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Comprehensive Screening Links Halogenated Organic Compounds with Testosterone Levels in Male *Delphinus delphis* from the Southern California Bight

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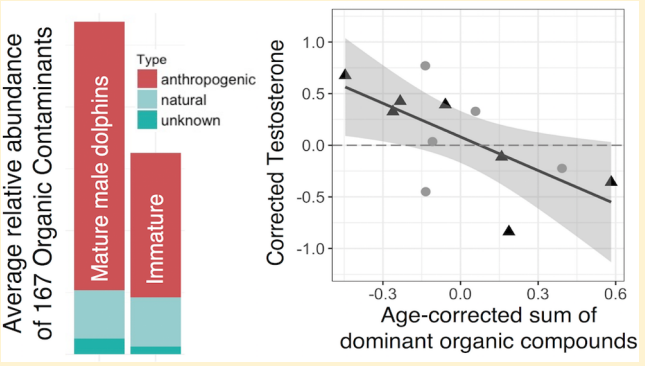
Supporting Information

ABSTRACT: While environmental pollutants have been associated with changes in endocrine health in cetaceans, efforts to link contaminant exposure with hormones have largely been limited to a list of known, targeted contaminants, overlooking minimally characterized or unknown compounds of emerging concern. To address this gap, we analyzed a suite of potential endocrine disrupting halogenated organic compounds (HOCs) in blubber from 16 male short-beaked common dolphins (*Delphinus delphis*) with known maturity status collected from fishery bycatch in the Southern California Bight. We employed a suspect screening mass spectrometry-based method to investigate a wide range of HOCs that were previously observed in cetaceans from the same region. Potential endocrine effects were assessed through the measurement of blubber testosterone. We detected 167 HOCs, including 81 with known anthropogenic sources, 49 of unknown origin, and 37 with known natural sources. The sum of 11 anthropogenic and 4 unknown HOC classes were negatively correlated with blubber testosterone. Evidence suggests that elevated anthropogenic HOC load contributes to impaired testosterone production in mature male *D. delphis*. The application of this integrative analytical approach to cetacean contaminant analysis allows for inference of the biological consequences of accumulation of HOCs and prioritization of compounds for future environmental toxicology research.



INTRODUCTION

Many known persistent organic pollutants (POPs), such as organochlorine pesticides, are halogenated organic compounds (HOCs) that are slow to degrade and biomagnify in food webs. Exposure to POPs poses a significant threat to the health of human and wildlife populations.[1,2] Because of the difficulty in measuring the large number of known contaminants in the environment and identifying potential unknown compounds, there is a paucity of information on the full extent of HOCs present in the environment and their potential effects[3] on wildlife.

Marine mammals are considered environmental sentinels of exposure to and effects of POPs in higher trophic level organisms,[4] serving as sensitive, early warning indicators of contaminant risks to aquatic environments.[5] Bioaccumulation and biomagnification of compounds in marine mammals allows for the detection of low-level compounds that are persistent and difficult to measure in lower trophic levels. POP exposure in pinnipeds and cetaceans has been associated with the disruption of endocrine hormones, reproductive failure, decreased immune function, and an increased risk of mortality in primarily observational studies in situ.[6−10] However, the majority of studies on HOC exposure in marine mammals focus on measuring a few selected compounds or compound classes, excluding additional known and unexpected contaminants, making it difficult to compare exposure effects to a broad set of HOCs.[3,11,12]

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Recent technological advances have enabled the nontargeted analysis of HOCs in biological samples, enhancing attempts to measure the total load of HOCs in wild individuals. Previous research has identified a broad suite of anthropogenic, natural, and unknown HOCs in fish oil, bird eggs, and dolphin blubber via nontargeted analysis using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC/TOF-MS).[11−15] Shaul et al.[12] identified 327 HOCs in blubber samples from eight bottlenose dolphins (*Tursiops truncatus*) stranded in the Southern California Bight (SCB), which revealed that 86% of detected compounds are not typically monitored. These included previously unrecognized anthropogenic HOCs, unknown HOCs, and halogenated natural products (HNPs). HNPs are compounds that have physical-chemical properties similar to anthropogenic POPs and are known to bioaccumulate in dolphins.

A high abundance of HOC compounds in mature individuals suggest that HOCs are persistent in the environment and difficult to metabolize, resulting in an increase in HOC body burden over time.[16] Given this body burden accumulation and biomagnification, HOC screening paired with biological metrics, including maturity status and hormone analysis can be used to identify potential biological effects of HOC compounds.[17] HOCs have been linked to disruption of endocrine hormones that are necessary for development, survival, and reproduction in humans and a range of wildlife species (e.g., birds, amphibians, and terrestrial and marine mammals),[2,18,19] including the disruption of testosterone, a hormone associated with male maturity. HOC exposure has also been associated with changes in sexual development and sperm quality in terrestrial mammals.[20−22] However, few studies, if any, have investigated the impact of HOCs on the endocrine system of marine mammals especially as they relate to androgen production.

