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

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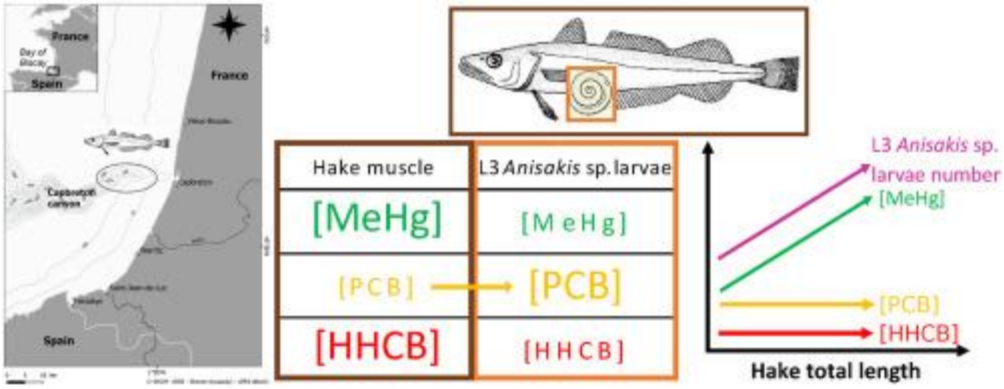
Abstract :

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

Introduction

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

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

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

Hake is an important demersal fishery resource. In the Bay of Biscay, this species is the first landed with 42 536 tons caught in 2017 (Ifremer Fisheries Information system, 2018). Its diet varies with ontogeny: juveniles mostly consume pelagic crustaceans, and adults are piscivores, with blue whiting, horse mackerel, sardine and also young hake being important preys (Mahe et al., 2007; Rault et al., 2017; Velasco and Olaso, 2000). Due to its diet, hake is one of fish species being an intermediate host for the L3 *Anisakis* sp. larvae (Aibinu et al., 2019). In addition, its trophic position drives biomagnification and high burdens in Hg (Chouvelon et al., 2014; Cossa et al., 2012) or PCB (Bodiguel et al., 2009). Understanding the contamination and parasitism levels and their combined effects on hake is thus of prime importance for ecological, economical and sanitary reasons.

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

Material and methods

Sampling and samples preparation

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

Anisakis sp. L3-larvae (referred as to *Anisakis* sp. hereafter) were systematically collected at the opening of the visceral cavity of all hake individual, before their leaking to the organs. Parasites were rinsed with distilled water, frozen at -20 °C for 48 h then decapsulated, counted and pooled to one sample. The amount of matter needed to perform contaminant analyses was high (> 2 g dry weight), *i.e.* a weight corresponding to ~200 *Anisakis* sp. individuals. Analyses were thus performed in pools of parasites collected in 20 hakes randomly selected among hakes with parasite's abundance higher than 200 individuals. All samples (*Anisakis* sp. pools and hake muscles) were stored frozen, freeze-dried and grinded.

