
Fish length, diet and depth drive Anisakis levels in a zooplankton-feeding fish

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Abstract :

Parasites play a key but overlooked role in the functioning of marine systems. Understanding the drivers of parasites infection in fish is thus crucial. *Anisakis* levels in the Atlantic horse mackerel *Trachurus trachurus* resulted from both biotic and environmental drivers. Ontogenic variation of the diet was the first driver of *Anisakis* level. Nonlinear pattern confirmed that the variation of parasitism was related to ontogenetic dietary shifts. The impact of depth on diet also explained the effect of depth on parasites. In deeper areas, fish only access pelagic preys, where *Anisakis* presence is high, consistently with the pelagic nature of the parasite. Finally, relative proportions of parasitic burden by tissue appeared to be driven by lipid content. In lipid-rich species like *T. trachurus*, *Anisakis* can find favorable conditions in the body cavity and in muscle and avoid liver. Parasitism levels in fish are thus the result of patterns at play at different levels and that these factors should be better considered before being able to include parasites in an integrated vision of marine ecosystem functioning.

Keywords : *Anisakis*, fish parasites, environmental drivers, ontogenic shift

23 Introduction

24 Parasites are nowadays recognized as key players of marine ecosystem functioning. They represent a
25 major part of biodiversity, up to half of the living species for some analyses (e.g. Dobson et al. 2008).
26 Their integration as actual component of ecosystems is thus crucial, as they are able to completely
27 transform our perception of ecosystem functioning when efficiently considered (Hudson et al. 2006;
28 Lafferty et al. 2008; Dobson et al. 2008; Frainer et al. 2018). Investigating the actual role of parasites
29 in ecosystem functioning has consequently been largely claimed in many papers and for decades
30 (Dobson and Hudson 1986; Marcogliese and Cone 1997; Hudson et al. 2006; Dobson et al. 2008; Timi
31 and Poulin 2020; Welicky et al. 2021) and is consistent with the current calls for more integrated
32 approaches in ecology (Seibold et al. 2018). Under the assumption that the more the interactions, the
33 more resistant and resilient the ecosystems, higher abundance of parasites is now proposed as a proxy
34 of healthier ecosystems. Indeed, higher abundance would result from increased transmission, related
35 to increased interactions. This is notably exemplified in marine protected areas, where lower fisheries
36 pressure results in higher host abundance, increased interactions and thus higher parasitic diversity
37 (Bartoli et al. 2005; Hudson et al. 2006; Wood et al. 2013).

38 Unifying ecology and parasitology remains consequently a challenge, and specifically in marine systems
39 (Poulin et al. 2016). The specific nature of marine environment precludes from a direct transposition
40 of concepts and mechanisms developed in terrestrial systems, as specific functioning (e.g. longer, more
41 complex and size-driven food webs) or physical properties of the aquatic environment shaped different
42 transmission pathways (Lopez and Duffy 2021). The vertical component of marine systems is notably
43 prevalent, as it can facilitate or impede the encounter between successive hosts of the parasite cycle.
44 More largely, investigating the importance of spatial drivers structuring networks of biological
45 interactions – whether considering predator-prey or host-parasites interactions – was called as a
46 promising avenue in community ecology (Ings et al. 2009; Valois and Poulin 2015; Seibold et al. 2018;
47 Lopez and Duffy 2021). The ability to perform important vertical migrations was by example observed
48 as a good predictor of the parasitic abundance in a mesopelagic fish assemblage (Woodstock et al.
49 2020). Similarly, limited interactions in pelagic system explained lower parasitic diversity in the pelagic
50 fish assemblage, while increased trophic interactions allowed higher transmission rates and explained
51 higher diversity for the benthic-demersal assemblage (Klimpel et al. 2006).