Quantification of hormones in marine mammal blubber specifically allows assessment of reproductive health in free-ranging marine mammal populations.[23,24] For most species, biopsies composed of skin and blubber are the only practical way to obtain biological material from wild dolphins in numbers sufficient for population-level analysis. Testosterone is primarily produced by the testes in males and is lipophilic therefore will partition into lipid-rich tissues, such as marine mammal blubber.[25] Previous studies have typically documented relationships between serum hormones and POPs in marine mammals (e.g., Schwacke et al.[10]) but hormone levels in blubber are less variable than serum providing a better measure of chronic rather than acute changes in endocrine biomarkers.[26] Thus, incorporating blubber hormone quantification with comprehensive HOC analysis enables assessment of potential long-term changes in reproductive hormones and provides baseline information for field-based studies on wild marine mammals.

Here, we performed contaminant suspect screening on the blubber of mature and immature male short-beaked common dolphins (*Delphinus delphis*) from the SCB using an established mass spectral library of 268 HOCs generated from a nontargeted analysis of bottlenose dolphins from the same region.[12] Marine mammals in the SCB have some of the highest levels of dichlorodiphenyltrichloroethane (DDT), polychlorinated diphenyls (PCB), and polybrominated diphenyl ethers (PBDEs) recorded globally due to historical deposition near Los Angeles and proximity to multiple urban areas.[12,27−29] *D. delphis* are likely to bioaccumulate high levels HOCs as they are frequently detected throughout the SCB[30] and feed at a high trophic level.[31] Blubber testosterone, a measure of reproductive health, was also quantified in each individual. We then paired testosterone measurements with comprehensive HOC screening for the first time to identify ecologically relevant HOCs and evaluate evidence of a potential biological response to HOC exposure and bioaccumulation in wild marine mammals.

MATERIALS AND METHODS

Samples. Sixteen male *D. delphis* individuals of known maturity (8 immature and 8 mature) were caught incidentally (presumed to be healthy) in gillnets and selected for HOC and hormone analysis: specimens were collected between 1999 and 2008 and archived at NOAA's Southwest Fisheries Science Center (Table S1). Sampling from incidental bycatch of nonthreatened marine mammals is permitted through the NMFS Marine Mammal Authorization Program under the Marine Mammal Protection Act (16 U.S.C. 1371(a)(5)). All blubber samples were collected, along with basic morphometric measurements, during nonbreeding season months (fall and winter) to avoid spurious correlations due to hormone changes from breeding activity during summer. Maturity was determined with reference to testes weight according to criteria defined in Kellar et al.[25] A complete cross section of blubber was taken from the dorsal region between 10 min and 4 h after death and stored at −20 °C until analysis. Kellar et al.[25] found no significant relationship between blubber testosterone level and time at −20 °C degrees thus it is assumed testosterone levels in blubber were relatively stable since collection. Two samples were designated as juveniles because they could not be appropriately classified. This was confirmed by obtaining ages determined via tooth histology, available for 14 of the 16 individuals. The two samples were eliminated from all maturity-related analyses because of their intermediate maturity status to focus on individuals within the two defined maturity states. All other immature individuals were presumed to be preweaning as all were younger than 16.5 months, the estimate of age-at-weaning for *D. delphis* in the Eastern Tropical Pacific.[32] All mature individuals are within the expected age range for peak reproduction (12−18 years of age) and not likely starting to senesce. According to Westgate and Read[33] males in this population reach sexual maturity by 11.9 years of age and can live up to 25+ years.

Suspect Screening for HOCs and Relative Abundance Quantification. Blubber samples for HOC analysis were prepared following the protocol of Shaul et al.[12] Two grams of blubber were extracted with dichloromethane using a pressurized liquid extractor (Dionex ASE 300, Dionex, Sunnyvale, CA, USA). The dichloromethane was evaporated from the sample. One gram of lipid extract was subsampled and spiked with a known amount of internal standard, $^{13}C_{12}$-PCB-169, because the final data were corrected by lipid weight rather than blubber weight. Lipid was removed by automatic gel permeation chromatography (GPC) (J2 Scientific, Columbia, MO). The eluent was evaporated to 100 μL in a water bath under a N_2 (g) stream a using a Zymark TurboVap. The extract was spiked with recovery standards ($^{13}C_{12}$-PCB-189 and 4′-fluoro-2,3,3′,4,5,6-hexabromodiphenyl ether) and concentrated to 100 μL under a N_2 (g) stream. The final extract of each sample was run on a Pegasus 4D GC × GC/TOF-MS system (LECO, St. Joseph, MI, USA) with the conditions described in Shaul et al.[12] Each batch included a procedural blank. Two compounds (a chlorinated benzene and a chlorophosphate)

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were detected in the procedural blank were discarded from final analysis.