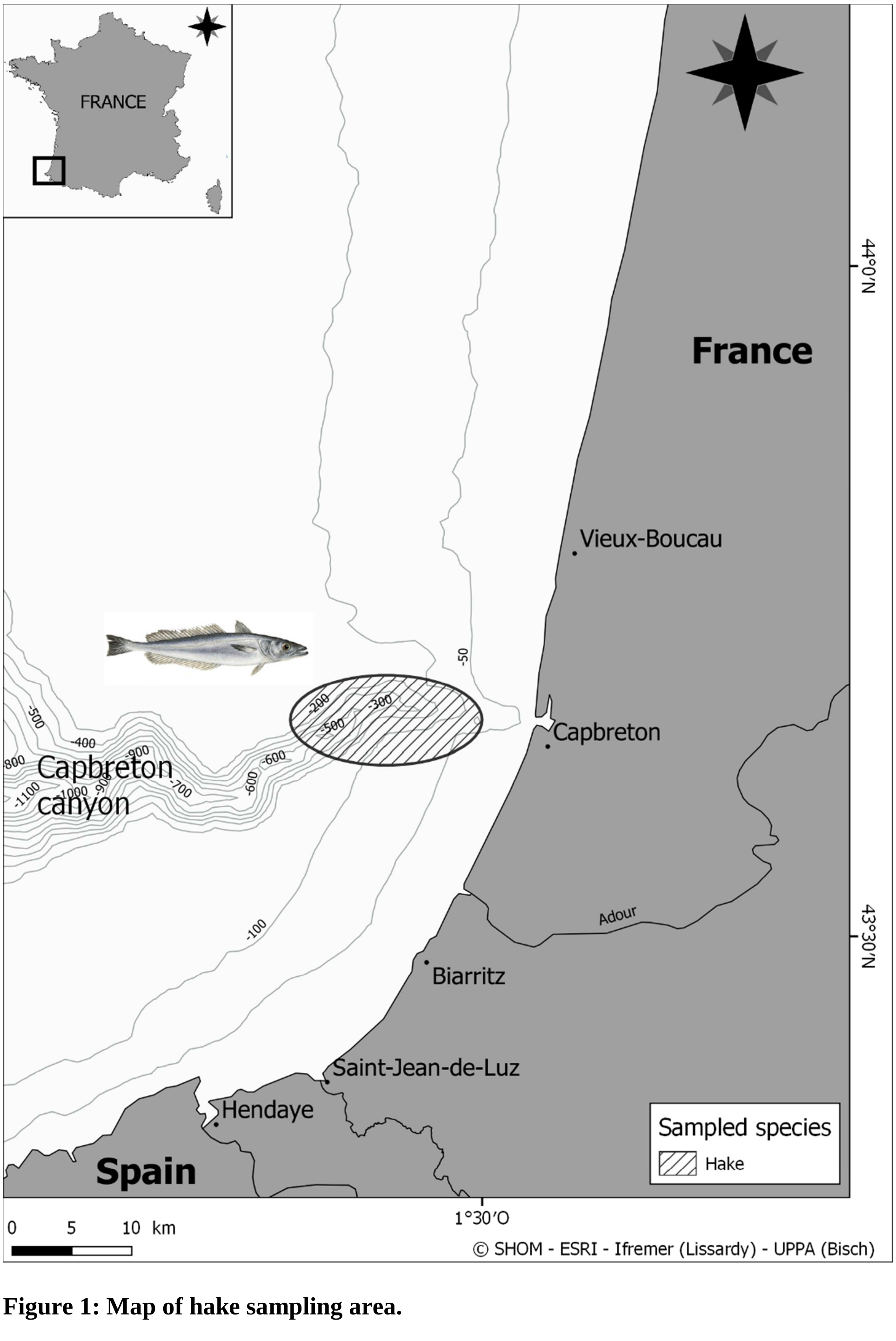


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Figure 1: Map of hake sampling area.

Carbon and nitrogen isotopic analysis

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

Mercury species analysis

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

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

Musk fragrances, UV-filters, and PCBs analyses

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

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

All concentrations were expressed relatively to dry weight. Concentrations measured for the 12 PCB congeners were summed and expressed with the usual ∑PCB notation hereafter.

