

1 **Supporting Information For**
2
3 **Comprehensive screening links halogenated organic compounds with**
4 **testosterone levels in male *Delphinus delphis* from the Southern**
5 **California Bight**

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34 **MATERIALS AND METHODS**

35

36 *Data analysis*

37

38 *Characterizing HOC profiles in Delphinus delphis*

39

40 We calculated the mean relative abundance and standard error for each compound class
41 according to maturity status and compound origin (anthropogenic, natural, and unknown).
42 To reduce the dimension of the data and to identify co-occurring compound classes, we ran
43 a PCA on all 16 individuals with the logged sum of the relative abundance of all 26
44 compound classes (167 individual total congeners) as variables.

45

46 *Identifying maturity-related HOC bioaccumulation*

47

48 We used Mann-Whitney U tests to evaluate significant differences in overall number of
49 compounds and total relative abundance between maturity status. We then created a
50 Random Forest model to examine differences in compound class abundance between
51 mature and immature individuals using the randomForest R package¹. Permutation tests
52 were conducted with the rfPermute package² to determine significance for each compound
53 class. Compound class abundances were calculated only using compounds found in 10 or
54 more individuals (76 compounds from 23 classes). Because Random Forest cannot be
55 conducted with missing data, we estimated the relative abundance for the five excluded
56 peaks from 3 different individuals by measuring the best available peak areas. These five
57 measurements were only used in random forest analyses and were excluded from all other
58 analyses and data summaries. A compound class was considered significant when
59 permutation tests calculated a p-value of 0.05 or below.

60

61 *Evidence of endocrine disruption*

62

63 Differences in log testosterone levels due to maturity status were evaluated with t-tests.
64 We then ran a linear regression between the first axis of principal component analysis
65 (PC1; which represented variation in 15 anthropogenic and unknown compound classes),
66 and blubber testosterone level to examine how endocrine health is related to HOC
67 exposure. We regressed the negative value of PC1 (because PC1 was negatively related
68 with prominent compound abundance) with age to control for age-related variation in HOC
69 load and extracted the residuals from the model as a proxy of the variation not explained
70 simply by age. Similarly, the log of blubber testosterone was regressed with average gonad
71 mass. If both gonad mass were unavailable, we included only the right gonad mass.
72 Residuals from each of the PC1-age and testosterone-gonad mass models were then used
73 in a linear model to compare age-corrected PC1 (as the predictor variable) with gonad
74 mass-corrected testosterone (the response variable). The logged sum of the congeners in
75 the PC1 group were similarly age-corrected. The age correction was done by building a
76 model with age and HOC load and extracting the residuals. The variation not explained
77 simply by the age model was then used in the linear regression with testosterone (corrected
78 by gonad mass) as above to confirm that this trend can be explained by the compound
79 classes of interest. In mature individuals, the non-age corrected sum of the PC1 compounds
80 were log transformed and regressed directly with gonad mass-corrected testosterone.

81 We constructed two additional Random Forest regression models on mature individuals
82 to explore the effects of HOCs on testes endocrine function (classes: mtry = 7, ntree =

83 4,000, nrep = 2,000; individual compounds: mtry = 13, ntree = 8,000, nrep = 4,000). For
84 both Random Forest analyses, we used only compounds found in 10 or more individuals
85 (76 compounds from 23 classes) in mature individuals to exclude confounding factors due
86 to bioaccumulation with age. The focus on mature individuals also reflects the greater
87 likelihood that impaired or compromised testes function can be detected in mammals
88 during near maximum testosterone production, i.e. when there is the greater physiological
89 demand to reproduce.³ The Random Forest models included gonad mass-corrected
90 testosterone as the response variable and either compound class abundance or individual
91 congener abundance as the predictor variable. Compound classes were considered
92 significant when permutation tests calculated a p-value of 0.05 or below. In case of non-
93 convergence (i.e. conflicting model results), we also assessed variable importance, or the
94 mean decrease in accuracy when removed from the model (%IncMSE), where a higher
95 variable importance represents a larger increase in predictive power with the inclusion of
96 the predictor variable.