A suspect screening was conducted for HOC analysis. The instrument software (LECO ChromaTOF software, version 4.50.8.0) was used to search for peaks of HOCs at $S/N \geq 50$. Each sample produced approximately 6,000 to 10 000 chromatographic features and associated mass spectra, which were manually identified by matching the mass spectra and the GC × GC retention times with the Pacific dolphin library produced by Shaul et al.[12] While the Pacific dolphin library contains 327 compounds, we excluded toxaphenes and polychlorinated terphenyls from the suspect screening list prior to analysis due to unclear mass spectra, as well as DDT, dichlorodiphenyldichloroethylene (DDE), and PCBs due to oversaturated peak areas. However, other known DDT-related compounds, including dichlorodiphenyldichloroethane (DDD), and methylsulfonyl-PCBs (PCB metabolites) were included. The resulting suspect screening list included 269 of the 327 compounds from 31 compound classes in the Pacific dolphin library. All compounds were named and classified according to the library produced by Shaul et al.[12] Relative abundance was quantified using peak area with the same ions as Shaul et al.[12] Peak areas for all compounds were corrected relative to the peak area of the internal standard in each sample, and further normalized by each sample's lipid weight. Isotope-labeled standards are not available for every class, such as unknown compounds or HNPs. In addition, we tested multiple internal standards in our previous project[12] and found no difference (using isotope labeled PCBs and PBDEs) for comparison of normalized relative abundances. Therefore, the reported values represent a normalized relative abundance of each compound in each sample, i.e. not absolute concentrations but a relative concentration of HOCs in each sample in the set.

Hormone Analysis. Approximately 100 mg of blubber was homogenized using an Omni BeadRuptor (Omni International, Kennesaw, GA), testosterone was isolated using a biphasic solvent extraction, and extraction efficiency was calculated according to the methods in Kellar et al.[34] and Trego et al.[35] The final extracts were dried and stored dry at −20 °C prior to analysis with a commercially available testosterone enzyme-linked immunosorbent assay (ELISA) kit from Enzo Life Sciences (Farmingdale, NY). Stored samples were reconstituted in 250 μL of phosphate buffered saline (pH 7.5) containing 1% bovine serum albumin prior to analysis on a 96-well ELISA plate. Samples were run in duplicate to account for assay variability. Final hormone concentrations were corrected according to extraction efficiency and blubber weight, and an average blubber hormone concentration (in nanograms per gram) was calculated for each individual.

Data Analysis. All data were analyzed using *R*, version 3.3.1.[36] Data analyses were broken down into three different steps: characterizing HOC profiles, identifying maturity-related HOC accumulation, and examining evidence of endocrine disruption. For the first step, we ran a principal component analysis (PCA) on compound classes to reduce the dimensionality of the data (167 compounds, 26 classes) and identify co-occurring compound classes accumulating in *D. delphis*. Next, we used Random Forest[37−39] and Mann−Whitney U tests to assess maturity-associated bioaccumulation of compounds classes. Finally, we used linear regression and Random Forest to investigate whether there was evidence of a biological response associated with higher HOC levels. Details on each quantitative analysis are provided in the Supporting Information.

Normalized relative peak abundances were natural log transformed prior to all statistical analyses except in Random Forest models, which make no assumption of normality. All nondetects were set to zero and all compounds were summed according to compound class as in Shaul et al.[12] The 5 individual peaks of 3 compounds were too saturated to get an accurate estimate of abundance and were excluded from all analyses except for the Random Forests: MBP-Cl_7 in two individuals and trans-nonachlor, *o,p′*-DDD, and *p,p′*-DDD each in one individual.

RESULTS

Characterizing HOC Profiles in *Delphinus delphis*. Of the 269 HOCs screened, we confirmed the presence of 167 HOCs from 26 out of 31 screened compound classes (see Table S2 for the complete list). Eighty compounds were of anthropogenic origin, 49 were of unknown origin, 37 were from natural compounds, and one was from mixed sources. In terms of abundance, anthropogenic compounds were the predominant type of HOC (61%, Figure 1a). While there were a greater number of unknown compounds detected compared to natural HOCs, unknown compounds represented 8% of total relative HOC abundance compared to 27% for natural HOCs. The compound classes in highest abundance were DDT-related compounds, PBDEs, dimethyl bipyrroles (DMBPs), tris(4-chlorophenyl)methane (TCPM), chlordane-related compounds, and tris(4-chlorophenyl)methanol (TCPMOH; Figure 1b). Of the 167 HOCs detected, only 28 are typically monitored (e.g., DDD, PBDEs, and chlordane), with the remaining 139 compounds identified as largely unmonitored (e.g., TCPM, TCPMOH, HNPs, and unknown compounds).