Data analyses

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

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

Results

Trophic ecology

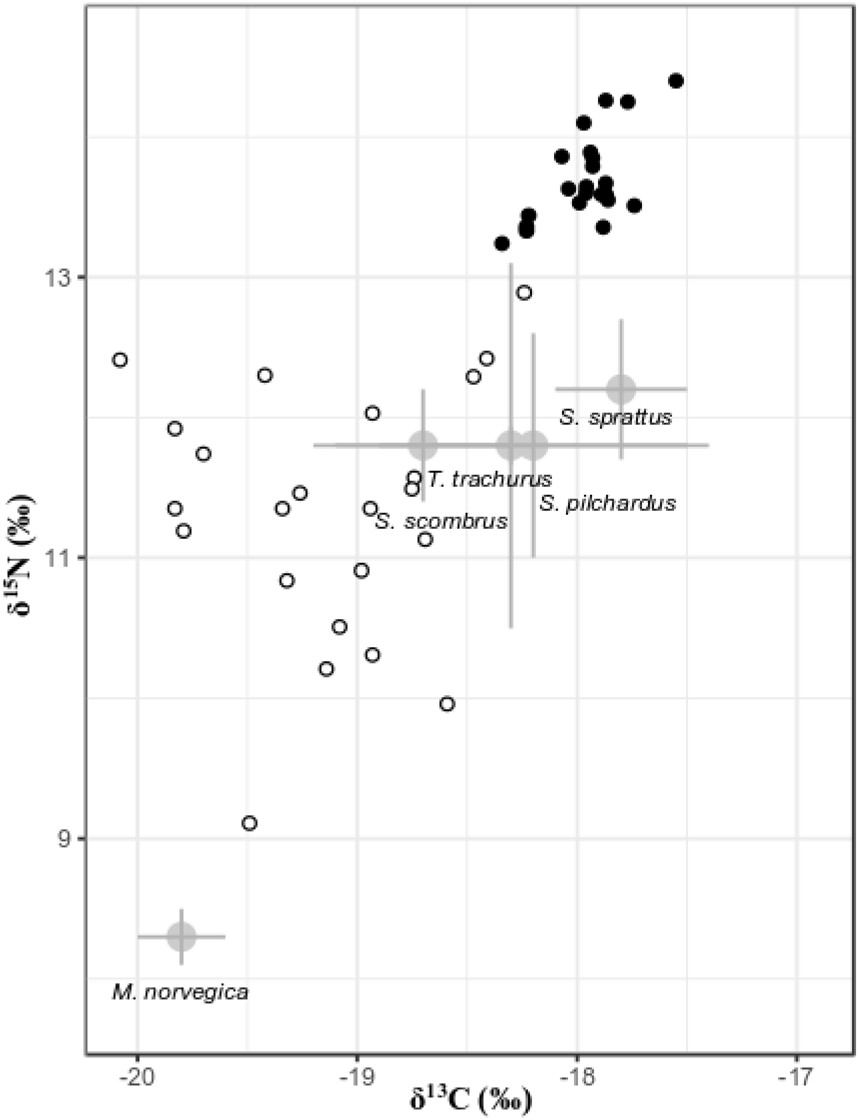

Figure 2: δ^{13}C and δ^{15}N signatures for hake (●) and *Anisakis* sp. (○) from the Capbreton canyon . In order to complete the food web, pelagic fishes and Euphausiids are displayed in grey and are based on literature values (Chouvelon et al., 2012).

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

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

	Merluccius merluccius	*Anisakis* sp.
Total length (mm)	553 ± 45 (425 – 640)	–
$\delta^{13}C$ (‰)	-17.9 ± 0.2 (-18.3 – -17.6)	-19.1 ± 0.5 (-20.8 – -18.2)
$\delta^{15}N$ (‰)	13.7 ± 0.3 (13.2 – 14.4)	11.4 ± 0.9 (9.1 – 12.9)
C/N	3.1 ± 0.1 (3.1–3.2)	5.4 ± 0.5 (4.6–6.1)