52 Nematodes of the genus *Anisakis* Dujardin, 1845 are usual parasites of pelagic fishes and cetaceans.
53 Eggs are emitted in cetaceans' feces, hatch and develop freely in the water. Larvae are able to float in
54 the water, allowing their consumption by zooplanktonic crustacean, generally euphausiids (Smith
55 1983a; Klimpel and Palm 2011; Busch et al. 2012; Gregori et al. 2015). Fish at several trophic levels are
56 infected through successive predation events. Fish are paratenic host, *i.e.* *Anisakis* do not undergo

57 morphological transformation in fish, but fish facilitate the encounter of larvae from first (zooplankton)
58 with the definitive host. Final transformation into adult reproductive stage occurs when cetaceans
59 consume fish or cephalopods, allowing the completion of the cycle (Smith 1983a; Klimpel and Palm
60 2011). Nonetheless, most studies focused on fish, and detailed information about other stages of the
61 cycle are largely missing, despite the importance of elucidating parasitic cycles (Poulin et al. 2016). The
62 actual occurrence of *Anisakis* larvae in other zooplanktonic groups is by example questioned, and few
63 information are available about level of adults in cetaceans. Human can ingest *Anisakis* after the
64 consumption of contaminated and undercooked or raw marine products, leading to substantial
65 visceral pathologies if *Anisakis* if consumed alive, or by allergic mechanism even after the consumption
66 of dead parasites (Audicana et al. 2002; Audicana and Kennedy 2008). Anisakidae is consistently ranked
67 amongst the ten most at risk foodborne parasites in Europe. About 500 cases are reported annually in
68 Europe, in countries with high fish consumption, namely the Netherlands, Germany, France and Spain
69 (Klimpel and Palm 2011 and references therein; Buchmann and Mehrdana 2016; Bouwknecht et al.
70 2018). Nevertheless, few studies actually investigated the drivers of individual parasitic abundance in
71 fish. This notably results from the predominance of human-health focusing studies, and that
72 understanding the drivers has limited interest to address and prevent risks for human health (Poulin
73 et al. 2016; Timi and Poulin 2020). Fish size is by example classically observed as a factor driving
74 parasitic abundance, but with limited investigation of the biotic or environmental drivers of this
75 pattern (Pulleiro-Potel et al. 2015; Cipriani et al. 2018; Gay et al. 2018; Levsen et al. 2018; Menconi et
76 al. 2022).

77 Previous studies in the English Channel demonstrated that *Anisakis* prevalence is high in several pelagic
78 species, notably Atlantic horse mackerel *Trachurus trachurus* (MacKenzie et al., 2008; Mattiucci et al.,
79 2008). This species is a key component of this ecosystem in autumn. It represents ~10 to 20 % of the
80 average fish biomass in the area, but can reach ~40 to 50 % some years (Fig. 1a). It is also an important
81 commercial species locally, with annual catches and landings higher than 10 000 tons since the 2000's
82 (Fig. 1b). Its level of biomass is a direct driver of the major organic matter fluxes locally (Timmerman
83 et al. 2021), consistently with the depth-driven variability of the benthic-pelagic coupling previously
84 observed in the area (Kopp et al. 2015; Giraldo et al. 2017; Travers-Trolet et al. 2019; Cresson et al.
85 2020).

86 Thus, this work aimed at understanding ecological and biological drivers of the abundance of *Anisakis*
87 in horse mackerel in the English Channel at three levels of ecological complexity. At the geographical
88 scale, the role of depth was investigated, as the effect of depth on trophic functioning previously
89 demonstrated was also expected to affect *Anisakis* burdens. At interindividual level, the role of diet
90 and length-related variation of diet were examined. The present case study was a powerful

91 opportunity to address the mechanisms originating the correlation between *Anisakis* levels and fish
92 length. Finally, at intraindividual level, the repartition of the parasitic burden between host tissues
93 allowed investigating some drivers of micro-niche availability for *Anisakis*.

