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## Differential micropollutants bioaccumulation in European hake and their parasites *Anisakis* sp.

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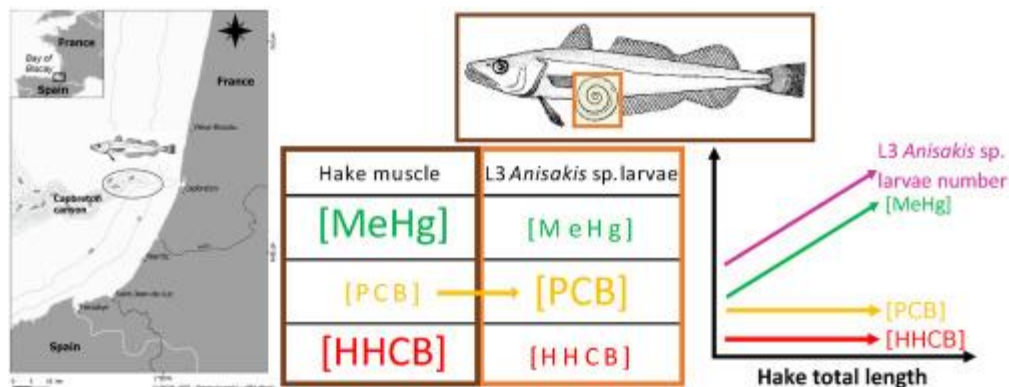
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### Abstract :

Organisms are exposed to various stressors including parasites and micropollutants. Their combined effects are hard to predict. This study assessed the trophic relationship, micropollutants bioaccumulation and infection degree in a host-parasite couple. Carbon and nitrogen isotopic ratios were determined in hake *Merluccius merluccius* muscle and in its parasite *Anisakis* sp.. Concentrations of both priority (mercury species and polychlorinated biphenyls congeners) and emerging (musks and sunscreens) micropollutants were also measured for the parasite and its host, to detect potential transfer of contaminants between the two species. The results showed partial trophic interaction between the parasite and its host, in accordance with the *Anisakis* sp. life encysted in hake viscera cavity. PCB transfer between the two species may result from some lipids uptake by the parasite, while no relation occurred for the two other contaminants. Finally, a positive correlation was found between the number of *Anisakis* sp. larvae and the methylmercury contamination for hake, emphasizing the assumption that the contamination level in methylmercury can weaken immune system of the host enough to affect parasite infection degree.

## Graphical abstract



## Highlights

► Partial trophic interaction between L3 *Anisakis* sp. larvae and its host, hake. ► PCB transfer may result from the uptake of lipids from hake by the parasites. ► Highly MeHg level in hake can weaken immune system and increase *Anisakis* sp. number.

**Keywords :** Parasite-host system, Mercury, PCB, Emerging contaminants, Nematode

## 35 **Introduction**

36 The role of parasites in marine food webs is now recognized as important but largely overlooked  
37 (Hudson et al., 2006). As they may have adverse effect on human health, too many parasites might  
38 seem as organisms with no ecosystemic role that simply should be eradicated. However, they do not  
39 necessarily have adverse effect on hosts nor cause economic or sanitary issues; they are important  
40 and integral components of all ecosystems. Healthy ecosystems have healthy parasite communities  
41 and assemblages (Marcogliese, 2005). They may be potentially used as indicators of species and  
42 ecosystem diversity (Balbuena et al., 1995; Sures et al., 2017) because they reflect the presence of  
43 organisms that contribute to their life cycles. They are also indicators of trophic relationships  
44 between their successive hosts (Pascual et al., 1996). By strengthening predator/prey links, they  
45 could be the glue that holds food webs together. In addition, since highly connected systems are  
46 considered more resilient to environmental perturbations (Rooney and McCann, 2012; Sanders et al.,  
47 2018; Thompson et al., 2012) and as parasitism results from biotic interactions, high level of  
48 parasitism may track ecosystems with higher resistance to environmental perturbations. Due to the  
49 contribution of parasites to population dynamics, Lafferty *et al.*, (2008) promote that they could be  
50 integrated into food webs models. Accumulating different heavy metals in aquatic systems, they  
51 could be used as bioindicators of pollution (Morsy et al., 2012; Sures, 2001). However, the  
52 association between parasitism (infection degree), pollution and host health is a complex subject.  
53 Cumulated effects of co-occurring parasites and pollutants can be either antagonists (contaminant  
54 affects parasites and limits infestation) or synergistics (contaminant affects hosts immune system  
55 and enhance infestation), precluding from easy understanding and prediction (Morrill et al., 2014).  
56 By example, Sures et al. (2006) suggested an antagonistic relation between parasites and pollution in

57 eels. Infection by the swim-bladder nematode *Anguillicola crassus* increased cortisol concentrations  
58 while simultaneously occurring metal and/or PCB contamination reduced plasma cortisol levels.

59 In the recent years, combining several trophic tracers was demonstrated efficient to understand  
60 trophic transfers of organic matter. Carbon and nitrogen stable isotopes have been largely used in  
61 trophic ecology, as predators' isotopic ratios are directly linked with isotopic ratios of their diet.  
62 Carbon isotopic ratio do not vary much at each trophic level (theoretically +1‰), allowing the use of  
63 this element as a tracer of organic matter source. On the contrary, nitrogen is gradually enriched  
64 (theoretically +3.4‰), leading to high  $\delta^{15}\text{N}$  values at high trophic levels (Layman et al., 2012).  
65 Nevertheless, stable isotopes are not suited when trophic sources are not isotopically distinct. In  
66 these cases, contaminants can also be used in combination (Pethybridge et al., 2018). By example, Hg  
67 level was powerful to elucidate trophic pathways in the deep Mediterranean. As fish species  
68 considered belonged to the same trophic pathway based on pelagic production, they exhibited  
69 comparable isotopic ratios, but had different Hg levels, as they feed whether in the water column or  
70 on the bottom, *ie* above or below the zone of Hg production (Cresson et al., 2014). Similarly, in the  
71 Bay of Biscay, spatial variation of Hg contamination was used as a tracer of stock segregation for  
72 hake, with higher Hg burdens in the southern individuals being interpreted as the occurrence of two  
73 stocks in the Bay of Biscay (Chouvelon et al., 2014). Combined measurement of PCB and Hg in fish,  
74 notably hake, is classical (e.g. Cresson et al., 2015; Dierking et al., 2009; Harmelin-Vivien et al., 2012),  
75 as these compounds may affect different systems of the organisms (reproductive or neurologic  
76 respectively) and results from different anthropic activities. As they have different chemical  
77 properties, they are also powerful tracers of different exchanges of organic matter in food web.  
78 Finally, investigating PCB content in different tissues allowed identifying intra-individual fluxes of  
79 matter. Lipid transfers between muscle and eggs during oogenesis explained lower PCB burden in  
80 females than in males (Bodiguel et al., 2009). Since isotopic relationship between host and parasites  
81 is not as straight forward as between predator and prey (Thieltges et al., 2019), coupling several

82 trophic tracers can be powerful to gain better understanding of the organic matter exchange  
83 between hake and *Anisakis* sp.