We identified a group of 15 co-occurring compound classes using PCA. PC1, which accounted for 44% of the total variance in the data set, represented several known anthropogenic compound classes (here referred to as the "AC group" to distinguish this set of compounds from general anthropogenic compounds, Figure 2), the majority of which are known to bioaccumulate. Several unknown compound classes also loaded strongly with this group, including unknown classes 2, 4, 7, and 8 (numbers as defined in Shaul et al.[12]). Unknown 2 compounds were recently identified as novel isomers of TCPMOH,[28] a metabolite of anthropogenic compound TCPM, (hereafter referred to as unknown 2 ($TCPMOH_N$)). Several HOCs with natural origin, including DMBPs, methyl bipyrroles (MBPs), and methoxy brominated diphenyl ethers (MeO-BDEs), were loosely grouped in the upper right of the biplot along with unknown compound classes 3 and 5 and a few additional anthropogenic compounds. A PCA biplot with the samples plotted based on maturity state is available in the Supporting Information (Figure S1).

Identifying Maturity-Related HOC Bioaccumulation. Overall, differences in HOC patterns were apparent between mature and immature individuals (Figure 3). Mature individuals had a significantly higher number of HOCs (104 ± 4.2 vs 81 ± 4.2, mean ± standard error respectively, $p = 0.01$, Mann−Whitney U), as well as the total relative abundance compared to immature animals (32.1 ± 3.5 vs 17.3 ± 1.2 respectively, $p = 0.001$). The two juvenile individuals of intermediate maturity level had a similar number of compounds as confirmed immature individuals (63 and 76) but differed greatly in total relative abundance (17.9 vs 88.3).

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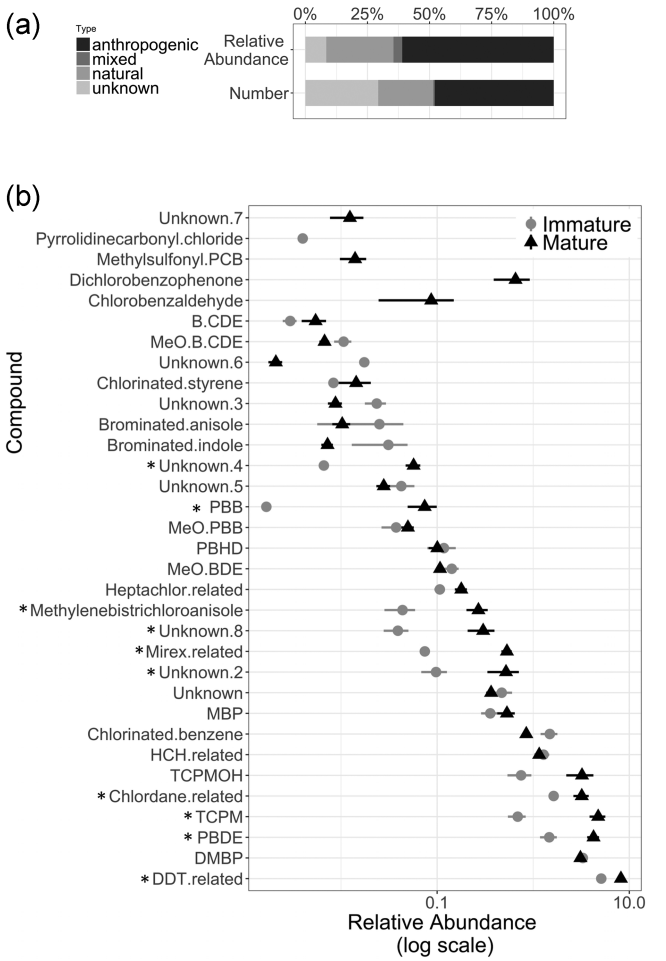

Figure 1. (A) The overall number and average relative abundance of halogenated organic compounds (HOCs) classified by origin (gray scale) among all samples. (B) The average relative abundance (in log scale) and standard error of all compound classes by maturity type (gray circles and black triangles represent data from immature and mature animal samples, respectively). The asterisk (*) denotes compound classes with significantly different abundance between mature and immature animals, as determined by randomForest permutation tests.