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TABLES AND FIGURES

Table S1: The month and year of sample collection, maturity, and age for each of the individuals that were included in this research.

ID	YEAR	MONTH	Maturity	Age
EPM0299	2005	9	I	0.3
MCM0005	2003	11	I	0.5
KAS0142	2006	11	I	0.6
CXG0035	2008	11	I	1.0
CYM0100	2008	11	I	1.2
JXA0060	2000	11	I	NA
SXC0035	1999	11	J	6.5
SMC0119	2002	1	J	7.2
JEA0085	2000	11	M	12.8
BXD0036	2004	11	M	13.0
PBW0002	2001	12	M	13.0
MKW0020	2003	12	M	13.2
SMH0021	1999	1	M	13.8
KSS0008	2008	10	M	14.3
TCL0090	2005	12	M	18.0
BXD0003	2003	10	M	NA

Table S2: A list of the compounds and corresponding group name detected across all 16 samples. For compound structure, identification, mass spectrum and chromatographic information, see Supplemental Material for Shaul et al⁴. The origin of each compound class is represented by the following: anthropogenic [A], natural [N], mixed [M], and unknown[U].

Brominated/chlorinated diphenyl ether (B/CDE) [U]

PBCDE Br₃Cl₃

Brominated-anisole [M]

2,4,6-tribromo anisole

Brominated-indole [N]

4,6-dibromoindole

5-bromoindole

Chlordane-related [A]

alpha chlordane
 chlordane related 2
 chlordane related 4
 chlordane related 6
 chlordane related 7
 chlordane related 9
 chlordane related 10
 chlordane related 13
 chlordane related 14
 cis nonachlor
 gamma chlordane
 oxychlordane
 trans nonachlor

Chlorinated-benzene [A]

1,2,3,4-tetrachlorobenzene
 hexachlorobenzene
 pentachlorobenzene

Chlorinated-styrene [A]

ethenyl benzene 3Cl
 polychlorinated styrene 7Cl

Chlorobenzaldehyde [U]

Chlorobenzaldehyde

Dichlorodiphenyltrichloroethane-related (DDT-related) [A]

DDMU 1
 DDMU 2
 DDMU 3
 DDMU 4
 DDT related 2
 DDT related 3
 DDT related 12
 DDT related 14
 DDT related 16
 DDT related 18
 DDT related 20
 DDT related 23
 o,p'-DDD
 p,p'-DDD

Dichlorobenzophenone [A]

4,4'-dichlorobenzophenone

Dimethyl bipyrroles (DMBPs) [N]

DMBP Br₂Cl₂ 1
 DMBP Br₂Cl₃
 DMBP Br₂Cl₄
 DMBP Br₃Cl₂ 1
 DMBP Br₃Cl₂ 4
 DMBP Br₃Cl₃
 DMBP Br₄Cl 1
 DMBP Br₄Cl 2
 DMBP Br₄Cl₂
 DMBP Br₅Cl
 DMBP 4Br 1

**Dimethyl bipyrroles (DMBPs) [N]
(cont'd)**

DMBP 4Br 2
DMBP 4Br 3
DMBP 5Br 1
DMBP 5Br 2
DMBP 6Br
DMBP 6Cl

**Hexachlorocyclohexane-related
(HCH-related) [A]**

alpha BHC
beta BHC
cyclohexene 4Cl 1

Heptachlor-related [A]

heptachlor epoxide
heptachlor related 1
heptachlor related 2
heptachlor related 3

Methyl bipyrroles (MBPs) [N]