84 The nematode genus *Anisakis* is a cosmopolitan endoparasite. *Anisakis simplex* (s.s.) is the most  
85 common species found in hake in NE Atlantic waters (Mattiucci et al., 2004). Its life cycle is  
86 heteroxenous with crustaceans -mostly Euphausiids (Smith and Wootten, 1978), but also amphipods  
87 or decapod larvae (Baird et al., 2014), being the first intermediate hosts. By the crustacean's  
88 consumption, *Anisakis* sp. at their second larval stage (L2) are transformed into L3 in 3 to 8 days in  
89 the hemocoel. The crustaceans are then ingested mainly by teleosts and cephalopods. *Anisakis* sp.  
90 larvae L3 become encysted, enter hypobiosis, and do not undergo transformation. Fish and  
91 cephalopods are thus considered as paratenic hosts. Cycle is completed in cetaceans' stomach, after  
92 the consumption of teleosts and cephalopods, where L3 larvae are transformed in L4 larvae and then  
93 mature adults. After sexual reproduction, eggs are expelled in the digestive tract of cetaceans and  
94 evacuated with the faeces. Eggs develop freely in the water. Hatching occur in 20 to 27 days at 5 - 7  
95 °C. The first larval stage (L1) develops inside the egg then after a second moult (L2), the larvae swims  
96 freely in the water to join a crustacean (Baird et al., 2014; Buchmann and Mehrdana, 2016). The life  
97 cycle of *Anisakis* is fairly well known, due to the importance of *Anisakis* sp. larvae L3 in food hygiene  
98 and public health. As *Anisakis* sp. cannot complete its cycle in humans, ingestion of raw, brined,  
99 marinated or undercooked fish fillet or cephalopods can lead to human health issues. Between 2010  
100 and 2014, ~80 human anisakidosis (human allergy caused by *Anisakis* sp. infestation) have been  
101 detected by French parasitology laboratories and hospitals, with 4 to 14 cases each year (ANSES,  
102 2017; Dupouy-Camet et al., 2016). In addition, a recent study observed an increased importance of  
103 *Anisakis* during the last decades with potential effects on fish and human health, and on fisheries  
104 (Fiorenza et al., 2020).

105 Hake is an important demersal fishery resource. In the Bay of Biscay, this species is the first landed  
106 with 42 536 tons caught in 2017 (Ifremer Fisheries Information system, 2018). Its diet varies with  
107 ontogeny: juveniles mostly consume pelagic crustaceans, and adults are piscivores, with blue

108 whiting, horse mackerel, sardine and also young hake being important preys (Mahe et al., 2007; Rault  
109 et al., 2017; Velasco and Olaso, 2000). Due to its diet, hake is one of fish species being an  
110 intermediate host for the L3 *Anisakis* sp. larvae (Aibinu et al., 2019). In addition, its trophic position  
111 drives biomagnification and high burdens in Hg (Chouvelon et al., 2014; Cossa et al., 2012) or PCB  
112 (Bodiguel et al., 2009). Understanding the contamination and parasitism levels and their combined  
113 effects on hake is thus of prime importance for ecological, economical and sanitary reasons.

114 As the actual magnitude of impact on the host, and a potential direct energy transfer between  
115 *Anisakis* sp. and hake is unknown, this paper is aimed at investigating the relationship between  
116 *Anisakis* sp. L3-larvae and hake. Combined use of stable isotopes and contaminants was employed to  
117 investigate the potential transfer of organic matter and of contaminant (mercury, PCB and emergent  
118 micropollutants) between host and parasite, and also the relationship between parasite infestation  
119 and contamination levels.

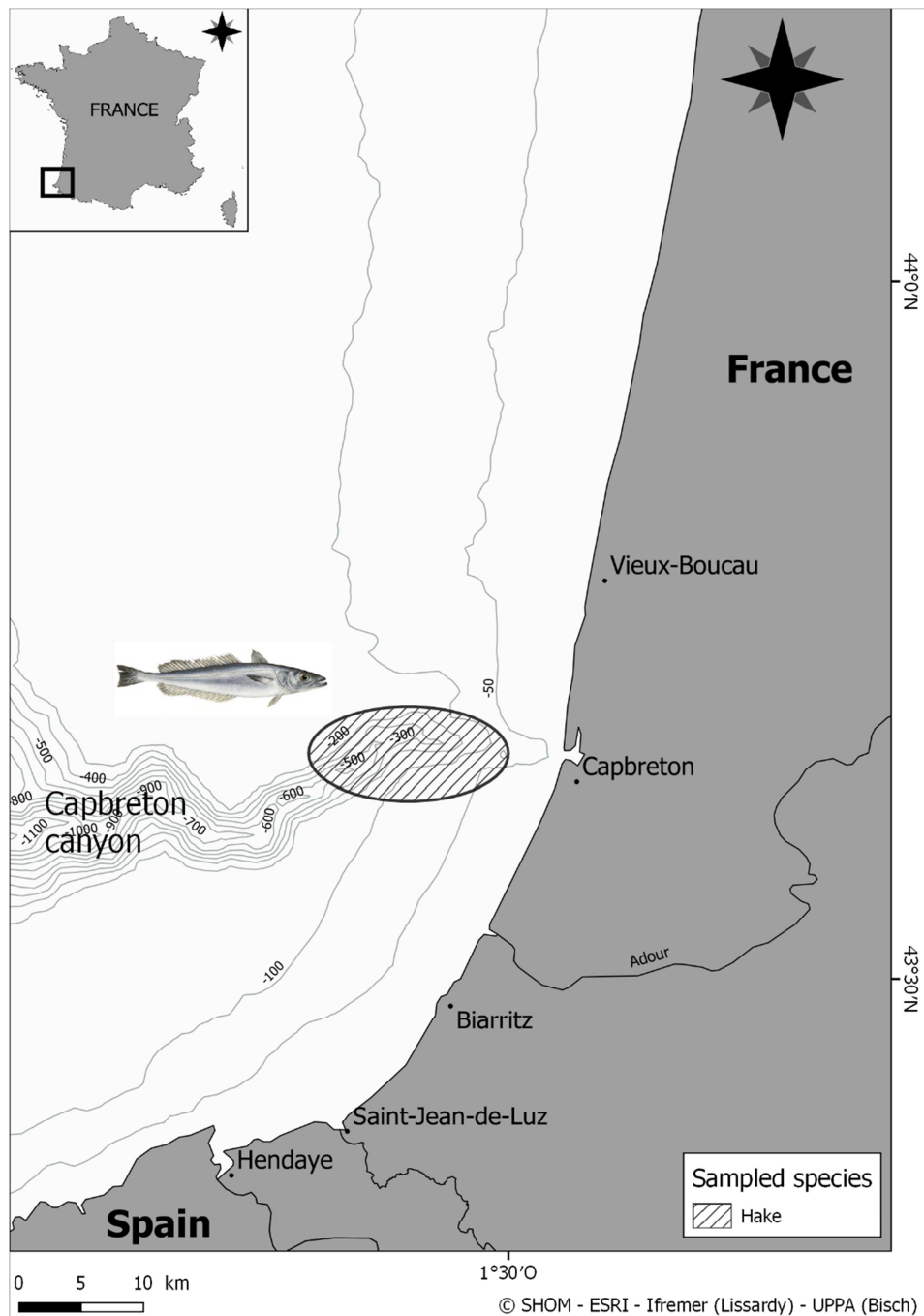
## 120 **Material and methods**

### 121 Sampling and samples preparation

122 A total of 114 hake individuals (*Merluccius merluccius*) were collected in 2017 to 2018 by fishermen  
123 operating in the south of the Bay of Biscay, on the continental shelf from the Capbreton canyon, at  
124 depth ranging between 150 and 200 m (Figure 1). Sampling occurred in February to June, ie before  
125 and during the main reproduction period of hake. Individuals were stored on ice until dissection. In  
126 the laboratory, each individual was identified, measured to the nearest mm (total length), weighted  
127 to the nearest g. Sex was identified from a macroscopic examination of the gonads. On each hake,  
128 muscle was collected for both stable isotope and contaminant analyses. A sample of white dorsal  
129 muscle without skin and bones was taken on all individuals. A small part was used for isotopic  
130 analysis and all remaining sample was used for contaminant analyses.