94 **Materials and methods**

95 **Field sampling** – Atlantic horse mackerel (*Trachurus trachurus*) individuals were collected during the
96 CAMANOC survey in October 2014 at 10 stations in the Western English Channel (Fig. 2). CAMANOC
97 was an ecosystemic survey dedicated to sample all components of the English Channel ecosystem
98 (Travers-Trolet and Verin 2014). Fishes were collected using a Grande Ouverture Verticale bottom
99 trawl, towed for 30 minutes at 4 knots. When onboard, all fish species were sorted and identified. At
100 each station, 10 to 20 horse mackerels belonging to the modal size class were collected and stored
101 frozen before further analyses. Modal size class was targeted, because it was considered as a proxy of
102 the most abundant size class at each station. Protocols were evaluated by the French research institute
103 for exploitation of the sea prior to the survey. In addition, survey's PIs received training about animal
104 well-being and ethics.

105 **Dissection** - Back in the laboratory, standard length of all individuals was measured to the nearest mm.
106 Standard length was chosen as the caudal tail of several individuals was damaged during trawling,
107 precluding from the measurement of total length. One small dorsal muscle sample (stable isotope
108 analyses) and stomach content were dissected out on all individuals and stored frozen (-20°C). Muscle
109 samples were then stored at -80°C for 24h, before being freeze dried and grinded. Liver, gonads,
110 abdominal cavity and remaining muscle were collected to analyze the presence of *Anisakis* sp.
111 parasites by the UV-press method (Karl and Leinemann 1993; Karl and Levsen 2011; Levsen et al. 2022).

112 **Isotopic analysis** – Analysis of stable isotope in fish is nowadays considered as a powerful tool to
113 determine position of organisms in food web (Fry 2006; Layman et al. 2012; Pethybridge et al. 2018).
114 Consumers integrate in their tissue the isotopic ratio of their diet, with an offset called discrimination.
115 Trophic discrimination factor (TDF hereafter) is higher for nitrogen (~3‰ per trophic level) than for
116 carbon (~1‰). This property allows the coupled use of these two elements to determine the trophic
117 level of organisms and the origin of the organic matter at the base of the food web the species belong
118 to. In addition, this tool is particularly suited to subsidized systems, like the English Channel, where
119 species can access to two types of organic matter, here benthic and pelagic pathways, that exhibit
120 markedly different isotopic ratios (Kopp et al. 2015; Giraldo et al. 2017; Cresson et al. 2020). Finally,
121 even if this method has some merits, it does not allow an accurate determination of the preys actually
122 consumed at fine taxonomic resolution, as species closely related from a taxonomic point of view
123 usually belong to a similar trophic guild, and thus cannot be differentiated based on their isotopic

124 ratios. Consequently, combining stable isotopes and stomach content became classical in trophic
 125 ecology. Their combined use has been revealed powerful, as they inform about different aspects of
 126 trophic features, *i.e.* integrated information about the main organic matter fluxes (stable isotopes) and
 127 detailed taxonomic information about the recently consumed preys (stomach content analyses)
 128 (Cresson et al. 2014; Pethybridge et al. 2018 and references therein).

129 C and N stable isotope ratios were measured in fish muscle with a Thermo Delta V isotope mass ratio
 130 spectrometer coupled with a Carlo Erba NC2500 elemental analyzer, used to analyze C and N elemental
 131 content. Accuracy of the isotopic ratios measurements was checked by repeated analyses of an in-
 132 house standard (one analysis of the standard after every 10 samples) with overall standard deviation
 133 of 0.07‰. Stable isotopes ratios were expressed following the classical δ notation:

$$134 \quad \delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3, \quad (1)$$

135 with X ^{13}C or ^{15}N respectively, and R the isotopic ratios ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ respectively) measured in
 136 fish muscle (sample) or in standard (Vienna Pee Dee Belemnite for C and atmospheric nitrogen for N).
 137 C:N ratios were measured to estimate and correct effect of high lipid content on $\delta^{13}\text{C}$, notably for a
 138 lipid-rich species like *T. trachurus* (Post et al. 2007; Cresson et al. 2017). $\delta^{13}\text{C}$ ratios measured in muscle
 139 with C:N ratios higher than 3.5 were corrected using the Post et al. (2007) formula:

$$140 \quad \delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{raw}} - 3.32 + 0.99 \text{ C:N} \quad (2)$$

141 Correction was required for 92 out of the 124 fish considered, and the individual $\delta^{13}\text{C}$ correction was
 142 of 0.60 ± 0.62 ‰ on average.