MBP 4Br 1
MBP 4Br 2
MBP 4Br 3
MBP 4Br 4
MBP 6Cl 1
MBP 6Cl 2
MBP 6Cl 3
MBP 7Cl

**Methoxy brominated/chlorinated
diphenyl ether (MeO-B/CDE) [N]**

MeOBCDE Br3Cl

**Methoxy brominated diphenyl ether
(MeO-BDE) [N]**

2'-MeOBDE-68
6-MeOBDE-47
MeOBDE 3Br
MeOBDE 4Br

**Methoxy polybrominated biphenyl
(MeO-PBB) [N]**

di-MeOPBB-80

Methylenebistrichloroanisole [A]

Methylenebistrichloroanisole

**Methylsulfonyl polychlorinated
biphenyl (Methylsulfonyl-PCB) [A]**

methylsulfonylPCB 5Cl 1
methylsulfonylPCB 5Cl 2
methylsulfonylPCB-101

Mirex-related [A]

mirex
mirex 1Cl
mirex 2Cl 1
mirex 2Cl 2
mirex related 1

Polybrominated biphenyl (PBB) [A]

BB-49
BB-52
BB-101
BB-153
BB 4Br 1
BB 5Br 2

**Polybrominated diphenyl ethers
(PBDEs) [A]**

BDE-17/25
BDE-28/33
BDE-47
BDE-49
BDE-66
BDE-75
BDE-99
BDE-100
BDE-116
BDE-153
BDE-154
BDE-155
BDE 5Br 1

**Polybrominated hexadroxanthene
derivative (PBHD) [N]**

PBHD 3Br 1
PBHD 3Br 2
PBHD 3Br 3
PBHD 4Br

Pyrrolidinecarbonyl-chloride [A]
pyrrolidinecarbonyl chloride

**Tris(chlorophenyl)methane
(TCPM) [A]**

TCPM
TCPM 1
TCPM 2
TCPM 3
TCPM 4
TCPM 4Cl 1
TCPM 4Cl 2
TCPM 4Cl 3
TCPM 5
TCPM 6

**Tris(chlorophenyl)methanol
(TCPMOH) [A]**

TCPME

Unknown [U]

unknown-11
unknown-13
unknown-14
unknown-15
unknown-17
unknown-21
unknown-22
unknown-27
unknown-32
unknown-34
unknown-35
unknown-36
unknown-38
unknown-39
unknown-41
unknown-43
unknown-44
unknown-46
unknown-47
unknown-49

Unknown [U] (cont'd)

unknown-55
unknown-56

Unknown-2 [U]

unknown-2-2
unknown-2-3
unknown-2-4
unknown-2-5
unknown-2-8
unknown-2-10

Unknown-3 [U]

unknown-3-1
unknown-3-2
unknown-3-3

Unknown-4 [U]

unknown-4-2
unknown-4-3
unknown-4-5
unknown-4-8
unknown-4-11
unknown-4-12
unknown-4-13
unknown-4-14
unknown-4-16
unknown-4-17

Unknown-5 [U]

unknown-5-2

Unknown-6 [U]

unknown-6-1
unknown-6-3

Unknown-7 [U]

unknown-7-1
unknown-7-2

Unknown-8 [U]