131 *Anisakis* sp. L3-larvae (referred as to *Anisakis* sp. hereafter) were systematically collected at the  
132 opening of the visceral cavity of all hake individual, before their leaking to the organs. Parasites were

133 rinsed with distilled water, frozen at  $-20\text{ }^{\circ}\text{C}$  for 48 h then decapsulated, counted and pooled to one  
134 sample. The amount of matter needed to perform contaminant analyses was high ( $> 2\text{ g}$  dry weight),  
135 *i.e.* a weight corresponding to  $\sim 200$  *Anisakis* sp. individuals. Analyses were thus performed in pools of  
136 parasites collected in 20 hakes randomly selected among hakes with parasite's abundance higher  
137 than 200 individuals. All samples (*Anisakis* sp. pools and hake muscles) were stored frozen, freeze-  
138 dried and grinded.



139

140 **Figure 1: Map of hake sampling area.**



## 141 Carbon and nitrogen isotopic analysis

142 Isotopic analyses were performed on a small subsample of grinded hake muscle or of *Anisakis* pools  
143 with a Thermo Scientific Delta V Advantage mass spectrometer coupled to a Thermo Scientific Flash  
144 EA1112 elemental analyzer. Results are presented in the classical  $\delta$  notation,  $\delta X = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times$   
145  $10^3$  where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R the ratio between heavy and light isotopes. Standard is Vienna-Pee  
146 Dee Belemnite for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ . Based on replicate measurements of  
147 internal laboratory standards, the experimental precisions were 0.13‰ and 0.12‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .  
148 Carbon and nitrogen concentration were measured with the elemental analyzer and used to  
149 calculate C/N ratios, classical proxy of lipid content in isotopic analyses. High C/N ratios are usually  
150 interpreted as high lipid content that bias isotopic measurement. All hake C/N ratios were lower than  
151 3.5, classically considered as the threshold values requiring lipid correction (Sweeting et al., 2006).  
152 Previously published isotopic values were also retrieved in the literature for other fish and  
153 invertebrate species living in the Bay of Biscay (Chouvelon et al., 2012).

## 154 Mercury species analysis

155 Methylmercury (MeHg) and inorganic mercury (IHg) were measured by capillary gas chromatography  
156 (Focus GC, Thermo Electron) connected to an inductively coupled plasma mass spectrometer (ICPMS  
157 X2 series, Thermo Electron). Due to its neurotoxicity, its ability to biomagnify along trophic network  
158 (Bryan et al., 1979), and as it the predominant Hg form in fish (Bloom, 1992; Chouvelon et al., 2018).  
159 MeHg has been used for the following analyses to represent mercury species. Methodology and  
160 analytical set-up for the GC-ICP-MS for Hg speciation analysis are detailed in Monperrus et al.(2005).  
161 Briefly, 100 mg of homogenized dry tissue (hake or *Anisakis* sp pool) were digested with 5 mL TMAH  
162 (Tetramethylammonium hydroxide) under a microwave field and then centrifuged to remove solid  
163 particles. The supernatant, stored at 4 °C, was then submitted to derivatization using sodium  
164 tetraethylborate (3%). Quantification of Hg species was performed by species specific isotope  
165 dilution, by adding the appropriate amount of isotopically enriched Hg standards ( $^{199}\text{IHg}$  and

166  $^{201}\text{MeHg}$ , and by applying isotope pattern deconvolution for data processing (Rodríguez Martín-  
167 Doimeadios et al., 2004). Total mercury (Hg) concentrations is equal to the sum of IHg and MeHg  
168 concentrations. Results quality was checked by repeated analyses of blanks and of reference material  
169 IAEA 407 (fish homogenate). Limits of quantification were set to 1.4 and 1.2  $\text{ng g}^{-1}$  dw for THg and  
170 MeHg, respectively.

171 Musk fragrances, UV-filters, and PCBs analyses

172 Personal care products such as musks and UV-filters are used in household products or cosmetic as  
173 fragrance ingredients and in sunscreens to protect the skin against the harmful effects of ultraviolet  
174 radiation, respectively. Because they are only partially eliminated by wastewater treatment plants,  
175 they enter aquatic systems leading to organisms exposure (Ternes et al., 2004). Moreover, due to  
176 their lipophilic nature and their great stability, these compounds can easily bioaccumulate and  
177 biomagnify in the marine trophic food web (Cunha et al., 2018; Reiner and Kannan, 2011; Zhang et  
178 al., 2013). On the contrary, PCB are historical contaminants in marine systems. Their toxicity drove  
179 their ban in the 1990's, but due to their persistence, they are still present with high concentrations in  
180 marine systems.

181 Therefore, concentrations of 9 musk fragrances (ADBI, AHMI, AHTN, ATII, HHCB, MA, MK, MM, MX),  
182 5 UV-filters (3-BC, EHMC, 4-MBC, OC, OD-PABA) and 12 PCB congeners (CB 18, 28, 31, 44, 52,  
183 101,118, 138, 149, 153, 180, 194) were determined in hake muscle and in *Anisakis* sp. pools by Gas  
184 Chromatography-Mass Spectrometry (GC-MS). A Quick, Easy, Cheap, Effective, Rugged and Safe  
185 (QuEChERS) method, combining an extraction and a clean-up step before the GC-MS analysis, has  
186 been used. Methodology and analytical set-up are detailed in Miossec *et al.* (2018). Briefly,  
187 micropollutants were extracted from an aliquot of 2 g of homogenized dry tissue (hake or *Anisakis* sp  
188 pool) by a QuEChERS method. Then, 20  $\mu\text{L}$  of the supernatant was injected and submitted to  
189 derivatization using helium as carrier gas in a HP-5MS UI capillary column (30 m length  $\times$  0.25 mm  
190 diameter and 0.25  $\mu\text{m}$  film thickness). Results were in good agreement with a sample spiked at 50  
191  $\text{ng g}^{-1}$  realized with all for target compounds and for each studied species (Supplementary material).

192 All concentrations were expressed relatively to dry weight. Concentrations measured for the 12 PCB  
193 congeners were summed and expressed with the usual  $\Sigma$ PCB notation hereafter.