Micropollutants level according to species

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

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

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

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

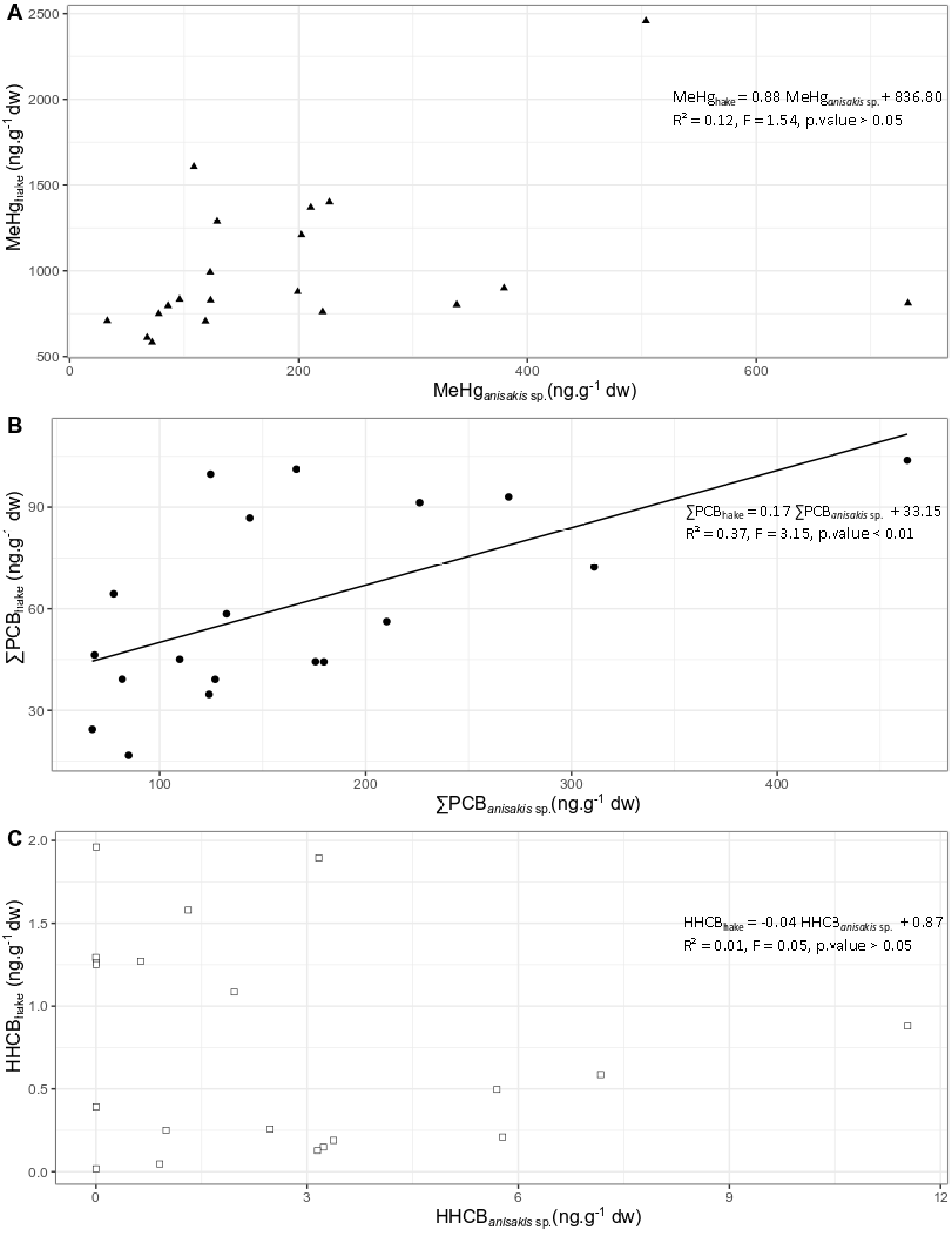

Figure 3: Relationship between hake contamination and its *Anisakis* sp. pool contamination in MeHg (A), ∑PCB (B) and HHCB (C). Regression line is plotted only when significant (*i.e.* between hake and *Anisakis* sp. ΣPCB only)