unknown-8-2

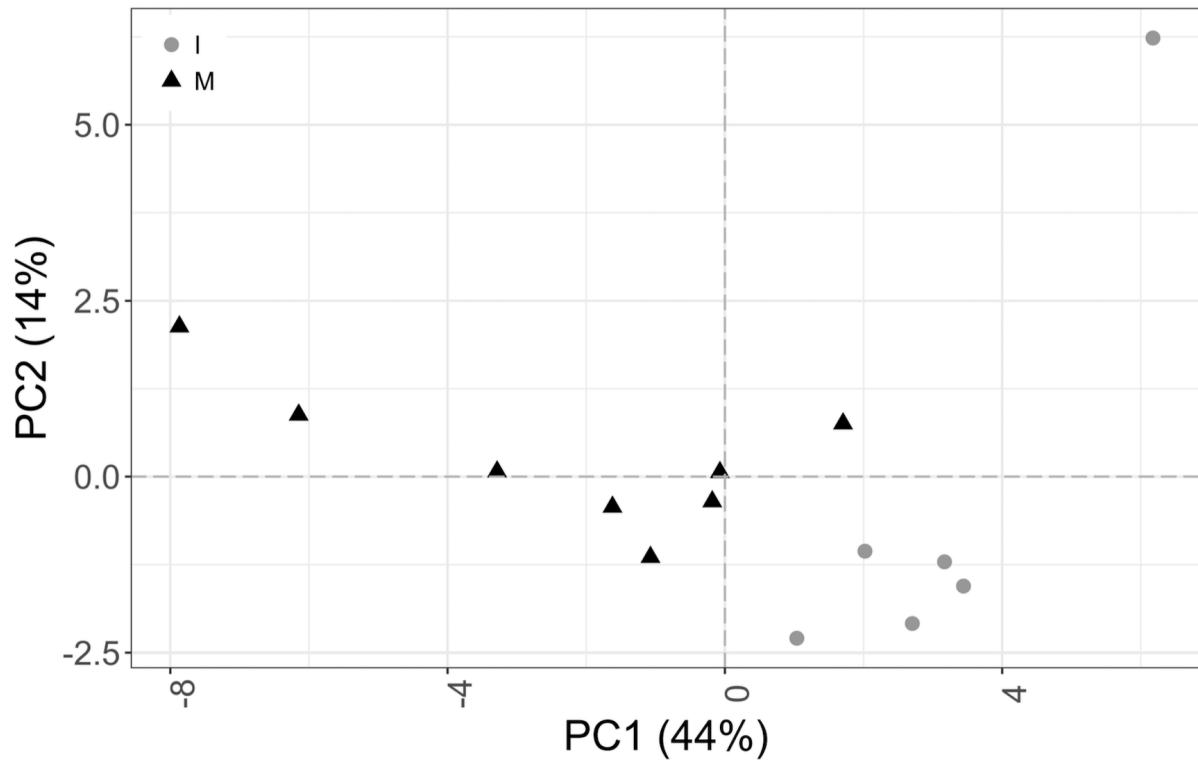


Figure S1: A principal components analysis (PCA) plot of the samples according to maturity (mature/M and immature/I).