194

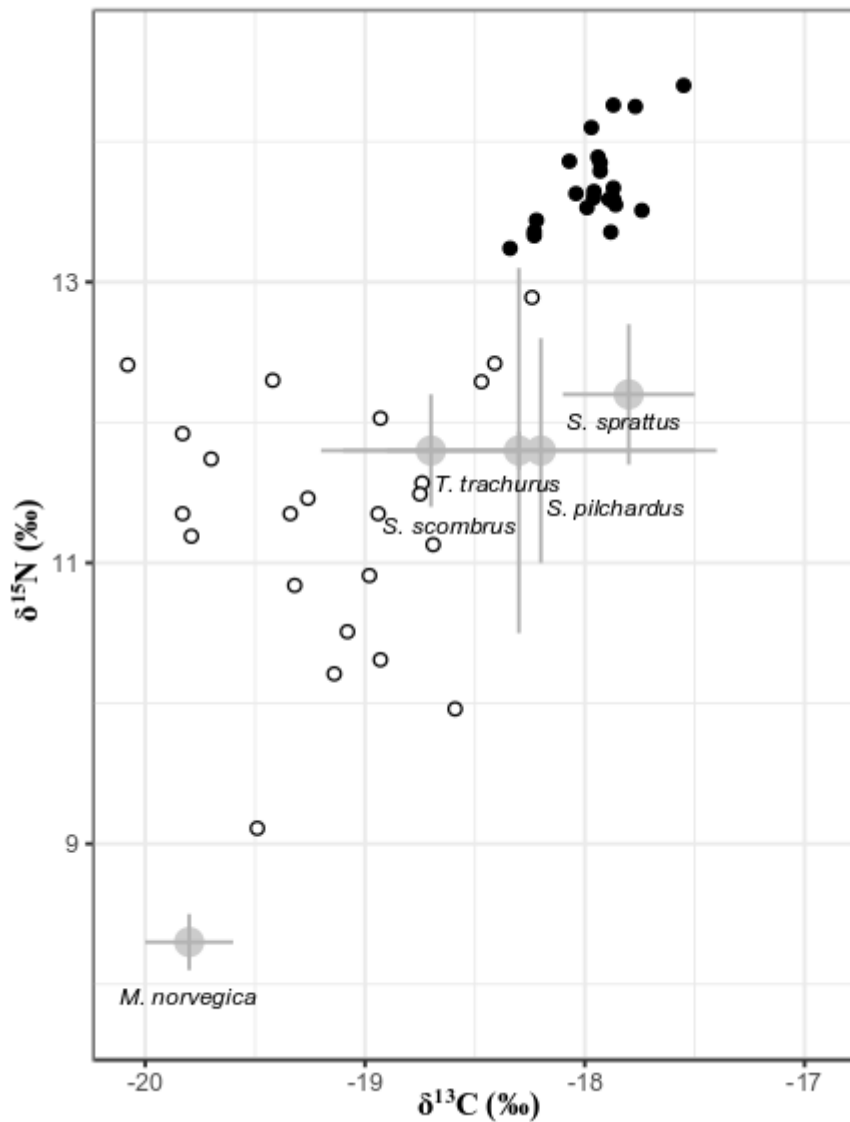
195 Data analyses

196 In order to investigate the potential contaminant transfer between the parasite and its host, the  
197 relationship between contaminant concentration in hake and *Anisakis* sp. have been performed by  
198 linear regressions.

199 Hake contamination level was related to its infection degree by *Anisakis* sp. in two steps. First, the  
200 relationship between the number of *Anisakis* sp. found in hake and its total length and sex have been  
201 investigate by an ANCOVA with total length as covariate and sex as factor, using data from all hake  
202 individuals. Second, since the fish contamination can also increase with fish size, the infection degree  
203 illustrated by the number of *Anisakis* sp. was fitted using a linear effect model as depending on total  
204 length and micropollutant concentration of hake as continuous variable. This analysis was performed  
205 on the subset of hakes the parasites of which were used for contaminant analyses. The model was  
206 reduced by a bidirectional elimination procedure based on the Akaike Information Criterion (Borcard  
207 et al., 2011). In order to determine active factors, significance of effects in the reduced model was  
208 tested by *F* tests between nested models respecting marginality of the effects (type 2 tests; Fox and  
209 Weisberg, 2011). The assumption of normality and homogeneity of variance were checked on  
210 residual data. All statistical analyses were performed to the error threshold of 5%, using the “car”  
211 package (Fox and Weisberg, 2011) in the statistical environment R Core Team (2018).

212 **Results**

## 213 Trophic ecology



214 **Figure 2:**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures for hake (●) and *Anisakis* sp. (○) from the Capbreton canyon . In order  
 215 to complete the food web, pelagic fishes and Euphausiids are displayed in grey and are based on literature  
 216 values (Chouvelon et al., 2012).  
 217

218  
 219 Average isotopic ratios for hake were of  $-17.96 \pm 0.18\text{‰}$  and  $13.70 \pm 0.31\text{‰}$  for carbon and nitrogen,  
 220 respectively, and of  $-19.13 \pm 0.51\text{‰}$  and  $11.34 \pm 0.90\text{‰}$  for *Anisakis* sp., but with a large range of  
 221 values for both  $\delta^{13}\text{C}$  (-20.8 – -18.2) and  $\delta^{15}\text{N}$  ( 9.1 – 12.9) (Figure 2). In addition, C/N ratios were  
 222 higher for *Anisakis* sp. ( $5.4 \pm 0.5$ ) than hake ( $3.1 \pm 0.1$ ) (Table 1).  
 223

224 **Table 1: Stable isotopic signatures in carbon and nitrogen measured in hake muscles and *Anisakis* sp.**  
 225 **pools (mean, standard deviation and range).**