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

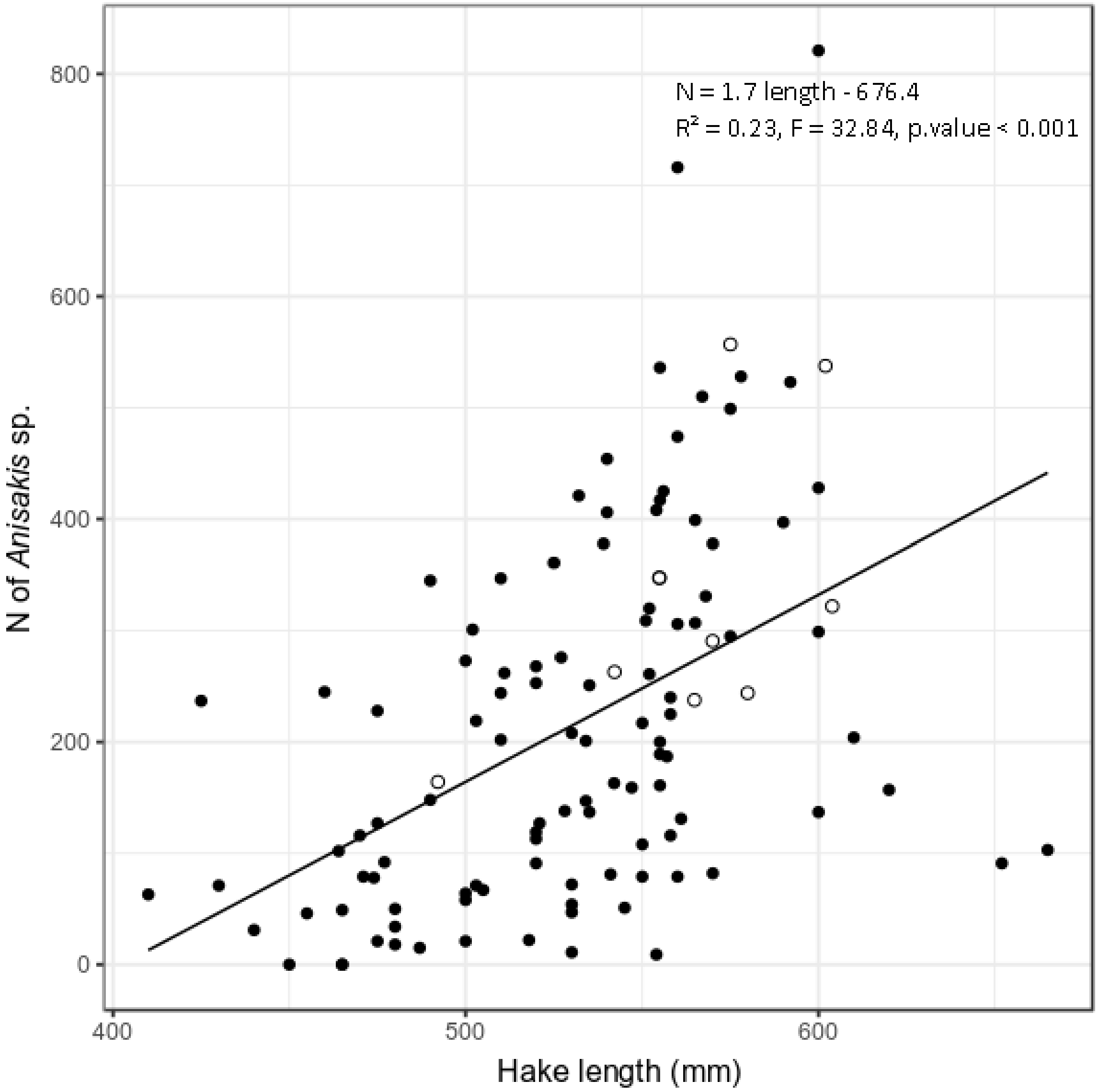

Figure 4: Relationship between the number (N) of *Anisakis* sp. (proxy of hake infection degree) and hake total length and sex (● female, ○ male).

A significant increase of the infection by *Anisakis* sp. with the total length of its host was found (F = 28.8, p.value < 0.05) (Figure 4). However, no difference according to fish sex on the infection degree has been observed (F = 1.4, p.value > 0.05), despite males had a higher number of parasite than females, (327 ± 134 and 209 ± 164 for males and females respectively). In addition, larger hakes were significantly more contaminated in MeHg (F = 6.8, p.value < 0.05) but not in PCB (F = 0.9, p.value > 0.05) and in HHCB (F = 6.0.10^{-4}, p.value > 0.05) (Figure 5A). The higher the MeHg concentration in hake muscle, the higher the number of *Anisakis* sp. (F= 10.5, p.value < 0.01) (Figure 5B). Similar relationships were not observed for other micropollutants tested (∑PCB and HHCB).