143 **Isotopic mixing model** – Trophic contribution of pelagic pathway in the food web of horse mackerel
 144 and trophic level were estimated at each station with the tRophicPosition isotopic mixing model
 145 (Quezada-Romegialli et al. 2018). Choosing the most accurate baseline is crucial when designing a
 146 mixing model, notably when considering spatial variability of isotopic ratios. Here, *Aequipecten*
 147 *opercularis* (bivalve) and *Calanus* sp. (zooplanktonic crustacean) were used as proxies of benthic and
 148 pelagic pathways respectively. As all Bayesian mixing models, tRophicPosition allows propagating
 149 uncertainties linked with isotopic ratios of fish, baseline and trophic enrichment factors. In addition, it
 150 allows calculating trophic level and contribution simultaneously, and do not require a priori
 151 assumptions on the trophic level (to estimate to most appropriate trophic enrichment factor) or the
 152 major baseline. As technical difficulties precluded from the sampling of bivalves and zooplankton at
 153 each station, and as to cope for spatial variation of isotopic ratios, a geostatistical interpolation method
 154 (namely kriging) was used to complete the dataset, using data from neighboring stations (P. Cresson
 155 and S. Dubois, unpubl. data). Details on the kriging method can be found in previously published papers
 156 (Kopp et al. 2015; Timmerman et al. 2020, 2021). Briefly, the method calculates the relationship
 157 between isotopic difference and spatial distance (variogram), and estimate isotopic values at sampling
 158 stations, based on this relationship and from isotopic values at neighboring stations. As
 159 tRophicPosition requires mean and distribution values, ten $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were simulated for
 160 both baseline at each station, considering that values are normally distributed and using kriged values
 161 as mean. Average standard deviation of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measured at each station (*i.e.* the average
 162 variability of isotopic ratios between individuals sampled at all stations of the ecosystem) was
 163 considered for *A. opercularis* (0.32 ‰ for and 0.33 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively) and *Calanus* sp.
 164 individuals (0.91‰ and 2.33‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively). Trophic discrimination factors (*i.e.* the
 165 isotopic difference between a consumer and its diet) were set to 3.4 ± 0.5 ‰ for nitrogen and $1.0 \pm$
 166 0.5 ‰ for carbon. Nonetheless, as tRophicPosition only generates a distribution of trophic level values
 167 for all individuals at each stations, individual trophic levels values (TL_{fish}) were calculated for each
 168 individual, using the following formula, that takes into account the relative importance of the pelagic
 169 and benthic pathways

$$170 \quad TL_{\text{fish}} = \frac{\delta^{15}N_{\text{fish}} - [\alpha \delta^{15}N_{\text{zoop}} + (1 - \alpha)\delta^{15}N_{\text{biv}}]}{TDF} + TL_{\text{baseline}} \quad (3)$$

171 with $\delta^{15}N_{\text{fish}}$, $\delta^{15}N_{\text{zoop}}$ and $\delta^{15}N_{\text{biv}}$ the nitrogen isotopic ratios measured or kriged at each station for
 172 *T. trachurus*, *Calanus* sp. or *A. opercularis* respectively, α the contribution of the pelagic pathway
 173 calculated by the mixing model at each station, TDF the nitrogen trophic discrimination factor (3.4‰),
 174 and TL_{baseline} the trophic level of the baseline, set to 2 for both bivalves and zooplankton.