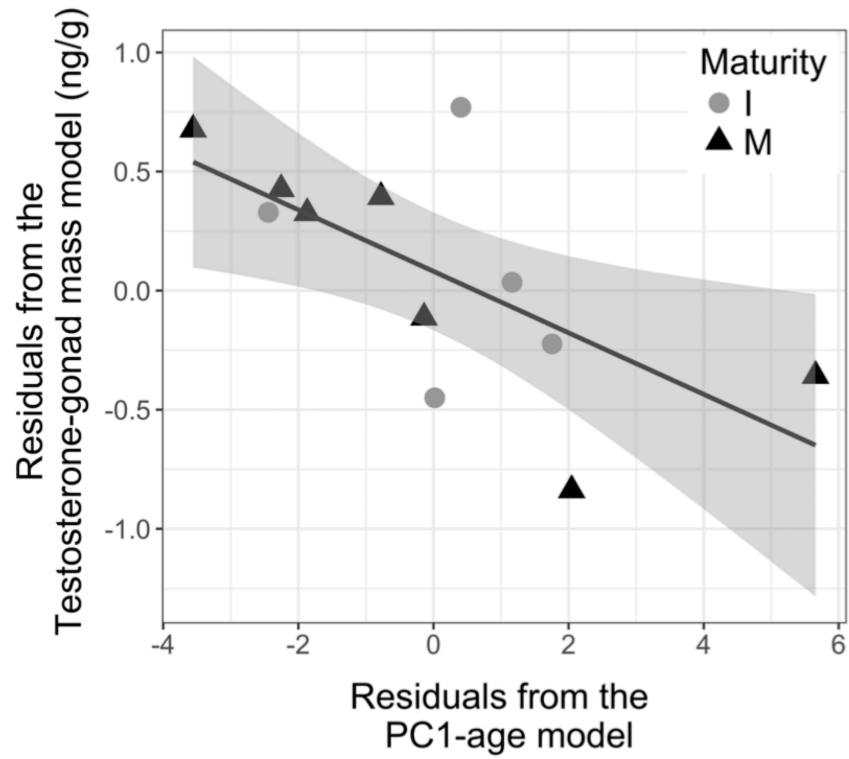


Figure S2: Regressions examining the relationship between PC1 (adjusted for age) and blubber testosterone (adjusted for gonad mass). Circles and triangles represent data from immature and mature animal samples, respectively.

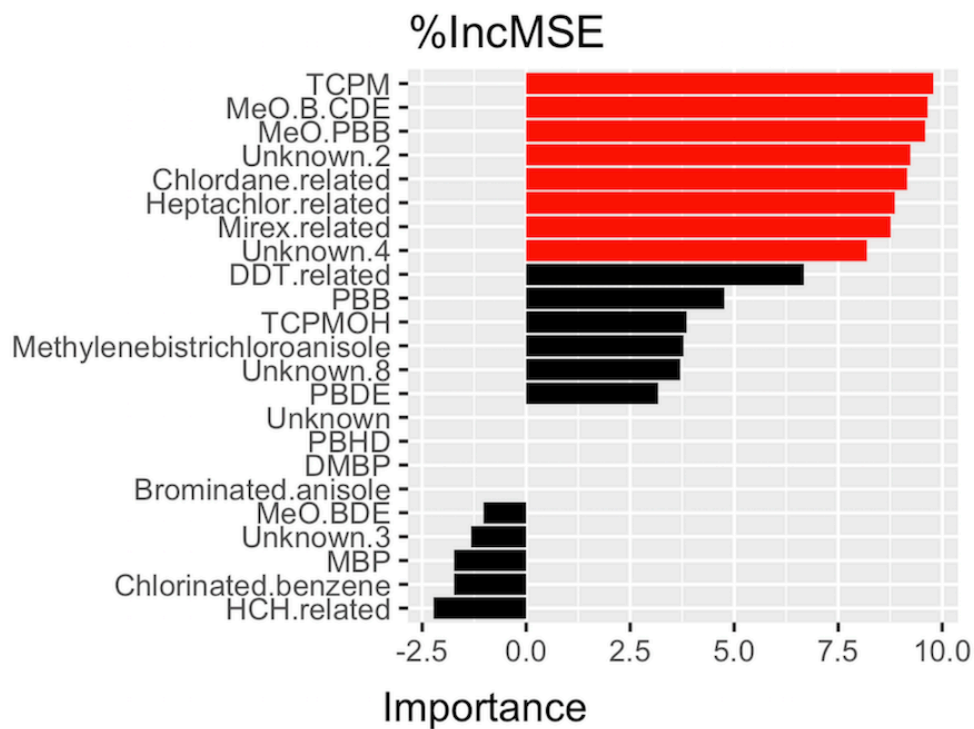


Figure S3: The mean decrease in accuracy when removed from the model (%IncMSE) for all compound classes. Red bars represent significant variables with $p < 0.05$.