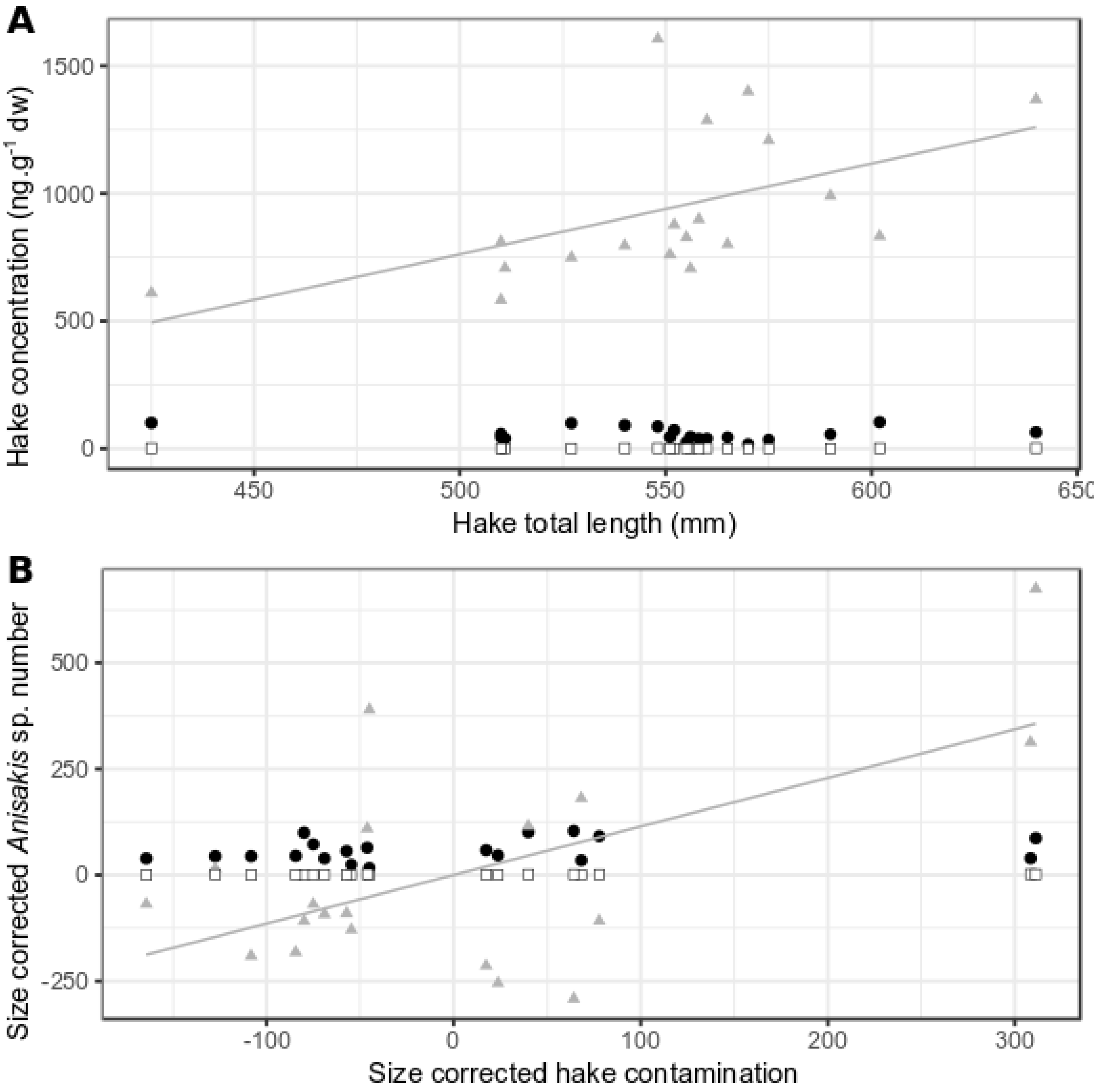

Figure 5: Relationship between hake contamination level (▲MeHg, •∑PCB, □HHCB) and its total length (A). Relationship between hake infection degree and its contamination level (▲MeHg, •∑PCB, □HHCB) corrected by hake total length (B).