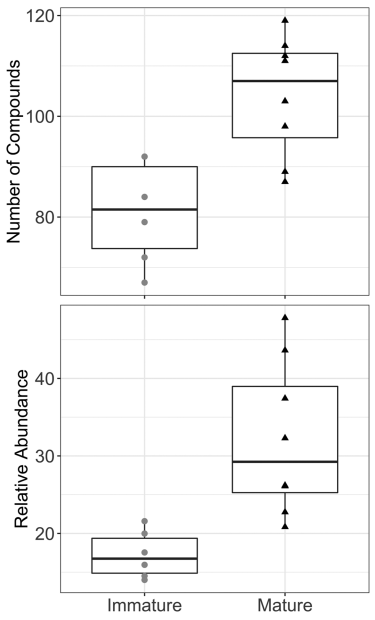

Figure 3. Difference in average total number and relative abundance of compounds detected in confirmed immature and mature individuals. Compound classes that differed between immature and mature individuals were mostly of anthropogenic origin and some of unknown origin. No compound classes of natural origin differed between maturity groups.

All compound classes that differed between maturity states were more abundant in mature individuals compared to immature individuals. The Random Forest classification model correctly distinguished between maturity states for all samples based on the relative differences in compound class abundance with zero out of bag error (i.e., all individuals were correctly classified). TCPM, polybrominated biphenyls (PBB), unknown 4, mirex-related, methylenebistrichloroanisole (MBTCA), unknown 2 ($TCPMOH_N$), PBDE, unknown 8, chlordane-related, and DDT-related compounds were significantly more abundant in mature individuals (Figure 1b). Additionally, chlorbenzylaldehyde, dichlorobenzophenone, methylsulfonyl-PCB (a metabolite of PCB), and unknown 7

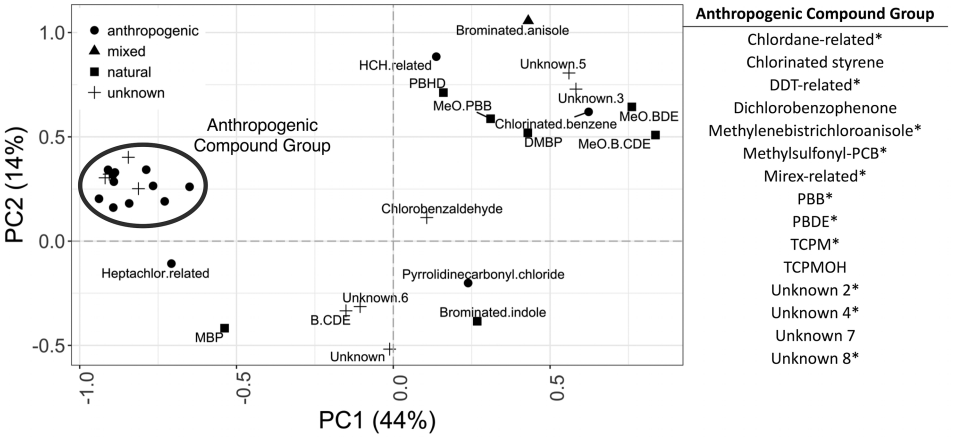

Figure 2. Principal components analysis (PCA) plot of the loadings for all compound classes according to origin (anthropogenic, mixed, natural, or unknown). The drawn circle highlights the anthropogenic compound group and the compound classes contributing to this group are listed on the right. Within the group, compound classes with significant higher relative abundance according to the Random Forest are marked with an asterisk (*).

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were only found in mature individuals, although at relatively low levels, but were not included in the Random Forest classification model because they were detected in fewer than 10 individuals (Figure 1b). Additionally, 14 of the 15 compound classes in the AC group demonstrated evidence of maturity-related bioaccumulation (Figure 2). There was no significant difference in the relative abundance of known natural compound classes between maturity states.

Evidence of Endocrine Disruption. As expected, blubber testosterone concentrations were significantly higher in mature individuals compared to immature individuals (3.7 ng/g ± 0.6, 1.2 ng/g ± 0.3, p = 0.004). Testosterone negatively correlated with both age-corrected PC1 (p = 0.02, r^2 = 0.44, Figure S2) and the age-corrected sum of the AC group (p = 0.02, r^2 = 0.42, Figure 4). This trend was stronger when mature individuals were analyzed separately without age-correction (p < 0.01, r^2 = 0.86, Figure 4). None of the linear models showed evidence of bias due to age, gonad weight, or maturity.

