

1 **Bioaccumulation of persistent organic pollutants in the deepest ocean fauna**

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12 **The legacy and reach of anthropogenic influence is most clearly evidenced by its impact on the most**
13 **remote and inaccessible habitats on Earth. Here we identify extraordinary levels of persistent**
14 **organic pollutants in the endemic amphipod fauna from two of the deepest ocean trenches (>10,000**
15 **metres). Contaminant levels were considerably higher than documented for nearby regions of**
16 **heavy industrialisation, indicating bioaccumulation of anthropogenic contamination and inferring**
17 **that these pollutants are pervasive across the World's oceans and to full ocean depth.**

18 The oceans comprise the largest biome on the planet with the deep ocean operating as a potential
19 sink for the pollutants and litter that are discarded into the seas¹. The spatial and bathymetric expanse
20 of the deep sea infers that there are still large areas untouched by anthropogenic activity, although
21 the intrinsic linkages between the deep sea and surface waters² would suggest this inference is ill-
22 conceived³. The hadal zone (6000 to 11,000 m deep) is comprised of trenches formed at tectonic
23 subduction zones and represents the least explored ecosystem on Earth and the last major marine
24 ecological frontier⁴. Trenches have been considered both as pristine environments, but also given their
25 locations and topography as likely sinks for contaminants that enter the marine environment². Of
26 particular concern are the persistent organic pollutants (POPs) that are highly detrimental to
27 organismal health through their endocrine disrupting properties⁵. POPs possess an inherent
28 hydrophobicity that confers a high binding affinity to organic or inorganic particles present in the
29 water column that through vertical transport will collect in the deep ocean. They also have inherent
30 lipophilicity, meaning these compounds readily bioaccumulate in organisms, with cumulative
31 increases at each trophic level⁶.

32 POPs were released into the environment through industrial accidents and discharges, leakage from
33 landfills or incomplete incineration⁷. Two key POPs are polychlorinated biphenyls (PCBs, used as
34 dielectric fluid) and polybrominated diphenyl ethers (PBDEs, used as flame retardants). From the
35 1930s to when PCB production ceased in the 1970s, the total global production was ~1.3 million
36 tonnes⁸. Approximately 65% is thought to be contained in landfills or still within electrical equipment,
37 with the other 35% residing in coastal sediments and open oceans⁹. These pollutants are invulnerable
38 to natural degradation¹⁰ and so persist in the environment for decades. Moreover they can spread
39 great distances, including to seemingly isolated environments, such as polar regions and open ocean³.

40 Pollutants entering the deep sea are deposited in sediments and can readily accumulate into the food-
41 chain¹¹. Studies on deep-sea organisms have reported higher concentrations than in nearby surface
42 water species^{11,12}. However, although these studies are described as 'deep sea' they rarely extend
43 beyond the continental shelf (<2000m), so contamination at greater distances from shore and the
44 extreme depths is hitherto unknown.

45 We measured the concentrations of key PCBs and PBDEs in multiple endemic and ecologically
46 equivalent Lysianassoid amphipod Crustacea from across two of the deepest hadal trenches - the
47 oligotrophic Mariana Trench in the North Pacific, and the more eutrophic Kermadec in the South
48 Pacific. Two endemic amphipods (*Hirondellea dubia* and *Bathycallisoma schellenbergi*) were sampled
49 from the Kermadec between 7227 and 10,000 m, and one (*Hirondellea gigas*) from the Mariana
50 between 7841 and 10,250 m. Samples were obtained using traps deployed on deep-sea landers¹³. The
51 concentrations of seven PCB congeners identified by ICES¹⁴ for marine pollution assessment and seven
52 PBDE congeners were measured both in sample dry weight (dw) and lipid weight (lw).

53 The salient finding was that PCBs and PBDEs were present in all samples across all species at all depths
54 in both trenches. The Σ PCB7 concentrations ranged from 147.3-905 ng g⁻¹ dw in the Mariana and
55 18.03-42.85 ng g⁻¹ dw in the Kermadec, with mean values of 382.28 ng g⁻¹ dw \pm 281.6 S.D and 25.24 ng
56 g⁻¹ dw \pm 9.1 S.D respectively. Across individual PCB congeners, PCB 153 was detected in the highest
57 concentration (mean 64.45; range 5.03–373.63 ng g⁻¹ dw). Congeners PCB 138 and 153 alone
58 accounted for 65% of the total PCB concentration, which suggested the heavier congeners were more
59 recalcitrant towards degradation.