Discussion

Trophic relationship between hake and *Anisakis* sp.

Stable isotopes are widely used to identify trophic interactions and to determine trophic positions of organisms in the food webs but for classical "prey-predator" or "primary producer - grazers" interactions. Direct applications of isotopic relationships calibrated for these interactions (*i.e.* fractionation factors of ~1‰ for carbon and of ~3‰ for nitrogen) to host-parasite relationship are called into question (Thieltges et al., 2019). The present study revealed that the *Anisakis* sp. were consistently depleted in $\delta^{15}N$ in relation to their host, as observed in Pinnegar et al. (2001) between *Merlangius merlangus* (Gadiforme) and its parasite *Hysterothylacium aduncum* (Nematode Ascaridoidea). Nitrogen isotopic ratios measured for *Anisakis* sp. was very close to literature values for small pelagic fish - themselves known for zooplankton predation behavior. This similarity could be interpreted like a classical of predator-prey relationship between *Anisakis* sp. and Euphausiids. Isotopic differences between *Anisakis* sp. and Euphausiids are of 2.36 ± 0.87‰ and 1.17 ± 0.53‰ for N and C, i.e. close to theoretical trophic enrichment factors between predator and preys, while lower values for *Anisakis* sp. than for hake can track the absence of trophic relationships. *Anisakis* sp. isotopic ratios would reflect thus a diet mostly based on the consumption of a previous host such as Euphausiids , first known intermediate host (Smith and Wootten, 1978). This hypothesis would be consistent with the fact that *Anisakis* sp. is encysted when in hakes' viscera cavity, potentially limiting matter exchanges. Nevertheless, large variability in *Anisakis* sp. isotopic ratios may demonstrate some variability in trophic behavior. In addition, isotopic relationship between host and parasites was questioned and may appear not straightforward as the relationship established between preys and predators (Pinnegar et al., 2001; Thieltges et al., 2019). The ability of parasites to use selectively some macromolecules from its host may explain odd factors. Parasites may be example selectively use ^{14}N-enriched ammonia excreted from host tissues for amino acid synthesis (Barrett, 1981). Similarly, as lipids have lower $\delta^{13}C$ values than proteins, their selective use may blur isotopic relationship, notably as isotopic ratios are measured for host muscle, a lipid poor tissue. Comparing tissue-specific and parasites isotopic ratios may be a pertinent research pathway to better address relationship established (Pinnegar et al., 2001). The isotopic variability measured in *Anisakis* sp. may also reflect that the nature of compounds uptaken may vary along with nutritional needs of the parasite. Future investigation of the actual pathway and macromolecules used by parasites, as well as the calibration of accurate isotopic fractionation factors are needed before being able to use stable isotope as tracers of host-parasites relationships.

Difference in micropollutants concentration between the parasite and its host

As diet constitutes the main contamination pathway for Hg and PCB, contaminant concentration in preys is an essential parameter in understanding bioaccumulation pattern in predators (Cresson et al., 2014; Harmelin-Vivien et al., 2012; Kenji et al., 2020). Since stable isotopes failed to ascertain the relationship between hake and *Anisakis* sp., contaminant could be powerful tools to look differently at this relationship. The absence of relationship between hake and *Anisakis* sp. for MeHg and HHCB concentrations can be viewed as consistent with the absence of link between these species. This hypothesis could nevertheless be formally confirmed in the absence of measurement in Euphausiids. Previous studies documented MeHg behavior in fish, notably driven by the link between Hg and some aminoacids (Harmelin-Vivien et al., 2012; Webb et al., 2006). The absence of relationship between Hg concentration in hake and parasites may track the absence of major protein exchanges between the two species. This assumption was confirmed by literature: nematodes such as *Anisakis* that used fish as paratenic host with an encapsulated visceral life had lowest ability to concentrate metals (Dural et al., 2011; Nachev and Sures, 2016)., notably when compared with other major parasitic groups, such as tapeworms and acanthocephalans that live within the host's intestine (Sures et al., 1999, 1997). Regarding other micropollutant tested, the pattern was somehow blurred. As these compounds are emergent, they have received less scientific interest so far. Since their behavior is less documented, mechanisms explaining the absence of a clear relationship between concentrations in hake and *Anisakis* sp. are hard to identify. In hake, muscle may not be the most suited organ to measure these compounds, and future studies could include measurement in other organs with involved in detoxication metabolism, such as liver, spleen or gallbladder.