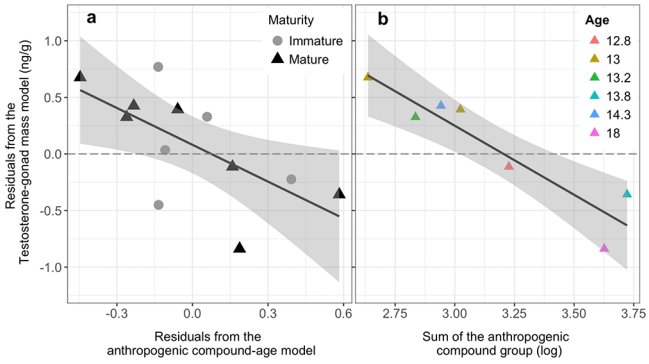

Figure 4. Regressions examining the relationship between the sum of the compound abundances in the anthropogenic contaminant group (AC group) compounds and blubber testosterone for age-corrected data combined (a) and the log-transformed mature-only, non-age-corrected contaminant sum (b). Circles and triangles represent data from immature (I) and mature (M) animal samples, respectively), and color represents the age of mature individuals. Individuals below the dotted line have less testosterone than expected given the same gonad weight (and age for the age-corrected graph).

The Random Forest models also independently demonstrated that relative abundance of both compound classes and individual compounds were linked to testosterone. The compound class and individual compound Random Forest models explained 59.02% and 43.74% of the variance in gonad-corrected testosterone, respectively. Of the 23 compound classes tested, four known anthropogenic and two unknown compound classes were negatively related to corrected testosterone in mature individuals and also belonged the AC group: TCPM, chlordane-related, heptachlor-related, mirex-related, unknown 2 ($TCPMOH_N$), and unknown 4. Two natural compound classes, methoxy brominated/chlorinated diphenyl ether (MeO-B/CDE) and methoxy polybrominated biphenyl (MeO-PBB), were positively correlated with corrected testosterone. DDT-related compounds, MBTCA, PBBs, PBDEs, TCPMOH, and unknown 8 compound classes also had positive variable importance (i.e., increased the predictive power of the model) but the p-values for these classes were above 0.05 (Figure 5, Figure S3).

The individual congener model did not converge (i.e., the significance level of a few compounds fluctuated between model runs) because of the small sample size therefore we also

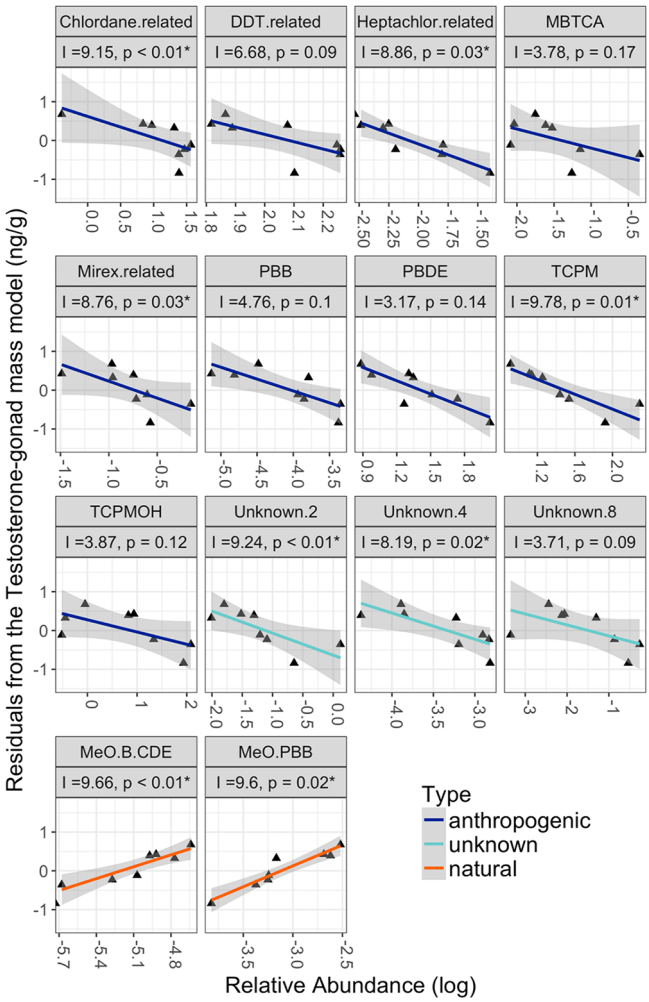

Figure 5. Relative abundance of compound classes that increased predictive power of gonad mass adjusted testosterone concentration. Variable importance metrics (I) and p-values (p) are provided for each compound, where p-values below 0.05 were considered significant (*). Color denotes the origin of the compound (anthropogenic, unknown, or natural).

took into account the variable importance. All compounds with a %Inc.MSE over 5 were consistently significant and variable importance dropped much more rapidly below this threshold (Figure S4). Of the 76 individual compounds tested, 16 individual compounds were significantly correlated with adjusted testosterone and had a variable importance greater than 5 (Figure S5). Of these, 10 anthropogenic and 2 unknown individual compounds, belonging to the same 6 compound classes identified by the compound class Random Forest, were negatively correlated with testosterone, while four natural compounds were positively correlated.

DISCUSSION

This study identified marine mammal exposure to a region-specific suite of HOCs and evaluated support for a corresponding biological impact, namely endocrine disruption, in a population of small cetaceans in the SCB. The suspect screening analysis successfully detected 167 HOCs in *D. delphis* on the southern California coast. Among the 167 HOCs, only 28, or 17%, are typically monitored in environmental analyses. We found an additional 139 HOCs that are largely unstudied

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and unmonitored in wildlife and the environment. A third of these additional HOCs have unknown origins. Several of the compounds we identified exhibit a pattern of bioaccumulation and potential endocrine disruption, including several unknown compound classes and individual compounds. These findings highlight the need for more attention to comprehensive HOC exposure, bioaccumulation, and potential endocrine disruption in higher trophic level organisms.

Characterizing HOC Profiles in *Delphinus delphis*. Anthropogenic compounds represented the largest proportion of HOCs detected in *D. delphis* within the study area (nearly 50%), suggesting that human activities have significantly influenced HOC accumulation in marine mammal populations off of the California coast. Consistent with other studies in the region, we found DDT-related compounds to be the most abundant and prevalent, closely followed by several other legacy contaminant classes that are no longer in use or production in the U.S., including TCPM (a component of DDT's technical mixture), TCPMOH, PBDEs, and chlordane-related compounds.[12,28,40,41] The predominance of these legacy contaminants in marine mammal blubber confirms the long-term persistence of these compounds in the region.

Halogenated natural products (HNPs), which can originate from marine sponges[42,43] or bacteria,[44] represented 22% (37 of 167) of all compounds detected in this study. The detected proportion of HNPs was similar to the proportion of natural compounds found in coastal *T. truncatus* in the SCB (24%, from Shaul et al.[12]). Natural HOCs are of particular interest given the chemical properties shared between anthropogenic and natural compounds.[43] Limited evidence suggests that DMBP can activate the aryl hydrocarbon receptor in chickens,[45] a pathway commonly associated with toxicity induced by anthropogenic contaminants.

Several unknown compounds or compound classes, such as unknown compound classes 2 ($TCPMOH_N$) and 8, were at similar relative abundance to other known, monitored POPs (e.g., mirex-related compounds). Our PCA indicated that unknown classes 2 ($TCPMOH_N$), 4, 7, and 8 loaded closely with an aggregation of known anthropogenic contaminants, suggesting these unknown compounds may be anthropogenic in origin, either as parent compounds or metabolites. Recent identification of class unknown 2 ($TCPMOH_N$) as novel isomers of TCPMOH by Mackintosh et al.[28] support the use of this approach to investigate preliminary accumulation patterns of unknown compounds. This HOC grouping includes mostly known bioaccumulating compounds, suggesting that unknown 2 ($TCPMOH_N$), 4, 7, and 8 may likewise be taken up at a rate faster than lost by catabolism and excretion. Unknown compound classes 3 and 5 loaded with several natural compounds, including DMBPs, MBPs, and MeO-BDEs, providing some evidence that these compounds could be correlated either by compound origin or by similar environmental exposure patterns, though anthropogenic sources cannot be ruled out.

Identifying Maturity-Related HOC Bioaccumulation. By examining the abundance of HOCs in animals with different maturity states, we were able to further characterize the long-term bioaccumulation of several different HOC classes. Overall, mature *D. delphis* had higher HOC loads compared to immature animals and anthropogenic compounds explained the majority of the bioaccumulation patterns, indicating these compounds pose a long-term bioaccumulation risk. Anthropogenic and unknown compound classes that were more abundant in mature individuals represent compounds that are less likely to be metabolized and thus more likely to bioaccumulate over a lifetime.[16] Several compound classes that were higher in mature individuals and are known to bioaccumulate, including TCPM, PBB, PBDE, mirex-related, chlordane-related, and DDT-related compounds, were abundant in the individuals sampled.[46−49] Furthermore, we observed lesser-known compounds that exhibited the same bioaccumulation pattern, including methylsulfonyl-PCB, MBTCA, chlorbenzylaldehyde, dichlorobenzophenone, and unknown compounds 2 ($TCPMOH_N$), 4, 7, and 8.

Interestingly, there was no difference in relative abundance of natural compound classes between maturity states, though several are thought to bioaccumulate over a lifetime in marine mammal tissues (e.g., DMBPs, MBPs, and MeO-BDEs).[47,50] Additionally, several unknown compounds, potentially of anthropogenic or natural origin, were not present in enough individuals to evaluate differences between maturity states. While several of the anthropogenic, natural, and unknown compounds detected here were found at relatively low levels, when combined, both low level and abundant compounds may contribute significantly to the overall HOC load.

Evidence of Endocrine Disruption. Our results suggest that, beyond characterizing HOC exposure and bioaccumulation in wild marine mammals, there is a continued need to understand how HOC compounds disrupt biological systems. By using an approach that integrates suspect HOC screening with biological markers, we identified a prominent group of bioaccumulating anthropogenic compounds (AC group, Figure 2) that was associated with a decrease in blubber testosterone when corrected for variation in age and gonad weight (Figure 4). Several other studies have also found evidence of reduced testosterone levels in mammals after exposure to several of the HOCs examined here, including DDT-related compounds (e.g., DDE), PBDEs, mirex, heptachlor, and chlordane.[19,21,22,51−54] Our analysis found additional supporting evidence that other unstudied compounds that bioaccumulate may also contribute to endocrine disruption, for example, TCPM, TCPMOH, methlysulfonyl-PCB, and unknown classes unknown 2 ($TCPMOH_N$), 4, 7, and 8. The relationship we observed was strongest in mature individuals, which is consistent with an increase in lifetime accumulation of anthropogenic HOCs contributing to inhibited production of testosterone in male *D. delphis*.

Within mature individuals, we found additional supporting evidence suggesting that multiple compounds, rather than one dominant compound class within the AC group, are likely contributing to endocrine disruption in *D. delphis*. Of this dominant group, six compound classes were identified as key predictor variables in the random forest analysis: chlordane-related, heptachlor-related, mirex-related, TCPM, unknown 2 ($TCPMOH_N$), and unknown 4 (Figure 5). In addition, 12 individual congeners from each of these classes were also identified as predictor variables: alpha-chlordane, chlordane-related 14, chlordane-related 9, cis-nonachlor, heptachlor epoxide, mirex, TCPM, TCPM 3, TCPM 4, TCPM 5, unknown 2−3 ($TCPMOH_N$), and unknown 4−3 (Figure S5). While one high abundance compound could possibly be driving the negative trend observed here, the congruence between results from the PCA and the random forest analyses indicates that the negative relationship between HOCs and testosterone is not likely to be explained by variation in one dominant compound class but rather several of the classes

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within the AC group. There also may be additional compounds or compound classes that are contributing to this relationship that were not included in this analysis, such as PCBs and DDE, due to extreme abundance causing GC peak saturation, both of which have been associated with lower testosterone levels in other mammals.[19,20,54,55] This study detected three PCB metabolites (methylsulfonyl PCBs) that covaried with the AC group suggesting PCBs may exhibit a similar pattern of accumulation.

Evidence of endocrine disruption from complex mixtures of anthropogenic contaminants has been found in mammals. A recent study of breeding Arctic foxes found a decrease in testosterone and sperm quality related to an increase in a mixture of HOC concentrations in their fatty tissue after a supervised diet of whale blubber,[22] similar to the pattern we detected in common dolphins. Mixtures of other known testosterone disruptors have been shown to alter reproductive development and function in a laboratory setting, even when each compound targets a different part of the testosterone pathway.[56−61] There is also limited evidence that PBDE congeners, TCPM, *p,p′*-DDD, and *β*-hexachlorocyclohexane (*β*-HCH) can inhibit sperm quality in humans or rats,[62−65] though the mechanism and effect levels are unclear. Thus, exposure to a suite of HOCs that potentially inhibit testosterone production could have important ramifications for the reproductive health and fitness of male marine mammals during the breeding season.

Our analyses indicate that anthropogenic sources, rather than HNPs, are the primary drivers of total HOC load in SCB *D. delphis* and that these levels are correlated with a measure of endocrine disruption that may pose a reproductive health risk to wild marine mammal populations. As the first study to relate exposure to a wide range of HOCs with an endocrine biomarker in marine mammals, this research highlights the need for continued attention to emerging and unmonitored HOCs in coastal oceans. The relationship between testosterone disruption and long-term accumulation of anthropogenic and unknown compounds supports continued monitoring of legacy HOCs, early detection of compounds of emerging concern, and a greater understanding of endocrine disruption in long-lived mammals.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04652.

Full list of detected compounds, individual sample information, and supplemental tables and figures (PDF)

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