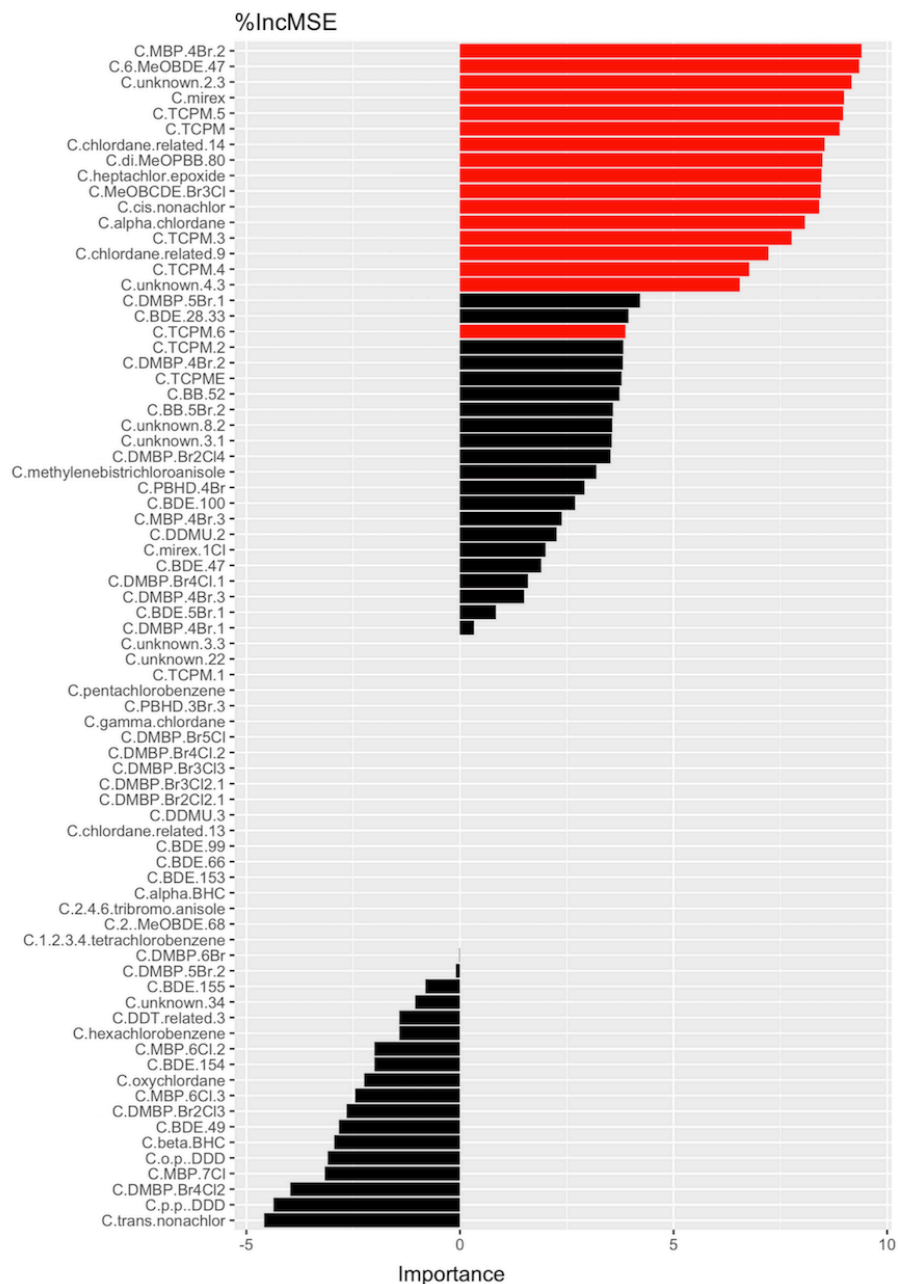


Figure S4: The mean decrease in accuracy when removed from the model (%IncMSE) for all compounds. Red bars represent significant variables with $p < 0.05$.

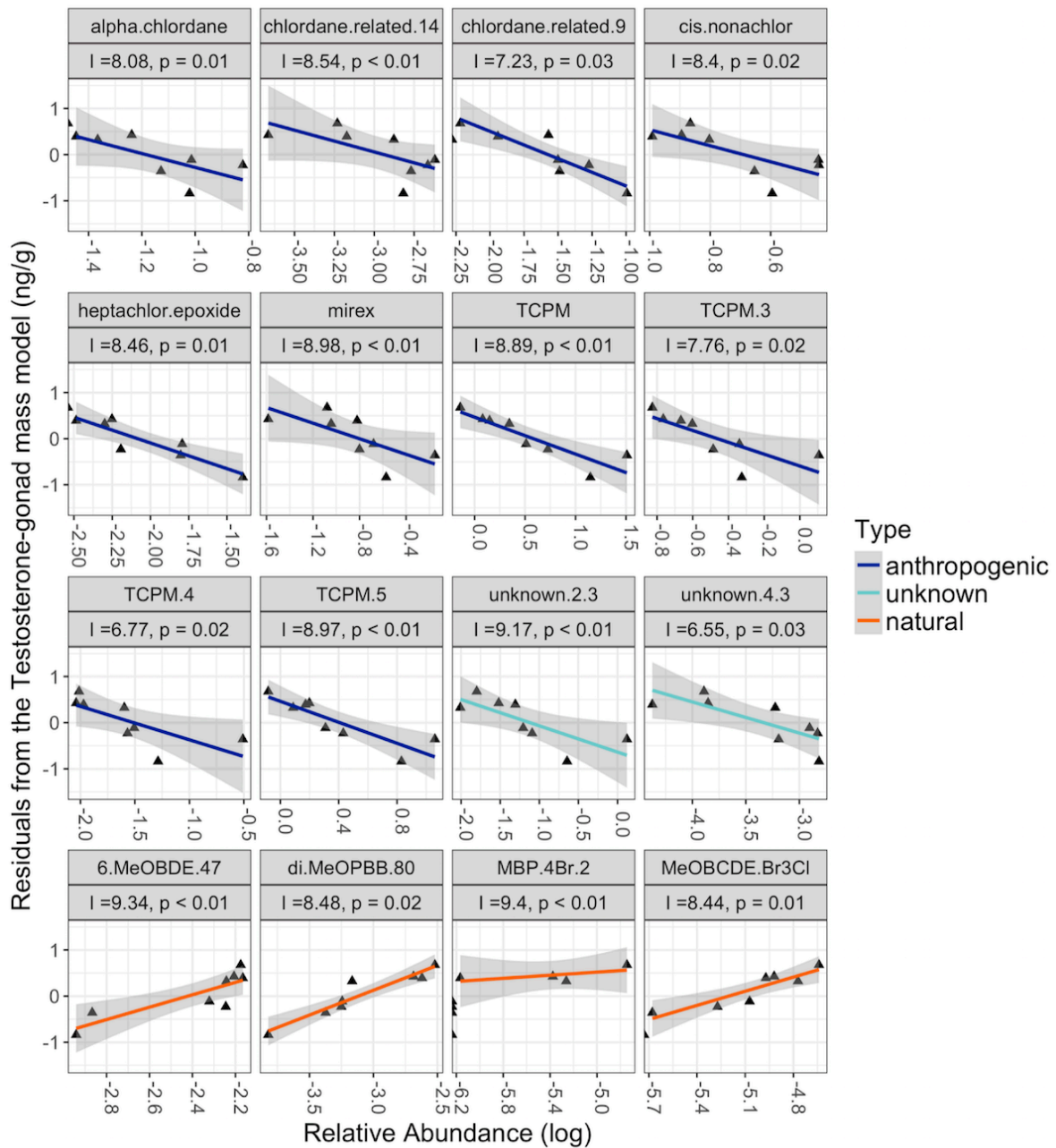


Figure S5: The relative abundance of individual compounds that significantly correlated with gonad mass adjusted testosterone concentration and had a variable importance greater than five. Variable importance in %IncMSE (I) and a p-value (p) is provided for each compound. Color denotes the origin of the compound (anthropogenic, unknown, or natural).

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