Fat content is usually low in most endoparasites. As they cannot produce their own long chain fatty acids, they rely on their host to take up lipids (Maule and Marks, 2006). As PCBs are lipophilic, they are usually transferred along with lipids. By example, oogenesis in hake is associated with PCB transfer, explaining sexual difference in PCB concentration in hake (Bodiguel et al., 2009). Therefore, the PCB concentration are usually lower in parasite than its host illustrating a low transfer between the two species In this study, the PCB content was nearly 3 time higher in *Anisakis* sp than in hake. Even if the exchanges of organic contaminants between host and parasites have been less investigated than the exchanges of metals most studies usually consider that parasites do not accumulate organic contaminants (Yen Le et al. 2014). However, the significant relationship between PCB concentrations in *Anisakis* sp. and hake could track some exchanges. Once ingested by its host, the parasite crosses the stomach wall and encysts in the abdominal cavity. Cuticle synthesis requires high fatty acids content, notably sterols, that *Anisakis* sp. presumably take up from its host (Mika et al., 2010). This lipid uptake can explain increase of PCB concentration between hake and *Anisakis* sp. Such a transfer would also be consistent with the high C/N ratio observed here, consistently with previous studies that demonstrated different lipid levels all along parasite life cycle (Abollo and Pascual, 2001). Nevertheless, this result is contradictory with the accepted hypothesis of an arrested development in paratenic host, and by the fact that *Anisakis* L3 larvae do not use hake resources, usually exemplified by lower PCB concentrations in parasites than in host (Sures, 2004; Yen Le et al. 2014). The results obtained here combing several trophic tracers may demonstrate that limited exchanges occur: blurred isotopic and Hg patterns may be consistent with no proteins exchange between hake and *Anisakis*; results based on PCB on the contrary may contrarily testify some exchanges of lipids and lipid-associated contaminants, even if of minor intensity. Assuming a PCB biomagnification factor of ~5 (*i.e.* a 5-time increase of PCB concentrations at each trophic level, (e.g. Fisk et al., 2001) and keeping in mind that *Anisakis* tissues are lipid-rich (highlighted by high C/N ratios), the high PCB concentration in *Anisakis* cannot be explained only by the consumption of Euphausiids. Even if exchanges of lipid occur between Euphausiids and *Anisakis*, considering other exchanges is needed to explain higher PCB concentration in *Anisakis* than in hake. If exchanges only occurred between *Anisakis* and Euphausiids, the parasite could be considered to be at a trophic level similar to that of zooplankton-feeding fish, *i.e.* one trophic level below hake. Thus, PCB concentrations should be somehow lower. Nevertheless, the low sample size of the present study and the impossibility to include Euphausiids and zooplankton-feeding fish in this study precluded from reaching a formal conclusion. Further measurements of isotopic ratios, lipids and contaminants concentrations is needed to accurately depict the complex exchanges of matter between host and parasites.

Relationship between fish contamination and degree of infection

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

In the present study, fish contamination (notably in MeHg) and degree of parasitism are correlated, even after removing size effect on both parameters. Such a result might be consistent with a synergistic effect of contaminant and parasites. Contaminant may presumably weaken hake's immune system, leading to enhanced parasitic infestation. Previous studies demonstrated that MeHg at concentrations lower than levels measured in the present study (300 to 400 ng g^{-1} in fish muscle) weaken immune system of marine fish species (Guardiola et al., 2016; Ren et al., 2019). In addition an increase of parasite load in highly contaminated fish was also observed in other studies (Sagerup et al., 2009; Sures and Knopf, 2012). Other studies also demonstrated some effect of PCBs on immune system, but at concentrations way higher than concentrations measured here (Sures and Knopf, 2012).

Conclusions

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

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Supplementary material

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

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

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

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: