2–4 mL) were taken from the dorsal heart of each lobster with 5-mL plastic syringes and 23-gauge needles, and then transferred to 15-mL Pyrex test tubes containing an ice-cold mixture of acetonitrile (2 mL) and an aqueous 4% NaCl solution (2 mL). Samples were mixed and

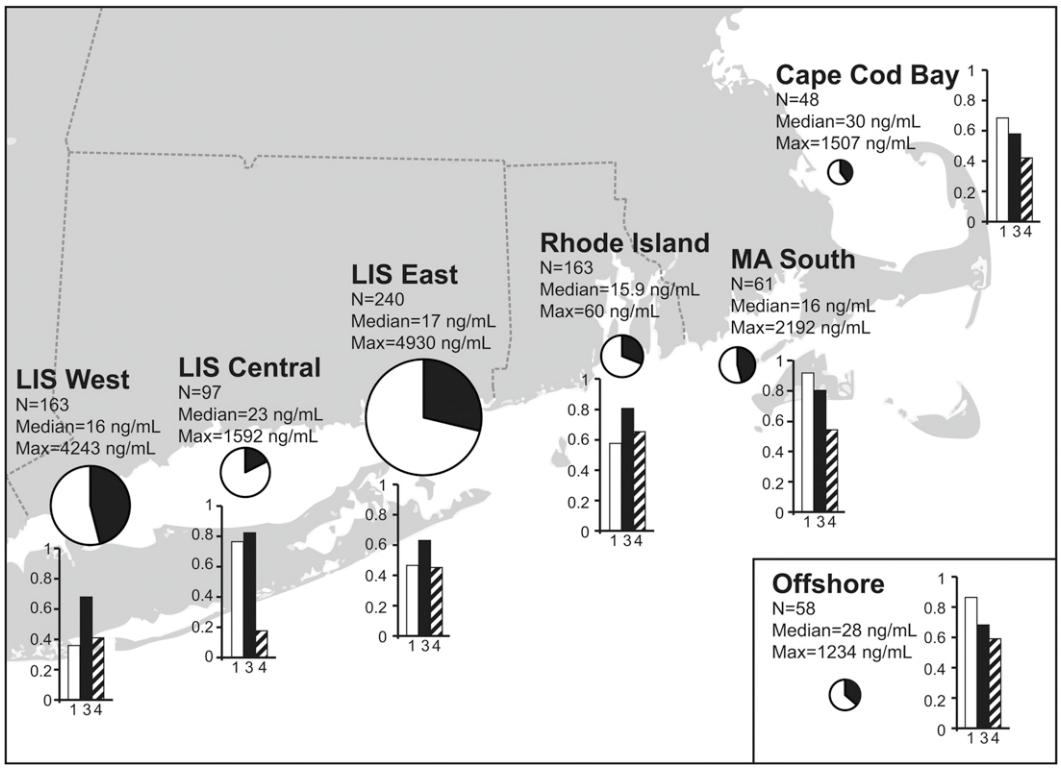

Figure 1. Prevalence (dark portions of pie charts) of alkylphenol contamination across southern New England and adjacent offshore areas. Pie charts are proportional to total sample size (*n*). Medians are for contaminated lobsters only. Bar charts show proportion of contaminated lobsters containing compounds 1, 3, and 4, labeled by number.

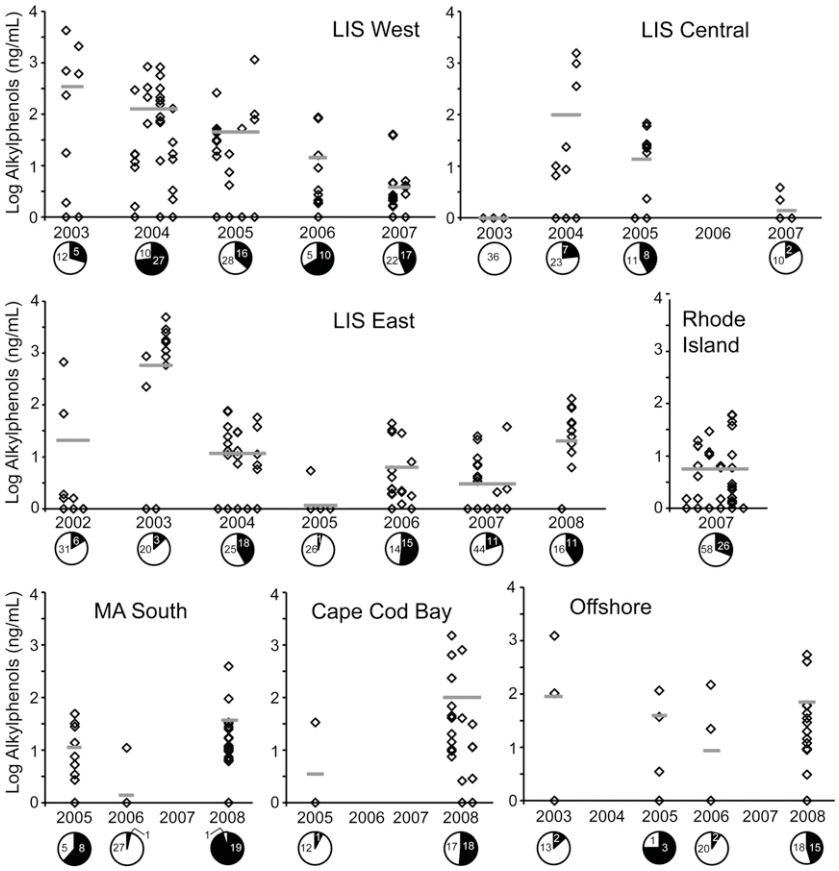

Figure 2. Log alkylphenol concentration (measured in nanograms per milliliter) plotted over time for each region. Each point represents an individual lobster. Collection trips within each year are offset. Pie charts below show the prevalence of alkylphenols and gray bars show mean contamination level for lobsters from that region in that year for all collection trips combined.

stored at –20°C before preparation for analysis. Many of the lobsters were bled multiple times after capture for the purposes of a separate experiment (Laufer et al. unpubl. data). To obtain an indication of how contaminant levels may change over time in lobster hemolymph, we searched our database of analyzed hemolymph samples to identify 14 lobsters that were initially contaminated and had been retested within 2 mo of capture. Of these, 1 was retested after 7 days, 7 were retested after 20 days, and 7 were retested after 40 days.

Chemicals and Materials

High purity standards of 4-cumylphenol (also known as 4-dimethylbenzylphenol), compound 3, and the chemical standard phenanthrene were purchased from Matheson, Coleman, & Bell; the chemical standard biphenyl was purchased from Sigma-Aldrich. Compounds 1, 2, and 4 were synthesized in the laboratory as described by Biggers & Laufer (2004). HPLC-grade acetonitrile, acetone, ether, hexane, methanol, and methylene chloride were purchased from Fisher Chemical Corporation. The cartridges used for solid-phase extraction (SPE) were Envi-Chrom P (Supelco/Aldrich) hydrophobic cartridges with 250 mg styrene divinylbenzene.

Analytical Procedures

Samples were prepared as described by Laufer et al. (2005). We processed 16 samples at a time using microwave-assisted extraction in 20 mL 1:1 methanol:methylene chloride. Extracts were filtered through Fisherbrand P5 filter paper (porosity, medium; flow rate, slow) to remove particulates, and the filtrate was collected in a 40-mL volatile organic analysis vial and vortexed with 5 mL aqueous 0.9% KCl to salt out the organic analytes into the organic solvent layer. The top (aqueous) phase of each sample was pipetted into a 100-mL volumetric flask and set aside, and the bottom (methylene chloride) phase was evaporated to dryness overnight under a stream of nitrogen gas. Dried samples were resuspended in 1.5 mL methanol, recombined with the aqueous phase, and diluted to 100 mL with pH 2 deionized water and 0.5g NaCl prior to SPE.

SPE cartridges were activated with 3×5 mL methanol and then conditioned with 3×5 mL pH 2 deionized water. We added each 100-mL extract to an SPE cartridge at a flow rate of about 1 mL/min under vacuum. Loaded cartridges were rinsed with 3×5 mL pH 2 deionized water and then dried for at least 30 min under a stream of nitrogen gas. We eluted each sample with 4×2 mL methanol into a 10-mL glass tube, then the eluates were evaporated to dryness overnight under a stream of nitrogen gas.

Dried samples were resuspended in 100 µL methanol plus 0.100 µg/mL of biphenyl and phenanthrene, both used as internal standards, and transferred carefully to 300-µL glass inserts with polymer feet (Agilent 5181–1270) placed in 1.5 mL amber GC/MS screw-cap vials (Agilent 5182–0716) with

Teflon-lined septae (Agilent 5182–0725), using 2 × 50-μL rinses of methanol for a total sample volume of 200 μL.

Gas Chromatography/Mass Spectroscopy

One microliter of the final extract was injected into either a Finnigan MAP/Thermo gas chromatograph/ion trap mass spectrometer (San Jose, CA) or a Hewlett-Packard (now Agilent, Wilmington, DE) 5890 Series II Gas Chromatograph paired with a 5970 Mass Selective Detector operating under HP Standard ChemStation, version A 0.300, 1986–1996. Separation of the compounds was achieved using either an Agilent DB-5MS capillary column or a Thermo/Fischer TR-1/MS capillary column. Columns measured 30 m × 0.25 mm × 0. 25-μm film thickness, and used either a methyl/phenyl or a methyl/silicone liquid phase. Helium was used as the carrier gas. The operating conditions for GC were an initial temperature of 50°C for 2 min, followed by a 15°C/min ramp to 250°C, followed by a second temperature ramp to 270°C at 5°C/min, and finally a 5-min hold at 270°C to bake the column. In each case, we used a splitless injection, set at 240°C with the M.S. interface maintained at 280°C.

Analysis by mass spectrometer was performed using the selective ion monitoring mode, and quantification was performed as described by Laufer et al. (2005), but with phenanthrene (M.S. 178) as the internal standard. The detection limit of the method (determined using the lowest concentration of each standard giving a signal-to-noise ratio of 3:1) was ≤ 1 ng/mL for each compound, and we recalibrated every 2–3 mo using known standards to ensure that results from different years and different GC/MS equipment were comparable. Percent recoveries were determined by spiking the 3 different compounds at various levels with the same concentration at each level of the internal standard using uncontaminated lobster blood. Percent recovery ±SD for the entire method (extraction + purification + GC/MS) based on positive controls with known standards was 21 ± 16% for compound 1, 27 ± 4% for compound 3, and 29 ± 15% for compound 4. Compound 2 was excluded from the study because percent recoveries were too low (<5%). Although recoveries from biological samples are typically lower than recoveries from water or sediment samples (e.g., Mouatassim-Souali et al. 2003) because of additional purification steps such as filtration, SPE clean-up and concentration, and blow-down steps (Gadzala-Kopciuch et al. 2008), our recoveries for compounds 1, 3, and 4 are still lower than would be considered ideal for this type of analysis. Thus, our findings for both the prevalence of and concentration levels of these alkylphenols represent extremely conservative estimates.

Statistical Analysis

All statistical tests were performed using JMP 6.0 (SAS Institute, Inc). We calculated the proportion of contaminated lobsters from each collection trip that returned at least 4 lobsters, and then analyzed prevalence of contaminants in lobster hemolymph as a function of year and region using a 2-factor nested analysis of variance on arc-sin square root-transformed proportions, with year and region nested within year as the fixed factors. We excluded MA South (all years), Offshore (all years), LIS West (2006), and Cape Cod Bay (2005) because we did not have data from more than 1 collection trip for those regions in those years. This left a total of 55 collection trips in the analysis, which we performed for total alkylphenols and also individually for each of the 3 compounds. Because we did not find an effect of year in any of these analyses (Table 1), we then compared prevalence of contaminants between all regions using a 1-factor analysis of variance, which allowed us to expand the analysis to include regions for which we only had data from a single collection trip each year (MA South and Offshore).

We compared variation in log-transformed alkylphenol concentrations recovered from hemolymph between years and collecting trips, and as a function of lobster size and shell disease status for each region separately using a 3-factor nested analysis of covariance on log-transformed data with year, collecting trip (nested within year), and shell disease status as fixed factors, and carapace length as a covariate. Within each region, years without at least 2 collection trips were excluded (Fig. 2, Table 2). We were not able to test the effects of year for Rhode Island or Cape Cod Bay because we did not have more than 1 y with multiple collection trips for either location. For RI, we included 6 collection trips from 2007, and for Cape Cod Bay we included 1 collection trip from 2005 and 3 from 2008. We were unable to test the effects of collection trip for MA South and Offshore because we only obtained lobsters from 1 collection trip per year for these regions.

We used a 3-factor nested analysis of variance to assess variation in log-transformed alkylphenol levels in hemolymph as a function of region (LIS West, LIS Central, or LIS East) and year (2003, 2004, 2005, and 2007). These were the only region × year combinations for which we had adequate sample sizes for a full factorial analysis. We also used nested 2-factor analyses of variance to compare log-transformed alkylphenol contaminant levels in lobsters between RI and LIS locations in 2007, and between Cape Cod Bay and LIS East in 2008. Because we obtained lobsters from only 1 collection trip per year in MA South and Offshore, we were unable to compare mean contaminant levels statistically in these lobsters with those from other regions.

Using a data set that included only contaminated lobsters, we compared the proportion of total contamination accounted for by compounds 1 and 3, the most widespread contaminants, as a function of year and region using an analysis of covariance. Lobsters that did not test positive for at least 1 contaminant, and region × year blocks that contained fewer than 3 lobsters, were excluded from the analysis, leaving a total sample size of

TABLE 1.

Analysis of variance in prevalence of contamination for each compound, and for all compounds lumped together, as a function of year and region with region nested within year.

Compound	*df*	Variable	F	*P*
All	4	Year	1.517	0.199
	12	Region(year)	1.335	0.248
C1	4	Year	2.217	0.062
	12	Region(year)	0.908	0.536
C34	4	Year	0.605	0.725
	12	Region(year)	1.593	0.146
C4	4	Year	1.489	0.208
	12	Region(year)	1.614	0.140

TABLE 2.

Analysis of variance in mean contamination levels as a function of year, collection trip, carapace length, and shell disease status, summarized separately for each region with collecting trip nested within year.

Region	Years Included	Source of Variation			
		Year	Collecting Trip	Carapace Length	Shell Disease
LIS west	2003–2005, 2007	$F_{3,142} = 10.89$, $\mathbf{P < 0.0001}$	$F_{7,142} = 4.699$, $\mathbf{P < 0.0001}$	$F_{1,142} = 0.204$, $P = 0.6520$	$F_{1,142} = 0.048$, $P = 0.827$
LIS central	2003–2005, 2007	$F_{3,96} = 4.117$, $\mathbf{P = 0.0054}$	$F_{6,96} = 8.812$, $\mathbf{P = 0.0003}$	$F_{1,96} = 0.004$, $P = 0.9042$	$F_{1,96} = 0.006$, $P = 0.9407$
LIS east	2002–2008	$F_{6,248} = 38.24$, $\mathbf{P = <0.0001}$	$F_{17,248} = 73.13$, $\mathbf{P < 0.0001}$	$F_{1,248} = 0.112$, $P = 0.5325$	$F_{1,248} = 0.059$, $P = 0.8089$
Rhode Island	2007	—	$F_{5,83} = 3.870$, $\mathbf{P = 0.0035}$	$F_{1,83} = 0.000$, $P = 0.9985$	$F_{1,83} = 0.426$, $P = 0.5162$
Massachusetts south	2005, 2006, 2008	$F_{2,59} = 35.42$, $\mathbf{P < 0.0001}$	—	$F_{1,59} = 1.081$, $P = 0.3030$	$F_{1,59} = 0.060$, $P = 0.8068$
Cape Cod Bay	2005, 2008	—	$F_{3,47} = 8.240$, $\mathbf{P = 0.0002}$	$F_{1,47} = 1.367$, $P = 0.2490$	$F_{1,47} = 2.145$, $P = 0.1505$
Offshore	2003, 2006, 2008	$F_{2,45} = 6.995$, $\mathbf{P = 0.0024}$	—	$F_{1,45} = 0.0001$, $P = 0.9920$	$F_{1,45} = 0.0155$, $P = 0.9016$

$F_{x,y}$ are the F statistic and the numerator and denominator degrees of freedom. P values in bold type are significant at $\alpha = 0.05$.

208 lobsters. The log-transformed contaminant level of compound 1 was the dependent variable, with year and region as fixed factors and the log-transformed level of compound 3 contamination as the covariate.

RESULTS

Alkylphenols Are Widely Distributed

We found alkylphenols in the hemolymph of lobsters from all areas examined. Overall, we found alkylphenols in the hemolymph of 252 of 766 lobsters (33%). Compound 3 was the most pervasive contaminant (24% of all lobsters sampled), followed by compound 1 (19%) and compound 4 (15%). Of the 252 contaminated lobsters, 111 (44%) were contaminated with 1 alkylphenol, 66 (26%) were contaminated with 2 alkylphenols, and 75 (30%) were contaminated with all 3 alkylphenols.

Prevalence of alkylphenols in lobster hemolymph was not significantly different between regions or years, either for all compounds combined or for individual compounds (Table 1). Overall prevalence of alkylphenols in hemolymph ranged from 18% in LIS Central to 46% in MA South (Fig. 1). Prevalence of compound 1 ranged from 13% (LIS East) to 43% (MA South), prevalence of compound 3 ranged from 14% (LIS Central) to 34% (MA South), and prevalence of compound 4 ranged from 3% (LIS Central) to 22% (Offshore). Although MA South and Offshore had some of the highest overall prevalence of contaminants, when these regions were included in a reduced analysis with ''year'' excluded (see Materials and Methods), prevalence of alkylphenols was still not significantly different between regions, either for all compounds combined ($F_{6,58} = 1.46$, $P = 0.21$) or for compounds 1 ($F_{6,58} = 1.08$, $P = 0.38$), 3 ($F_{6,58} = 1.25$, $P = 0.29$), or 4 ($F_{6,58} = 1.03$, $P = 0.41$).

The number of lobsters per collection trip varied between 4 and 29, with a median value of 10 lobsters per trip. We did not observe a relationship between sample size of a trip and the proportion of lobsters from that trip that were contaminated ($R^2 = 0.004$), suggesting that small sample sizes did not underestimate or overestimate systematically the prevalence of contamination.

Alkylphenol Concentrations Vary Spatially and Temporally

Levels of alkylphenols in lobster hemolymph were extremely variable and ranged from our detection limit of 1 ng/mL to 4,930 ng/mL (Figs. 1 and 2). Within regions, mean alkylphenol levels varied significantly as a function of year and collection trip, but not carapace length or shell disease status (Table 2). Temporal trends were different for different regions (Figs. 2 and 3). In LIS West, mean alkylphenol levels declined steadily between 2003 and 2008. We did not detect any alkylphenols in LIS Central in 2003, but observed a decline between 2004 and 2007. In LIS East, alkylphenol levels fluctuated between 2002 and 2008. Interestingly, mean alkylphenol levels increased in 2008 for all regions sampled in that year (LIS East, MA South, Cape Cod Bay, and Offshore).

Alkylphenol levels also varied significantly between regions. Within LIS, total contaminant levels ($F_{2,364} = 16.70$, $P < 0.0001$) and levels of compound 3 ($F_{2,364} = 16.32$, $P < 0.0001$) were highest in LIS West, intermediate in LIS East, and lowest in LIS Central. Similarly, levels of compound 4 were higher in LIS West and LIS East compared with LIS Central ($F_{2,364} = 14.66$, $P < 0.0001$). Levels of compound 1, however, did not vary significantly as a function of region within LIS ($F_{2,364} = 0.06$, $P = 0.95$).

There was no significant difference in the level of total contamination ($F_{3,175} = 1.86$, $P = 0.14$) or in the level of compound 3 ($F_{3,175} = 0.67$, $P = 0.57$) between lobsters from RI and lobsters from LIS locations in 2007 (the only year in which RI was sampled). However, in that same year, RI lobsters had significantly higher levels of compound 1 compared with those from LIS East and LIS West (with LIS Central as intermediate; $F_{3,175} = 6.36$, $P = 0.0004$), and there was also a strong trend for higher levels of compound 4 in RI lobsters compared with LIS lobsters ($F_{3,175} = 2.38$, $P = 0.07$). Levels were not significantly different between Cape Cod Bay and LIS East in 2008 for total alkylphenols ($F_{1,57} = 0.12$, $P = 0.73$), compound 1 ($F_{1,57} = 1.15$, $P = 0.29$), compound 3 ($F_{1,57} = 1.27$, $P = 0.27$), or compound 4 ($F_{1,57} = 2.47$, $P = 0.12$).

Among contaminated lobsters, the median level of alkylphenols was highest in Cape Cod Bay (31 ng/mL) and lowest in

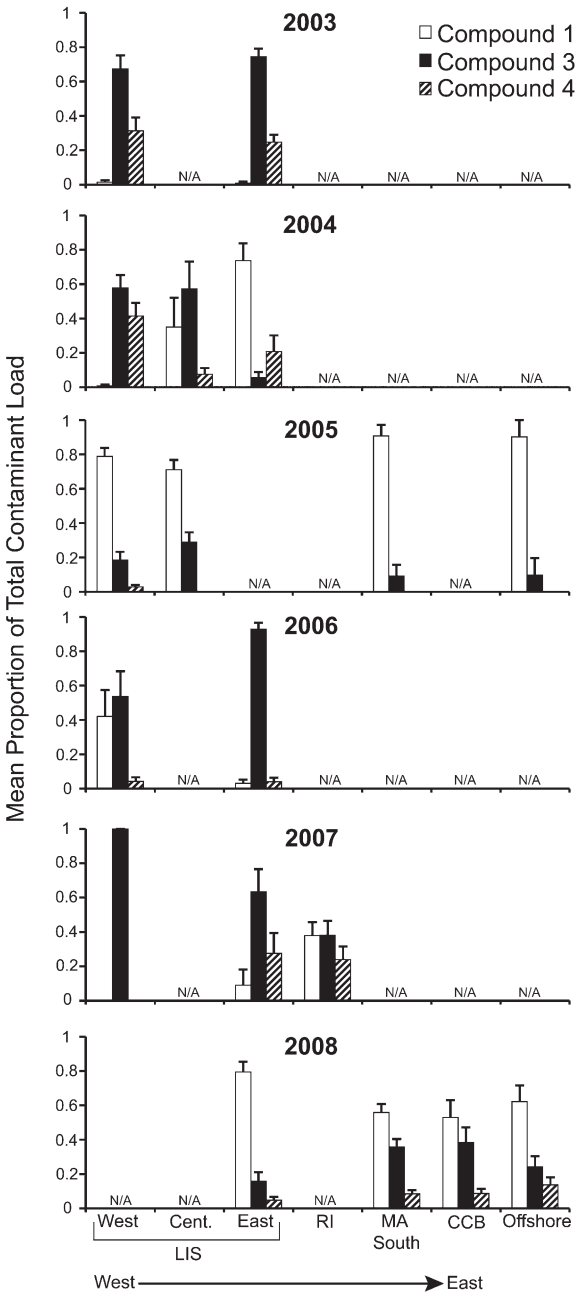

Figure 3. Regional variation in the composition of the total alkylphenol contaminant load, shown separately for each year. Lobsters without any detectable contaminant were excluded, and region × year combinations with fewer than 3 lobsters were excluded, for a total sample size of 208 lobsters. Regions are arranged from west to east, and error bars are SEs.

RI (5.2 ng/mL; Fig. 1). Maximum alkylphenol level was highest in LIS East (4,930 ng/mL) and lowest in RI (60 ng/mL; Fig. 1). However, all these spatial trends varied greatly from year to year (Fig. 2). Contamination by compound 1 was not significantly correlated overall with contamination by compound 3 ($F_{1,208} = 2.72$, $P = 0.10$), but we did observe significant interactions between compound 3 levels and year ($F_{5,208} = 3.16$, $P = 0.01$) and between compound 3 levels and region ($F_{6,208} = 4.69$, $P = 0.0002$). Compound 1 displayed significantly higher levels of contamination in 2005 and 2008 compared with other years ($F_{5,208} = 19.82$, $P < 0.0001$), and constitutes the highest proportion of contaminants recovered from lobsters in those years (Fig. 3). In contrast, compound 3 was found in the highest proportions among contaminated lobsters in 2003, 2006, and 2007 (Fig. 3). Regional patterns are difficult to interpret because different regions were sampled in different years, but compound 3 generally constituted a higher proportion of the contamination load for lobsters collected from western regions compared with lobsters collected from eastern regions (Fig. 3).

TABLE 3.

Change in alkylphenol contamination levels in the hemolymph of initially contaminated lobsters held in the laboratory for 7, 20, or 40 days.

Initial Hemolymph (ng/mL)				Final Hemolymph (ng/mL)		
C1	C3	C4	No. of Days	C1	C3	C4
0	15	51	7	0	0	0
0	1	0	20	0	6	0
3	1	0		0	1	0
5	1	1		0	0	0
5	1	1		0	0	0
0	772	554		0	0	0
312	562	529		0	0	0
338	651	630		0	0	0
0	1	0	40	0	1	0
0	3	0		0	0	0
2	2	1		0	3	0
0	6	0		0	0	0
3	13	1		0	0	0
0	13	89		0	0	0
320	646	679		224	578	333

C1, C3, and C4 denote compounds 1, 3, and 4, respectively.

Alkylphenols Do Not Accumulate in Hemolymph

Alkylphenols disappeared from the hemolymph of many initially contaminated lobsters after they were held in the laboratory. We found that alkylphenols disappeared from the hemolymph of the single lobster tested after 6 days, 4 of 7 lobsters tested after 20 days, and 4 of 7 lobsters tested after 40 days (Table 3).

DISCUSSION

We detected all 3 alkylphenolic compounds in the hemolymph of lobsters from every region and in every year sampled. This suggests that these pollutants are ubiquitous and may be difficult to trace to any particular source. Although both prevalence and level of alkylphenols were extremely variable, some broad temporal and geographical patterns do emerge.

Lobsters from the easternmost areas (MA South, Cape Cod Bay, and Offshore) had higher concentrations of compound 1 compared with compounds 3 and 4, and compound 1 was also the compound detected most frequently from these areas. However, lobsters from these regions were collected primarily in 2005 and 2008, years in which we also observed much higher proportions of compound 1 in LIS. Lobsters from western areas (LIS) had higher concentrations of compound 3 compared with

compounds 1 and 4 in all years except 2005 and 2008, and compound 3 was the most frequently detected compound from these areas. Within LIS, compound 3 also displayed significantly higher overall contamination levels in western regions. Contamination levels observed for compounds 1 and 3 were not significantly correlated overall, but the relative proportions of these compounds did vary significantly with both year and region. Taken together, these data provide evidence of spatial variation in total alkylphenol contamination and contamination by individual compounds over intermediate scales, although additional sampling of eastern and western regions in the same year are required to determine whether these patterns hold outside of LIS. However, the sources of alkylphenolic pollutants in marine habitats may vary over broad geographical scales, and it is reasonable to hypothesize that the LIS basin may present a barrier to movement of environmental contaminants between the Sound and other regions.

Our collecting trip data show that contamination is extremely variable; the prevalence of alkylphenols in hemolymph was often dramatically different between collecting trips conducted in the same location and the same year (e.g., 0% vs. 100% for the two 2008 trips in LIS East; see Fig. 2). We did not detect any monthly pattern in prevalence or level of alkylphenol contamination (data not shown), although our sampling was not designed to test for this effect, and we cannot eliminate the possibility that monthly patterns are masked by spatial and annual variation in our data set.

Alkylphenol contamination in lobster hemolymph disappeared or (in all but 2 cases) decreased when lobsters were held in the laboratory for 20–40 days. We also found no correlation between alkylphenol contamination and lobster size (this study) or sex (Jacobs et al. 2009), despite the fact that large lobsters molt less often than small lobsters, and female lobsters molt less often than males. Taken together, these results suggest that alkylphenols do not persist in lobster hemolymph during intermolt. Lobsters may be able to clear contaminants by excreting them through the gills or other excretory organs, or sequestering them in tissues such as the hepatopancreas, epidermis, gonads, or even the cuticle during molting, although these potential pathways for the metabolism and excretion of alkylphenols remain untested. Consistent with this hypothesis, Laufer et al. (2005) observed female lobsters with uncontaminated hemolymph carrying contaminated embryos. Egg-bearing female lobsters do not molt, so assuming the alkylphenols passed to the ovaries from the hemolymph, this observation suggests that the lobsters were able to clear the contaminants from their hemolymph (but not their eggs) during intermolt.

We were surprised to find that the prevalence and level of alkylphenol contamination in the hemolymph of lobsters from offshore canyons equaled or exceeded contamination levels for inshore lobsters. If alkylphenols are transported passively offshore in sediments, then we would expect sites hundreds of miles offshore to have lower contamination levels in sediment, as has been shown for other environmental contaminants (Rees et al. 1999, Boonyatumanond et al. 2006, Zoller 2006, Rato et al. 2008). Many offshore marine organisms are known to accumulate environmental contaminants, and thus carry contamination loads that are surprisingly high relative to ambient levels (e.g., Scott et al. 2007, Rato et al. 2008). Lobsters may be exposed to alkylphenols directly through sediment or water, or through prey items with elevated levels of contaminants.

We were also surprised to find no correlation between the prevalence or severity of alkylphenol contamination and shell disease signs (Jacobs et al. 2009), despite the fact that contaminated lobsters have weaker cuticles (Laufer et al. 2012b). However, shell disease takes some time to develop (Smolowitz et al. 2005), and we have shown that alkylphenols in hemolymph disappear relatively quickly. If weaker cuticles increase vulnerability to shell disease, then we would expect the alkylphenol content of cuticle to be correlated with shell disease status. It would, however, be technically difficult to test this because such an assay would require breaking apart the cross-linked proteins of the fully hardened cuticle.

Our data set shows that alkylphenol contamination is a persistent, widespread, but environmentally heterogeneous problem in lobster populations in southern New England and adjacent offshore areas. Lobsters in this region are likely repeatedly exposed to these endocrine-disrupting compounds throughout the course of their lifetime. We do not understand all the ways in which these compounds affect the biology of lobsters, but have good reason for concern: Exposure to very low levels of alkylphenols disrupted metamorphosis significantly and was severely toxic for larval lobsters (Laufer et al. 2012a), and weakened cuticles of adult lobsters (Laufer et al. 2012b). Earlier work on other crustaceans (Forward & Costlow 1976, Costlow 1977, Borst et al. 1987, Abdu et al. 1998) and mosquitoes (Sacher 1971) also suggests that alkylphenols may disrupt behavior, metamorphosis, and cuticle hardening of lobsters and related organisms.

We hypothesize that alkylphenol contamination levels in lobster hemolymph represent recent exposure, through sediment or food. Our data suggest that lobsters do not retain alkylphenols in their hemolymph, but unless lobsters are different from most other marine organisms examined, accumulation likely does occur in other tissues, including the edible portions such as the tail muscle and hepatopancreas. Additional work is urgently required to assess the sources and environmental distribution of these and other endocrine-disrupting compounds, and to understand their biological consequences for lobsters and other organisms. It will be particularly important to assess the risk to human health from these and similar contaminants in commercially harvested lobsters.

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