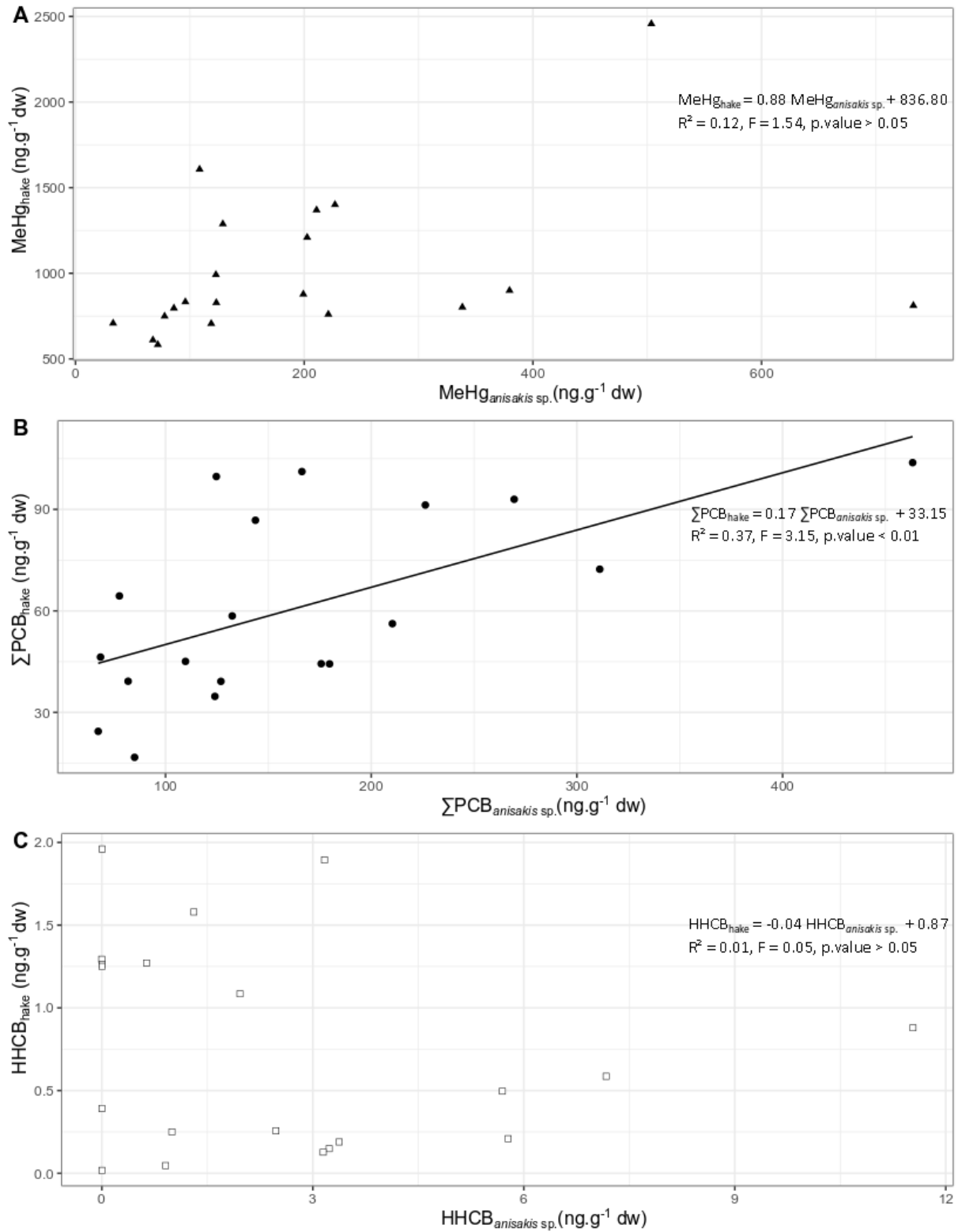
	<i>Merluccius merluccius</i>	<i>Anisakis</i> sp.
<b>Total length (mm)</b>	553 ± 45 (425 – 640)	–
<b>δ<sup>13</sup>C (‰)</b>	-17.9 ± 0.2 (-18.3 – -17.6)	-19.1 ± 0.5 (-20.8 – -18.2)
<b>δ<sup>15</sup>N (‰)</b>	13.7 ± 0.3 (13.2 – 14.4)	11.4 ± 0.9 (9.1 – 12.9)
<b>C/N</b>	3.1 ± 0.1 (3.1–3.2)	5.4 ± 0.5 (4.6–6.1)

226  
 227 Micropollutants level according to species  
 228 THg and MeHg concentrations were on average 6.4 and 4.8 times higher in hake than in *Anisakis* sp.  
 229 pools, respectively (Table 2). No significant relationship between mercury concentration in hake and  
 230 *Anisakis* sp. could be observed (Figure 3A). Among the 12 investigated PCB, congeners 101,118, 138,  
 231 149, 153, and 180 were measured in all hake and parasite samples tested whereas PCB 194 was  
 232 quantified in *Anisakis* sp. only. Unlike Hg, PCB concentrations were ~3 times higher in *Anisakis* sp.  
 233 than in hake (Figure 3B). Three emergent compounds, *i.e.* one musk (ATNH) and two UV-filters  
 234 (EHMC and OC) have been detected but in few individuals. Contrarily HHCB have been detected in  
 235 almost all hake and *Anisakis* sp. samples tested and consequently been used to look at size and  
 236 parasite effects. No relationship between HHCB concentration in host and parasite could be detected  
 237 (Figure 3C).

238 **Table 2: Micropollutants concentrations measured in hake muscles and *Anisakis* sp. pools (mean,**  
 239 **standard deviation and occurrence).**

	<i>Merluccius merluccius</i>	<i>Anisakis</i> sp.
<b>Mercury species (ng g<sup>-1</sup> dw)</b>		
<b>THg</b>	1543 ± 795 (20/20)	239 ± 196 (20/20)
<b>MeHg</b>	1000 ± 450 (20/20)	206 ± 176 (20/20)
<b>PCB congeners (ng g<sup>-1</sup> dw)</b>		
<b>PCB 101</b>	2 ± 1 (20/20)	11 ± 5 (19/20)
<b>PCB 149</b>	5 ± 2 (20/20)	17 ± 10 (20/20)

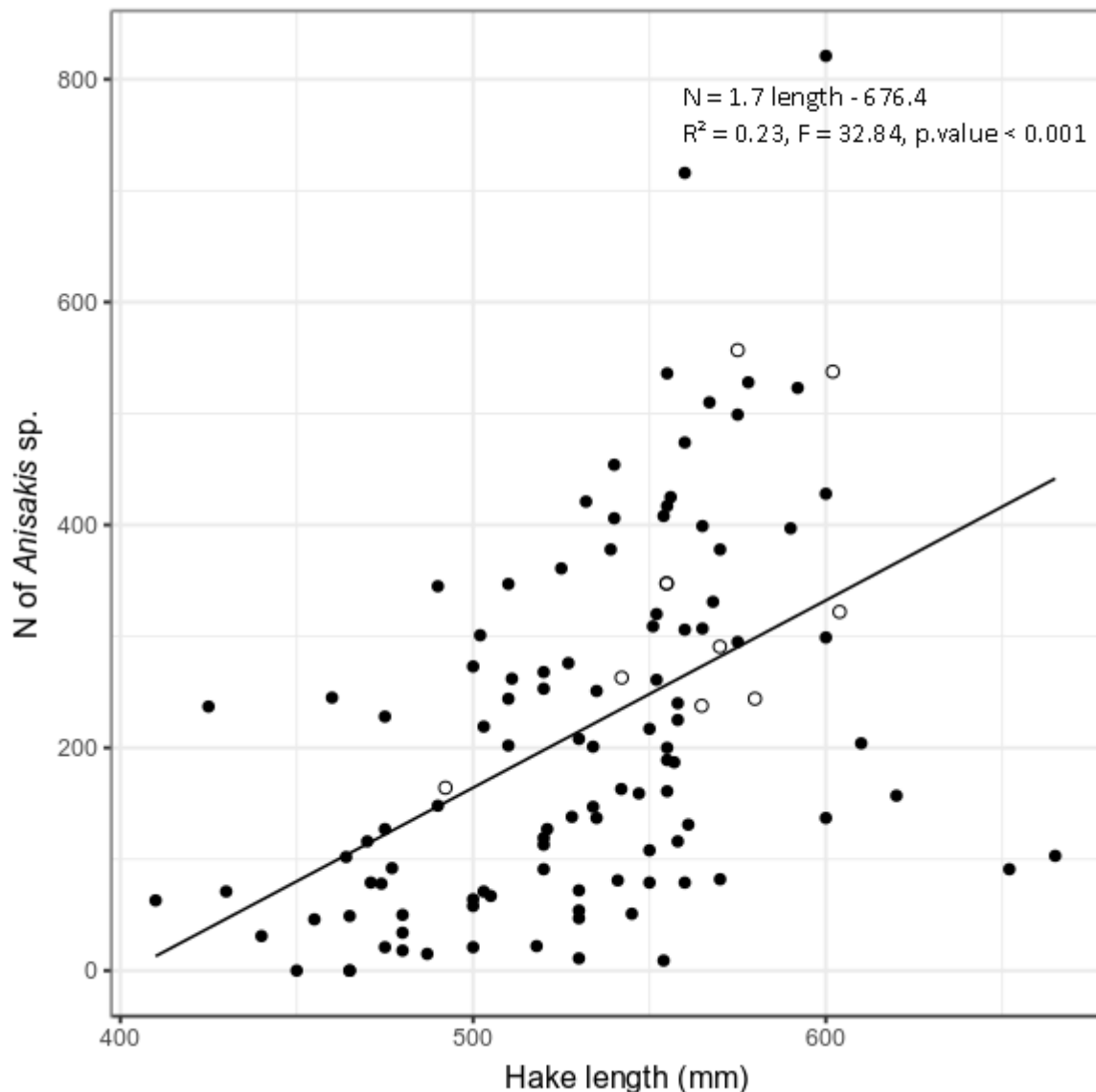
<b>PCB 118</b>	1 ± 1 (17/20)	8 ± 4 (20/20)
<b>PCB 153</b>	21 ± 9 (20/20)	51 ± 36 (20/20)
<b>PCB 138</b>	17 ± 8 (20/20)	46 ± 27 (20/20)
<b>PCB 180</b>	13 ± 7 (20/20)	32 ± 18 (20/20)
<b>PCB 194</b>	1 ± 1 (10/20)	<LOQ (0/20)
<b>∑PCB</b>	61 ± 28 (20/20)	165 ± 99 (20/20)
<b>Musk fragrances (ng g<sup>-1</sup> dw)</b>		
<b>ATNH</b>	1 ± 1 (4/20)	1 ± 2 (9/20)
<b>HHCB</b>	1 ± 1 (20/20)	3 ± 3 (14/20)
<b>UV-filters (ng g<sup>-1</sup> dw)</b>		
<b>OC</b>	<LOQ (0/20)	15 ± 22 (12/20)
<b>EHMC</b>	1 ± 1 (5/20)	<LOQ (0/20)