60 Σ PBDE7 concentrations ranged from 5.82-28.93 ng g⁻¹ dw in the Mariana Trench and 13.75-31.02 ng
61 g⁻¹ dw in the Kermadec. Concentrations of congeners PBDE 153 and 154 were below limit of detection
62 (LOD) in some samples, while PBDE 183 was not detected. Congener PBDE 47 and 99 were found the
63 higher concentrations (mean: 8.81, range 2.55–21.36 ng g⁻¹ dw; mean 3.31, range 0.78-8.38 ng g⁻¹ dw
64 respectively) and accounted for 71% of the total PBDE concentration.

65 For both Σ PCB7 and Σ PBDE7 there were significant correlations between dry and lipid weights
66 (Pearson's $r = 0.98$ $p = 8 \times 10^{-9}$ and $r = 0.70$, $p = 0.012$, respectively). There was no statistically significant
67 relationship between concentration and depth within either trench (see supplementary information).
68 In both trenches the highest values were found in the upper trench; 7841 m in the Mariana and 7227
69 m in the Kermadec.

70 These data clearly indicate that potent anthropogenic contamination and bioaccumulation has
71 occurred in a dominant macrofaunal group inhabiting the complete depth range of two of the deepest
72 marine trenches. Placing the levels of contamination into a broader comparative context with values
73 published from the western Pacific is complicated by variations in POP congeners assayed across
74 different studies and level of replication. Notwithstanding, some insightful comparisons are possible.
75 World baseline levels for Σ PCBs arising from atmospheric transport found in clean coastal sediments
76 are $<1 \text{ ng g}^{-1} \text{ dw}^{15}$. In grossly polluted areas, levels can be far higher¹⁶, reaching up to $314 \text{ ng g}^{-1} \text{ dw}$ in
77 in Guam, $240 \text{ ng g}^{-1} \text{ dw}$ in Japan and $160 \text{ ng g}^{-1} \text{ dw}$ in Australia. Indeed, in the Mariana, the highest
78 level of PCBs were fifty times more contaminated than crabs from paddy fields fed by the Liaohe River,
79 one of the most polluted rivers in China¹⁸. The only NW Pacific location with values comparable to the
80 Mariana Trench is Suruga Bay (Japan), a highly industrialised area with historically heavy usage of
81 organochlorine chemicals¹⁹.

82 Contamination by PBDEs did not reach the magnitude of PCB levels. The closest data for PBDE levels
83 to the Kermadec Trench are estuarine sediments from New Zealand's North Island (0.55 to 573 ng g^{-1}
84 dw^{23}). These results are however highly variable with some locations, such as Puketutu Island, being
85 exceptionally high ($>500 \text{ ng g}^{-1} \text{ dw}$). However, the median value of $10.3 \text{ ng g}^{-1} \text{ dw}$ is considerably lower
86 than the Kermadec Trench ($18.4 \text{ ng g}^{-1} \text{ dw}$) and close to that in the Mariana Trench ($10.44 \text{ ng g}^{-1} \text{ dw}$).
87 The levels of PBDEs from New Zealand estuaries were not considered excessively high²³.
88 Notwithstanding, the salient finding is that PBDEs are present in the hadal samples at comparable or
89 higher levels than in coastal waters.

90 PCB concentrations were notably higher in the Mariana than in the Kermadec Trench. There are
91 several possible explanations that are not mutually exclusive and at this juncture speculative. First,
92 the high levels of the Mariana PCBs may originate from proximity to the industrialised regions in the
93 NW Pacific²¹ and the North Pacific Subtropical Gyre, famed for its reputation as the 'Great Pacific
94 Garbage Patch'²². As such, it is located beneath a mass accumulation of trapped plastic debris which
95 ultimately sinks as the plastics degrade and fragment, transporting POPs to depth. Second, the
96 amphipods may be accumulating POPs through surface derived carrion-falls that have experienced
97 contrasting levels of contamination in the surface. This however appears unlikely given the consistent
98 patterns of contamination at different depths and the oligotrophic nature of the Pacific around the

99 Mariana. Third, it may also be likely that the different species bioaccumulate these contaminants
100 differently through physiological differences, lipid turnover rates, metabolic capacity, and ingestion
101 throughput. Again, this is somewhat less likely given the ecological equivalence of the species though
102 there is a lack of information on their respective physiologies.

103 The most parsimonious explanation for PCB and PBDE accumulate in these remote and deep trenches
104 is via long range oceanic and atmospheric transport and association with particulate matter and
105 carrion-falls sinking through the water column. The potentially rapid rate of vertical transportation of
106 surface derived material was clearly demonstrated following the 2011 Fukushima Dai-ichi nuclear
107 disaster where the detection of ^{134}Cs radiation at 7553 m deep in the Japan Trench suggested a sinking
108 rate of 64 to 78 m day⁻¹²⁴. Therefore the travel time between the surface and seafloor at full ocean
109 depth is between ~110 and 170 days, and likely shorter for carrion-falls. Given that 370×10^3 t of PCBs
110 are estimated to now reside in the oceans⁹, a rapid influx of surface material suggests little
111 bathymetric variation of seafloor concentrations. Indeed, topographically closed trench morphologies
112 may hamper contaminant dispersal.

113 The extreme hydrostatic pressures that characterise the hadal zone require non-trivial evolutionary
114 adaptations for survival, and present major engineering challenges in accessing full ocean depth. Such
115 statements underpin the perspective that the hadal zone is remote and inaccessible, with popularist
116 analogies generally reinforcing this view, such that if Mount Everest was placed into the Mariana
117 Trench its summit remains a mile below the surface. However, the distance from the surface to full
118 ocean depth is actually only equal to the widest point of the Mississippi River, and half the length of
119 Manhattan Island. These alternative views emphasize that our proximity to these extreme locations is
120 far from remote, which is why even the deepest chasms of the ocean are no longer pristine. The
121 challenge moving forward is to determine the physiological consequences of such contamination and
122 understand knock-on effects on ecosystem function.

123 **Acknowledgements.** The Kermadec and Mariana 'HADES' expeditions (RV *Thomas G. Thompson*
124 TN309, and RV *Falkor* FK141109) were funded through the National Science Foundation (NSF-OCE
125 #1130712, 1140494) and the Schmidt Ocean Institute. SBP was supported by a Fellowship from the
126 Leverhulme Trust. The analytical costs were supported by the Total Foundation (France) and the
127 Marine Alliance for Science and Technology, Scotland (MASTS) through a Deep Sea Forum small grant
128 award.

129 **Author contributions.** A.J.J. conceived the experiment, designed the sampling equipment and was
130 awarded the analytical costs. A.J.J. and S.B.P. performed the sampling at sea. S.B.P identified species,

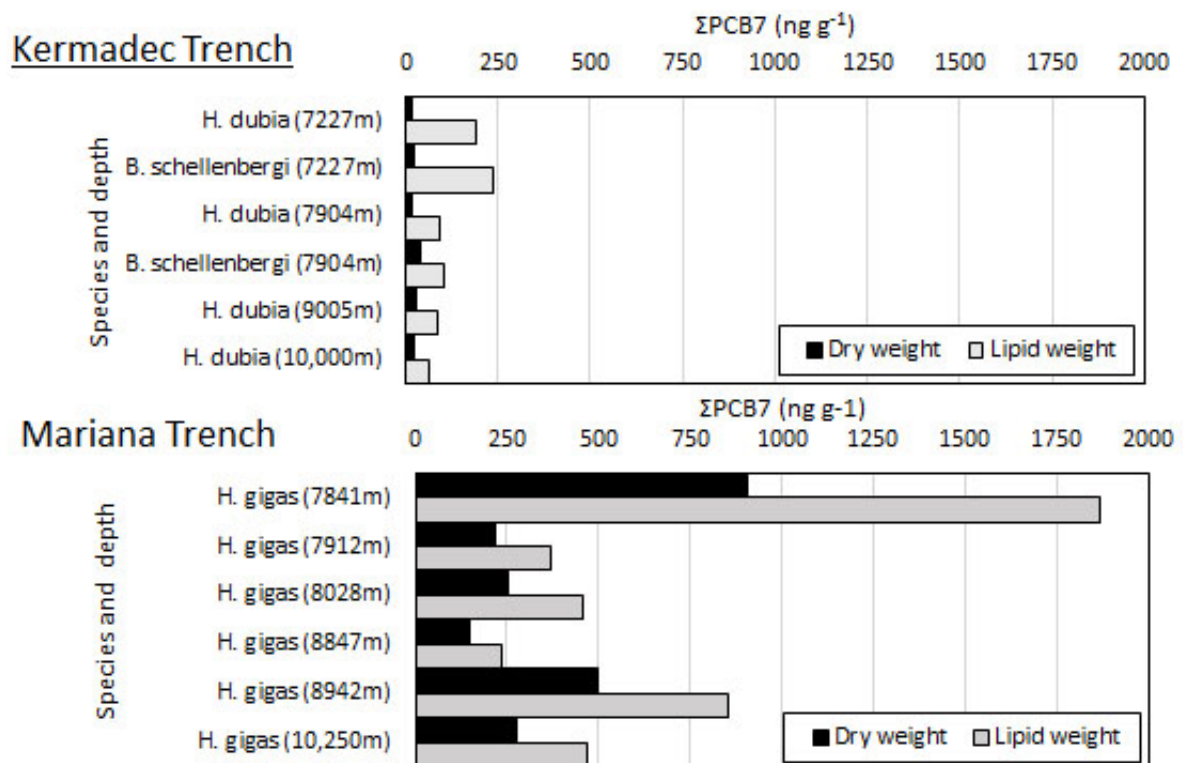
131 and T.M. performed the laboratory analysis under the supervision of Z.Z. and A.J.J. T.M. and T.F.
132 performed the statistical analyses and the manuscript was written by A.J.J., T.M., S.B.P. and Z.Z.

133 **Author Information.** The authors declare no competing financial interests. Correspondence and
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135 (s.piertney@abdn.ac.uk) or Z.Z. (Zulin.Zhang@hutton.ac.uk).

136 **Data availability.** The data that support the findings of this study are available from the
137 corresponding author upon reasonable request.

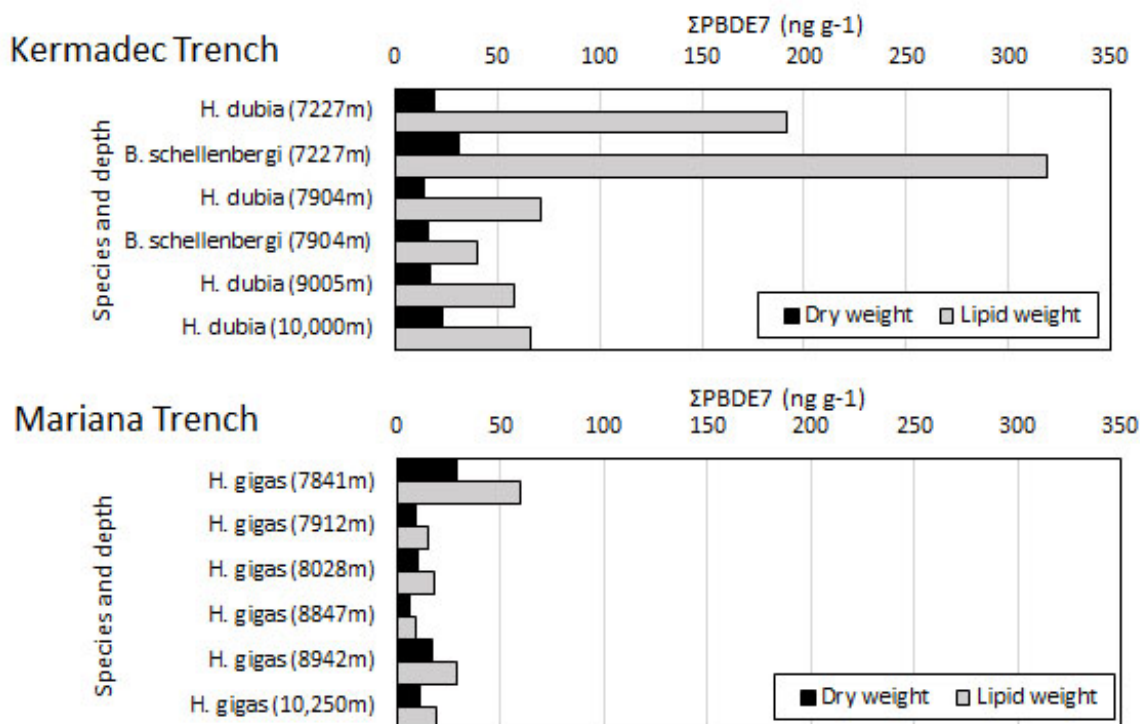
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139 **Figures**



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141 **Figure 1.** Polychlorinated biphenyl (PCB) concentration (ng g⁻¹) for both dry and lipid weight found in
142 endemic hadal amphipods across the bathymetric ranges of the Kermadec and Mariana trenches.



143

144 **Figure 2.** Polybrominated diphenyl ether (PBDE) concentration (ng g⁻¹) for both dry and lipid weight
 145 found in endemic hadal amphipods across the bathymetric ranges of the Kermadec and Mariana
 146 trenches.

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179 **METHODS**

180 **Sampling** - Samples were collected using a full ocean depth rated lander vehicle²⁵ deployed to the
181 seafloor by free-fall and acoustically recalled by jettisoning ballast weights. The lander typically
182 remained on the seafloor for 8 to 12 h. Coupled to the lander footpads were a cluster of three small
183 funnel traps (30 cm length by 6 cm diameter with trap openings of approximately 2.5 cm diameter).
184 The traps were baited with ~100 g of mackerel that was enclosed in a mesh bag to allow the
185 development of an odour plume, but prevent the amphipods consuming any of the bait that might
186 otherwise affect POP levels in downstream assays. Once the samples were landed on deck they were
187 not exposed to any plastics during preparation or preservation. The samples were transferred to a
188 cold room (4 °C) on glass plates and sorted using metal implements before being transferred into pre-
189 muffled (450 °C) foil wrapped tightly in several layers and frozen at -80 °C in natural fibre pouches.

190 **Study sites and species** – Samples of Lysianassoid amphipods were obtained from across two of the
191 deepest hadal trenches - the Mariana Trench that underlies the oligotrophic surface waters of the
192 North Pacific, and the Kermadec Trench situated under moderate primary productivity off New
193 Zealand in the South Pacific. Two endemic species of amphipod (*Hirondellea dubia* and *Bathycallisoma*
194 *schellenbergi*) were sampled from the Kermadec (32° S 177° W), at depths of 7227, 7904, 9005 and
195 10,000 m. One species of endemic amphipod (*Hirondellea gigas*) was sampled from the Mariana (12°
196 N 145° E), at depths of 7841, 7912, 8028, 8847, 8942 and 10,250 m. In all cases, samples were obtained
197 using traps deployed on autonomous deep-sea lander vehicles²⁵.

198 **Contaminant analyses.** The concentrations of seven PCB congeners (28, 52, 101, 118, 138, 153 and
199 180) identified by ICES¹⁴ for marine pollution assessment and seven PBDE congeners (28, 47, 99, 100,
200 153, 154 and 183) with a wide range of bromination. Concentrations of PCBs and PBDEs in amphipods
201 were determined using gas chromatography/mass spectrometry (GCMS), following extraction and
202 sample clean-up, using method described previously²⁶. Procedural blanks were run in parallel with
203 each batch of samples and the results were corrected accordingly. The mean recoveries of the method
204 were 40-77% for PCBs and 71-93% for PBDEs, respectively. Limits of detection (LOD) were 0.02 ng g⁻¹
205 for all PCBs and PBDE congeners 28, 47, 99 and 100, and 0.50 ng g⁻¹ for PBDE congeners 153, 154 and
206 183.

207 **Lipid content.** Total lipid content was assessed using a single step extraction method²⁷. 90 mg of
208 sample were extracted with 2:1 chloroform-methanol (v/v) mixture in a glass centrifuge tube. After
209 adding NaCl water solution, the phase separation was facilitated by centrifugation to recover the
210 chloroform phase into a clean, weighed scintillation vials. Chloroform was evaporated and the vials
211 were re-weighed to calculate total lipid content (%) for each sample.

212 **Statistical Methods.** Simple linear regression was performed to examine the possible effect of depth
213 on the measurements of each pollutant (i.e. PCB7 dw, PCB7 lw, PBDE7 dw and PBDE7 lw) in both
214 trenches (supplementary information Table 3). The pollutant measurements were highly skewed and
215 therefore natural-log transformation was applied to normalise and stabilise the variance. Estimated
216 regression model residuals were examined for statistical adequacy using standard graphical tools to
217 verify the assumption of normality and homogeneity. In addition, Welch's t-test was performed to
218 test for the difference in the respective pollutant levels between the two trenches (supplementary
219 information Table 4). For the results of both linear regression analysis and Welch's t-test, adjusted
220 significance levels ($\alpha = 0.05$ divided by $n=8$ and 4 , respectively) were applied using the Bonferroni
221 inequality correction. All analyses were performed using R v3.2.3 package (R Development Core
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