240

241 **Figure 3: Relationship between hake contamination and its *Anisakis sp.* pool contamination in MeHg (A),**  
 242  **$\Sigma\text{PCB}$  (B) and HHCB (C). Regression line is plotted only when significant (*i.e.* between hake and *Anisakis***  
 243 ***sp.*  $\Sigma\text{PCB}$  only)**

244 Hake's contamination and degree of *Anisakis* sp. infection



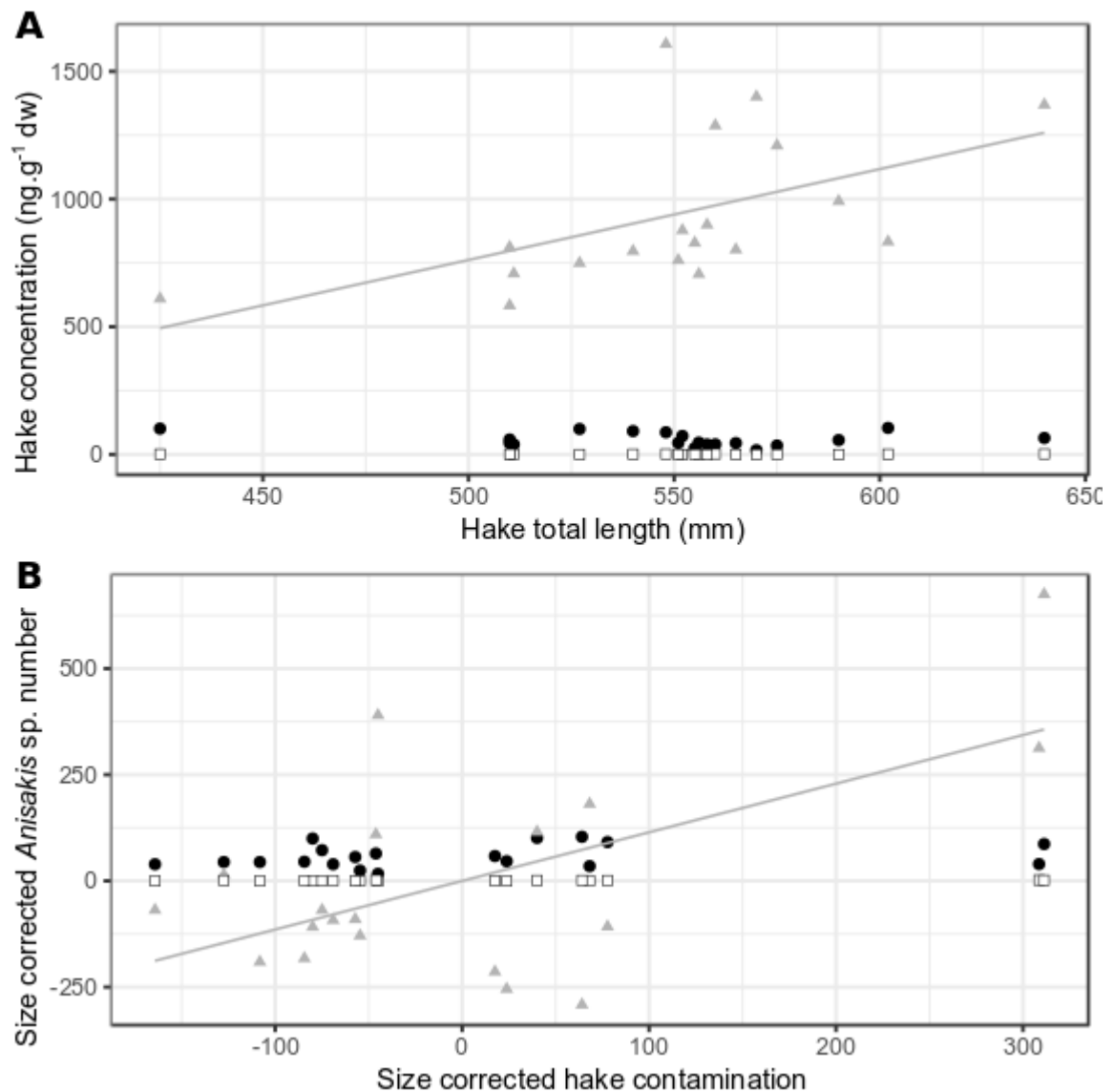
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**Figure 4: Relationship between the number (N) of *Anisakis* sp. (proxy of hake infection degree) and hake total length and sex (● female, ○ male).**

249 A significant increase of the infection by *Anisakis* sp. with the total length of its host was found  
 250 ( $F = 28.8, p.\text{value} < 0.05$ ) (Figure 4). However, no difference according to fish sex on the infection  
 251 degree has been observed ( $F = 1.4, p.\text{value} > 0.05$ ), despite males had a higher number of parasite  
 252 than females, ( $327 \pm 134$  and  $209 \pm 164$  for males and females respectively). In addition, larger hakes  
 253 were significantly more contaminated in MeHg ( $F = 6.8, p.\text{value} < 0.05$ ) but not in PCB ( $F = 0.9,$   
 254  $p.\text{value} > 0.05$ ) and in HHCB ( $F = 6.0 \cdot 10^{-4}, p.\text{value} > 0.05$ ) (Figure 5A). The higher the MeHg



255 concentration in hake muscle, the higher the number of *Anisakis* sp. ( $F= 10.5$ ,  $p.value < 0.01$ ) (Figure  
 256 5B). Similar relationships were not observed for other micropollutants tested ( $\Sigma$ PCB and HHCB).



257  
 258 **Figure 5: Relationship between hake contamination level (▲MeHg, ● $\Sigma$ PCB, □HHCB) and its total**  
 259 **length (A). Relationship between hake infection degree and its contamination level (▲MeHg, ● $\Sigma$ PCB,**  
 260 **□HHCB) corrected by hake total length (B).**

## 261 Discussion

262 Trophic relationship between hake and *Anisakis* sp.

263 Stable isotopes are widely used to identify trophic interactions and to determine trophic positions of  
 264 organisms in the food webs but for classical “prey-predator” or “primary producer - grazers”  
 265 interactions. Direct applications of isotopic relationships calibrated for these interactions (*i.e.*  
 266 fractionation factors of  $\sim 1\text{‰}$  for carbon and of  $\sim 3\text{‰}$  for nitrogen) to host-parasite relationship are

267 called into question (Thieltges et al., 2019). The present study revealed that the *Anisakis* sp. were  
268 consistently depleted in  $\delta^{15}\text{N}$  in relation to their host, as observed in Pinnegar et al. (2001) between  
269 *Merlangius merlangus* (Gadiforme) and its parasite *Hysterothylacium aduncum* (Nematode  
270 Ascaridoidea). Nitrogen isotopic ratios measured for *Anisakis* sp. was very close to literature values  
271 for small pelagic fish - themselves known for zooplankton predation behavior. This similarity could be  
272 interpreted like a classical of predator-prey relationship between *Anisakis* sp. and Euphausiids.  
273 Isotopic differences between *Anisakis* sp. and Euphausiids are of  $2.36 \pm 0.87\text{‰}$  and  $1.17 \pm 0.53\text{‰}$  for  
274 N and C, i.e. close to theoretical trophic enrichment factors between predator and preys, while lower  
275 values for *Anisakis* sp. than for hake can track the absence of trophic relationships. *Anisakis* sp.  
276 isotopic ratios would reflect thus a diet mostly based on the consumption of a previous host such as  
277 Euphausiids, first known intermediate host (Smith and Wootten, 1978). This hypothesis would be  
278 consistent with the fact that *Anisakis* sp. is encysted when in hakes' viscera cavity, potentially limiting  
279 matter exchanges. Nevertheless, large variability in *Anisakis* sp. isotopic ratios may demonstrate  
280 some variability in trophic behavior. In addition, isotopic relationship between host and parasites  
281 was questioned and may appear not straightforward as the relationship established between preys  
282 and predators (Pinnegar et al., 2001; Thieltges et al., 2019). The ability of parasites to use selectively  
283 some macromolecules from its host may explain odd factors. Parasites may be example selectively  
284 use  $^{14}\text{N}$ -enriched ammonia excreted from host tissues for amino acid synthesis (Barrett, 1981).  
285 Similarly, as lipids have lower  $\delta^{13}\text{C}$  values than proteins, their selective use may blur isotopic  
286 relationship, notably as isotopic ratios are measured for host muscle, a lipid poor tissue. Comparing  
287 tissue-specific and parasites isotopic ratios may be a pertinent research pathway to better address  
288 relationship established (Pinnegar et al., 2001). The isotopic variability measured in *Anisakis* sp. may  
289 also reflect that the nature of compounds uptaken may vary along with nutritional needs of the  
290 parasite. Future investigation of the actual pathway and macromolecules used by parasites, as well  
291 as the calibration of accurate isotopic fractionation factors are needed before being able to use  
292 stable isotope as tracers of host-parasites relationships.

293

294 Difference in micropollutants concentration between the parasite and its host

295 As diet constitutes the main contamination pathway for Hg and PCB, contaminant concentration in

296 preys is an essential parameter in understanding bioaccumulation pattern in predators (Cresson et

297 al., 2014; Harmelin-Vivien et al., 2012; Kenji et al., 2020). Since stable isotopes failed to ascertain the

298 relationship between hake and *Anisakis* sp., contaminant could be powerful tools to look differently

299 at this relationship. The absence of relationship between hake and *Anisakis* sp. for MeHg and HHCB

300 concentrations can be viewed as consistent with the absence of link between these species. This

301 hypothesis could nevertheless be formally confirmed in the absence of measurement in Euphausiids.

302 Previous studies documented MeHg behavior in fish, notably driven by the link between Hg and

303 some aminoacids (Harmelin-Vivien et al., 2012; Webb et al., 2006). The absence of relationship

304 between Hg concentration in hake and parasites may track the absence of major protein exchanges

305 between the two species. This assumption was confirmed by literature: nematodes such as *Anisakis*

306 that used fish as paratenic host with an encapsulated visceral life had lowest ability to concentrate

307 metals (Dural et al., 2011; Nachev and Sures, 2016)., notably when compared with other major

308 parasitic groups, such as tapeworms and acanthocephalans that live within the host's intestine (Sures

309 et al., 1999, 1997). Regarding other micropollutant tested, the pattern was somehow blurred. As

310 these compounds are emergent, they have received less scientific interest so far. Since their behavior

311 is less documented, mechanisms explaining the absence of a clear relationship between

312 concentrations in hake and *Anisakis* sp. are hard to identify. In hake, muscle may not be the most

313 suited organ to measure these compounds, and future studies could include measurement in other

314 organs with involved in detoxication metabolism, such as liver, spleen or gallbladder.

315 Fat content is usually low in most endoparasites. As they cannot produce their own long chain fatty

316 acids, they rely on their host to take up lipids (Maule and Marks, 2006). As PCBs are lipophilic, they

317 are usually transferred along with lipids. By example, oogenesis in hake is associated with PCB

318 transfer, explaining sexual difference in PCB concentration in hake (Bodiguel et al., 2009). Therefore,

319 the PCB concentration are usually lower in parasite than its host illustrating a low transfer between  
320 the two species. In this study, the PCB content was nearly 3 times higher in *Anisakis* sp than in hake.  
321 Even if the exchanges of organic contaminants between host and parasites have been less  
322 investigated than the exchanges of metals most studies usually consider that parasites do not  
323 accumulate organic contaminants (Yen Le et al. 2014). However, the significant relationship between  
324 PCB concentrations in *Anisakis* sp. and hake could track some exchanges. Once ingested by its host,  
325 the parasite crosses the stomach wall and encysts in the abdominal cavity. Cuticle synthesis requires  
326 high fatty acids content, notably sterols, that *Anisakis* sp. presumably take up from its host (Mika et  
327 al., 2010). This lipid uptake can explain increase of PCB concentration between hake and *Anisakis* sp.  
328 Such a transfer would also be consistent with the high C/N ratio observed here, consistently with  
329 previous studies that demonstrated different lipid levels all along parasite life cycle (Abollo and  
330 Pascual, 2001). Nevertheless, this result is contradictory with the accepted hypothesis of an arrested  
331 development in paratenic host, and by the fact that *Anisakis* L3 larvae do not use hake resources,  
332 usually exemplified by lower PCB concentrations in parasites than in host (Sures, 2004; Yen Le et al.  
333 2014). The results obtained here combining several trophic tracers may demonstrate that limited  
334 exchanges occur: blurred isotopic and Hg patterns may be consistent with no proteins exchange  
335 between hake and *Anisakis*; results based on PCB on the contrary may contrarily testify some  
336 exchanges of lipids and lipid-associated contaminants, even if of minor intensity. Assuming a PCB  
337 biomagnification factor of  $\sim 5$  (i.e. a 5-time increase of PCB concentrations at each trophic level, (e.g.  
338 Fisk et al., 2001) and keeping in mind that *Anisakis* tissues are lipid-rich (highlighted by high C/N  
339 ratios), the high PCB concentration in *Anisakis* cannot be explained only by the consumption of  
340 Euphausiids. Even if exchanges of lipid occur between Euphausiids and *Anisakis*, considering other  
341 exchanges is needed to explain higher PCB concentration in *Anisakis* than in hake. If exchanges only  
342 occurred between *Anisakis* and Euphausiids, the parasite could be considered to be at a trophic level  
343 similar to that of zooplankton-feeding fish, i.e. one trophic level below hake. Thus, PCB  
344 concentrations should be somehow lower. Nevertheless, the low sample size of the present study

345 and the impossibility to include Euphausiids and zooplankton-feeding fish in this study precluded  
346 from reaching a formal conclusion. Further measurements of isotopic ratios, lipids and contaminants  
347 concentrations is needed to accurately depict the complex exchanges of matter between host and  
348 parasites.

349

350 Relationship between fish contamination and degree of infection

351 Previous studies demonstrated that combined effects of contaminants and parasites on host are hard  
352 to identify and predict. In the present study, parasites number and Hg contamination were both  
353 correlated with hake length. Size effect of Hg increase is classical in hake and explained by  
354 bioaccumulation and chronic exposure to contaminant (Cossa et al., 2012; Cresson et al., 2015;  
355 Harmelin-Vivien et al., 2012). The same mechanism were proposed to explain higher parasite  
356 number in large individuals (Morsy et al., 2012). In addition, the parasite number in hake was  
357 generally higher in males than in females despite being non-significant. This may be explained by the  
358 sexual dimorphism in fish size. A slower growth rate of males (de Pontual et al., 2006) implies a  
359 longer expose time to parasite. Moreover, larger fish increase the capacity to have larger number,  
360 size and various species of prey generating more interaction in the ecosystem and thus in turn  
361 increasing the probability of exposition to infected prey (Hudson et al., 2006; Morsy et al., 2012).  
362 Such an effect of sexual dimorphism and differential growth rate was already proposed to explain  
363 difference in contamination in hake (Cossa et al., 2012; Harmelin-Vivien et al., 2012).

364 In the present study, fish contamination (notably in MeHg) and degree of parasitism are correlated,  
365 even after removing size effect on both parameters. Such a result might be consistent with a  
366 synergistic effect of contaminant and parasites. Contaminant may presumably weaken hake's  
367 immune system, leading to enhanced parasitic infestation. Previous studies demonstrated that MeHg  
368 at concentrations lower than levels measured in the present study (300 to 400 ng g<sup>-1</sup> in fish muscle)  
369 weaken immune system of marine fish species (Guardiola et al., 2016; Ren et al., 2019). In addition  
370 an increase of parasite load in highly contaminated fish was also observed in other studies (Sagerup

371 et al., 2009; Sures and Knopf, 2012). Other studies also demonstrated some effect of PCBs on  
372 immune system, but at concentrations way higher than concentrations measured here (Sures and  
373 Knopf, 2012).

## 374 **Conclusions**

375 The present data indicated that average contamination levels in parasite were lower than in its host  
376 for Hg and emergent micropollutants but not for PCB and that isotopic relationship was not  
377 straightforward. These results testified some complex trophic transfers, consistent with functional  
378 optimization of the parasite, and its encysted nature in hake. In addition, parasites number increases  
379 in highly Hg contaminated hake individual, presumably as a result of a weakened immune system of  
380 the host. Present results call for extended analyses of the complex relationship established between  
381 hosts and parasites, notably regarding the nature and the intensity of the organic matter fluxes. They  
382 are nevertheless consistent with the current idea of the importance of parasites as important drivers  
383 of marine ecosystems.

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## 611 Supplementary material

612 Analytical performances of the method used including limits of quantification (LOQ), recoveries (%) and  
 613 precisions.

	LOQ	<i>Merluccius merluccius</i>		<i>Anisakis</i> sp.	
		Recovery values (%)	Precision (%)	Recovery values (%)	Precision (%)
<b>PCB congeners (ng g<sup>-1</sup> dw)</b>					
<b>PCB 18</b>	0.1	46.2	0.2	70.7	13.0
<b>PCB 28</b>	0.2	68.3	13.1	82.7	10.6
<b>PCB 31</b>	0.2	68.3	13.1	82.7	10.6
<b>PCB 44</b>	0.1	57.5	15.2	81.5	7.8
<b>PCB 52</b>	0.1	65.9	20.0	84.1	8.1
<b>PCB 101</b>	0.1	62.8	21.8	84.1	4.4
<b>PCB 118</b>	0.1	69.3	17.1	84.7	2.9
<b>PCB 138</b>	0.2	51.7	6.2	68.8	21.6
<b>PCB 149</b>	0.1	59.1	16.5	79.8	6.1
<b>PCB 153</b>	0.2	59.6	5.3	77.1	24.4
<b>PCB 180</b>	0.3	53.9	7.6	79.6	8.9
<b>PCB 194</b>	0.5	61.4	11.3	67.4	17.6
<b>Musk fragrances (ng g<sup>-1</sup> dw)</b>					
<b>ADBI</b>	0.1	102.1	2.9	87.4	3.3
<b>AHMI</b>	0.1	98.2	2.6	83.2	20.4
<b>AHTN</b>	0.1	110.0	12.8	105.9	32.2
<b>ATII</b>	0.1	108.8	11.5	102.1	15.1
<b>HHCB</b>	0.1	111.6	14.7	100.8	7.5
<b>MA</b>	0.9	102.9	4.0	75.0	8.9

<b>MK</b>	0.2	104.0	5.5	92.1	9.1
<b>MM</b>	0.1	110.1	13.0	91.7	1.8
<b>MX</b>	0.1	108.0	10.5	94.1	12.1
<b>UV-filters (ng g<sup>-1</sup> dw)</b>					
<b>3-BC</b>	1.4	127.0	30.1	108.4	11.2
<b>EHMC</b>	0.2	111.7	14.8	123.8	11.3
<b>4-MBC</b>	0.7	108.3	10.8	102.1	20.6
<b>OC</b>	1.9	104.0	5.4	93.2	1.2
<b>OD-PABA</b>	0.1	112.8	16.0	67.0	29.3

Highlights:

- Partial trophic interaction between L3 *Anisakis* sp. larvae and its host, hake.
- PCB transfer may result from the uptake of lipids from hake by the parasites.
- Highly MeHg level in hake can weaken immune system and increase *Anisakis* sp. number.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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