175 **Stomach content analysis** – Atlantic horse mackerel's diet was estimated by the identification of the
176 preys in stomach content. Preys were identified at the lowest taxonomic level possible, using visual
177 observation under stereomicroscope. Preys were then numbered, estimating the most plausible
178 number of individuals consumed. Paired items (*e.g.* eyes or claws) were counted and this number
179 divided by two to estimate the number of individuals consumed. Other items (*e.g.* degraded parts of
180 antennae or legs), the number of which cannot be related to the number of preys actually consumed,
181 were not counted, and it was considered that one individual was consumed. Preys were attributed to
182 the benthic or pelagic realm depending on their life history traits. Unidentified preys items, or preys
183 with no affinity for benthic or pelagic realm were included in the "other" group (Duffill Telsnig et al.
184 2019). Preys actually included in each group are detailed in Table S1. Diet was expressed as the
185 percentage of abundance of each prey, calculated as the number of individuals of this taxa counted in
186 stomachs, divided by the total number of preys in all stomachs. In addition, *Anisakis* individuals
187 observed in the stomach during stomach content analysis were also recorded, whether observed
188 within the preys or fixed to the stomach wall.

189 **Parasite detection and identification**

190 Each nematode was rinsed in physiological water and cleaned. The morphological features described
191 by Berland (1961) were observed under a microscope and the nematodes were divided to the genus
192 level. A subsample of each genus was molecularly identified as described in Gay et al. (2018) with the
193 former use of the qPCR for *Anisakis simplex* developed in Paoletti et al. (2018).

194 Parasite distribution descriptors rely on the definitions from Bush et al. (1997). In particular,
195 prevalence is "the number of hosts infected with one or more individuals of a parasite species (or of a
196 taxonomic group) divided by the number of hosts examined for that parasite species". The abundance
197 is "the number of individuals of a particular parasite in/on a single host regardless of whether or not
198 the host is infected". The intensity (of infection) is "the number of individuals of a particular parasite
199 species in a single infected host".

200 At each station, proportion of parasites from each tissue in the total intensity (hereafter parasitic
201 profile) was calculated as the tissue intensity (*i.e.* the sum of all *Anisakis* larvae observed in one tissue
202 of all individuals hosting at least one parasite) divided by the total intensity (*i.e.* the total number of
203 *Anisakis* in all tissues of all fish hosting at least one parasite) at this station. Stations with similar profile
204 were grouped together with a hierarchical clustering based on Euclidean distance metric and Ward
205 method, using the *cluster* package (Maechler et al. 2021).

206 **Data analysis** – Differences in length, isotope ratios and *Anisakis* abundance between stations were
207 checked with ANOVA, followed by Tukey posthoc tests, using the *multcomp* package (Hothorn et al.

208 2008). Effect of fish length on *Anisakis* abundance was assessed with a piecewise linear regression, to
209 identify a potential breakpoint in the pattern, using the package *segmented* (Muggeo 2008). As a
210 significant relationship was observed, the effect of depth was then estimated on the residuals of the
211 length vs. abundance relationship, *i.e.* on the pattern corrected for length effect. Linear models
212 assumptions (normality of residuals, homoscedasticity and homogeneity of variance) were checked
213 with visual examinations of the plots produced by the *check_model()* function from the *performance*
214 package (Lüdtke et al. 2021). *Anisakis* abundance was log+1 transformed to reach these assumptions.
215 All analyses were run using R statistical software version 4.0.2 (R Core Team 2020). Plots were
216 produced using the *ggplot2* package (Wickham 2009).

217 Results

218 **Length, stable isotopes and mixing model outputs** – All tested variables significantly differed between
219 stations, but with different patterns. Average length ranged between 131 and 250 mm, but with
220 individuals of 150 mm or less at four stations, and of more than 200 mm at five stations. Average
221 isotopic ratios varied between -19.12 ± 0.71 ‰ (S0613) and -18.10 ± 0.38 ‰ (S0648) for $\delta^{13}\text{C}$ and
222 between 12.32 ± 0.60 ‰ (S0575) and 13.75 ± 0.92 ‰ (S0705) for $\delta^{15}\text{N}$ (Tables 1, S2, Fig. S1). Pelagic
223 contribution were always important, *i.e.* higher than 80% for all but the shallowest station (S0754),
224 where pelagic modal contribution was still of 69% (Fig. 3c). Trophic level of *T. trachurus* was close to 3
225 at all stations, with modal values ranging between 2.88 and 3.52. Range of trophic levels calculated
226 with mixing models were larger than values calculated at individuals level, consistently with the ability
227 of Bayesian models to propagate all sources of variability (Fig. S1). Nonetheless, ranges of values
228 calculated with both methods largely overlapped, allowing the use of individual trophic level values to
229 investigate correlations with length. Length had a very limited negative effect on $\delta^{15}\text{N}$ ($\delta^{15}\text{N} = 13.43 -$
230 $.003 \text{ SL}$, $r^2=0.02$, $p\text{value} = 0.06$) and trophic level ($\text{TL} = 3.50 - 0.001 \text{ SL}$, $r^2= 0.06$, $p\text{value}=0.008$; Fig. S2).

231 **Stomach content analysis** – Diet of Atlantic horse mackerel is almost exclusively based on zooplankton,
232 with euphausiids (43%), copepods (24%) and other crustaceans (29%) being the major dietary items.
233 *Nyctiphanes couchii* is the predominant (94%) species of euphausiids consumed, along with
234 *Meganyctiphanes norvegica* (4%). An ontogenic change in the diet was nonetheless observed: before
235 20 cm, copepods were the main dietary items, representing 76% of the preys, while euphausiids
236 represented 62% of the diet of the larger individuals (Fig. 3a). Preys belonging to all other taxa had
237 almost no importance in the diet (2.4% when all fish are considered; 1.7% for small individuals, 0.7%
238 for large individuals)

239 Depth also affected diet, as preys of benthic and pelagic origin had almost the same importance at the
240 shallowest station (S0754), while diet comprises quite exclusively pelagic preys at the deepest (Fig. 3b).

241 **Parasitic infection** – Parasite abundance was largely variable between stations (Fig. 4a). Individuals'
242 burden ranged between 0 and 312 *Anisakis* larvae per fish, with two thirds of the individual fish hosting
243 less than 10 parasites in their tissues. Average prevalence was generally high (68%), non-null at all
244 stations and with difference between them, as it ranged between 30 and 100% (Table 2). Average
245 abundance – whether considering total or tissue-specific abundance – and prevalence discriminated
246 three groups of stations (Tab. S2): fish from more than half on the stations (S0613, S0648, S0639, S0601
247 and S0754) hosted less than three larvae, and had the lowest prevalence (30 to 60 %). Station S0718
248 had an intermediate pattern, with slightly higher burden (8.5 larvae per fish) and prevalence (70%)
249 than at the previous stations. High burdens – more than 40 larvae per fish – were then observed in
250 four stations (S0577, S0705, S0670, and S0575), where all individual fish (but one at station S0705)
251 hosted *Anisakis*. Burden was markedly higher at station S0575, explaining why this station formed a
252 single-station cluster. All fish individuals but one at this station hosted more than 70 larvae. Individual
253 trophic level had a poor and negative effect on *Anisakis* abundance [$\log(\text{Abundance} + 1) = 3.5 - 0.001$
254 TL , $r^2=0.05$, $p\text{value}=0.008$; Fig. S2].

255 More than 3500 nematodes were isolated from the fish originating from the different stations. 95% of
256 them were morphologically identified as *Anisakis* spp. One hundred and seventy four parasites were
257 molecularly identified: 149 belonged to *Anisakis simplex*, 6 to *A. pegreffii*, 12 to *Hysterothylacium* spp.
258 and 7 were not identified.

259
260 **Tissue-specific parasitic burden** – *Anisakis* from the abdominal cavity represented the majority of the
261 total *Anisakis* abundance at all stations (Fig. 4b). Prevalence in the abdominal cavity was also the
262 highest (58% - Tab. 2) but highly variable between stations (15 - 100%). Importance of the abdominal
263 cavity in the total burden also differed between stations, and was the first variable driving the
264 clustering. Stations S0601 and S0613 clustered together (cluster 3 in Fig. 4b) due to their lower burden
265 in abdominal cavity. These two stations had a very specific profile, as they hosted *Anisakis* in abdominal
266 cavity and stomach only. Stomach was the other tissue with non-null prevalence at all stations, but
267 with generally low importance at all other stations but S0754 (15%). Then, the third most infected
268 tissue was muscle. Prevalence in muscle was comparable with stomach, even if *Anisakis* did not occur
269 in mackerel muscle at three stations (S0613, S0639 and S0601). Proportion in muscle was also the
270 second driver of the clustering, separating stations with high proportions (14-22%) in muscle (S05777,
271 S0718, S0705 and S0670 – cluster 1) from stations with low (0-8%) proportions (S0675, S0648, S0754
272 and S0639 – cluster 2). Proportion in muscle is poorly related with fish length (Proportion= $-0.08 + 7.7$
273 10^{-4}SL ; $p\text{value}=0.04$, $r^2=0.04$; Fig. S3). Finally, gonads and liver were tissues of minor importance for

274 *Anisakis*. Parasites were not observed in these tissues at five and three stations respectively, and
275 represented less than 9% of the total abundance at the others.

276
277 **Combined size and depth effect on parasite abundance** – Fish standard length (SL) significantly
278 affected log-transformed parasite abundance [$\log(\text{Abundance}+1) = -4.06+0.03 \text{ SL}$, $R^2=0.65$, $p\text{value}$
279 $< 2.2 \cdot 10^{-16}$]. The relationship could nevertheless be divided between two sections, with an inflexion
280 point at 193 ± 10 mm, as illustrated by the loess regression (Fig. 5). A poorly significant relationship
281 [$\log(\text{Abundance}+1) = -0.96 + 9.92 \cdot 10^{-3} \text{ LS}$, $R^2=0.06$, $p\text{value}=0.03$] was observed for fish smaller than 193
282 mm, while the effect of size was markedly more important for larger individuals [$\log(\text{Abundance}+1) =$
283 $9.38 + 0.05 \text{ LS}$, $R^2=0.40$, $p\text{value}=6.59 \cdot 10^{-7}$]. After removing the effect of length, depth also affected
284 residuals of the parasite abundance vs. length relationship (Residuals = $-1.30 + 0.02 \text{ depth}$, $R^2=0.08$,
285 $p\text{value}=3.39 \cdot 10^{-3}$).

286 Discussion

287 The main feature of the parasitic burden revealed by the examination of the results here is the large
288 variability at all levels, from intraindividual to geographic areas. This pattern, commonly observed in
289 other studies of parasitism in fish, calls for an accurate investigation of the underlying drivers, such as
290 depth, diet and tissue-related variation for the present study.

291 Depth drives spatial variation of *Anisakis* levels

292 Depth is a classical factor structuring fluxes of organic matter in marine systems, whether through
293 passive fluxes of particles or active interactions between predators and preys (Walters et al. 2021; van
294 Denderen et al. 2021). In the English Channel, depth variation exerts a paramount control on the
295 dependency of fish community on benthic or pelagic pathways (Kopp et al. 2015; Giraldo et al. 2017;
296 Cresson et al. 2020; Timmerman et al. 2021). Despite the importance of trophic interactions in parasitic
297 transmissions, the effect of depth on parasites was poorly examined, and for assemblages only
298 (Klimpel et al. 2006; Woodstock et al. 2020). At the genus level, depth affected infection levels of two
299 species of the Anisakidae family, namely *Anisakis* (“whale-worm”) and *Pseudoterranova* (“seal-
300 worm”). In the Mediterranean Sea, *Anisakis* prevalence increased with depth for pelagic species
301 (sardine and anchovy; Pulleiro-Potel et al. 2015) while *Pseudoterranova* levels were higher in cod
302 caught in coastal and shallow stations (Hauksson 2011). In both cases, effect of final host was proposed,
303 and the opposite effect related with their preferential habitat (cetaceans for *Anisakis* and pinnipeds
304 for *Pseudoterranova*). Parasite and host should match to allow the encounter between parasite and
305 its definitive host. The crucial role of definitive hosts as drivers of parasites cycles is thus largely
306 recognized, even if accurate data are scarce (e. g. Levsen et al. 2022). Their behavior and the location

307 of eggs production and release constrain the ecology of the parasite (Klimpel and Palm 2011; Nuwer
308 2022). Negative effect of depth on *Pseudoterranova* levels in cod was thus related to the proximity of
309 seals' resting places (Hauksson 2011), while higher *Anisakis* levels at deeper stations highlight the
310 importance of cetaceans (Fiorenza et al. 2020). Interestingly, a similar effect of depth on *Anisakis*
311 abundance was previously observed for euphausiid, *i.e.* higher prevalence at deeper stations (beyond
312 100 m depth), while shallow samples were not infested (Smith 1983b). Effect of depth seem thus to
313 be conserved throughout the cycle of *Anisakis*. In the English Channel, the most abundant cetacean,
314 the harbor porpoise (*Phocoena phocoena*), is by example known to host *Anisakis* (Mattiucci et al. 2014;
315 Laran et al. 2017). Methodological and ethical constraints preclude from the simple collection of
316 quantitative data for this definitive host, notably regarding spatial variability of *Anisakis* burdens. In
317 addition, when available, parasites levels are estimated during necropsies of dead stranded mammals.
318 Chemical-related immunosuppression is largely reported as a cause of death, but lower immunity may
319 also lower defense against parasites. Number recorded in dead animals may consequently
320 overestimate the levels in healthy individuals (Bull et al. 2006). Investigating the levels in cetaceans
321 should thus be a crucial research avenue in the future. On the contrary, the effect of depth on diet
322 provides another fruitful research avenue to explore, notably through the powerful combination of
323 stable isotopes and stomach content analyses.

324 Stable isotopes confirmed here the prime importance of the pelagic production in the horse mackerel
325 food web (75 to 90%), regardless of the depth, and consistently with previous results (Kopp et al. 2015;
326 Cresson et al. 2020; Timmerman et al. 2021). On the contrary, the effect of depth on preys actually
327 consumed illuminates the effect of depth on *Anisakis* abundances. At shallowest stations, benthic and
328 pelagic compartments are not distinct. *T. trachurus* and benthic preys co-occur vertically, whether
329 because *T. trachurus* can reach the bottom to feed or benthic invertebrates use the totality of the
330 water column. The similar importance of benthic and pelagic preys may appear unexpected for a
331 pelagic fish and the result of a methodological artifact, as many preys were too digested to be
332 identified at species level. Nevertheless, benthic and pelagic preys were previously estimated to have
333 a similar importance in the diet at shallow stations (Giraldo et al. 2017). In addition, these unidentified
334 preys were all crustaceans. Digestibility can thus be considered similar, and the similar proportion
335 between benthic and pelagic identifiable preys could be extrapolated to all remains. Then, when depth
336 increases, benthic and pelagic systems decouple, and benthic preys are no longer accessible to
337 mackerels. As *Anisakis* occurs mostly in pelagic crustaceans and is almost absent in benthic ones (Busch
338 et al. 2012), the exclusive dependency of mackerel on pelagic preys at deep stations makes them highly
339 susceptible to the ingestion of larvae, as *Anisakis*-free benthic preys are no longer consumed.

340 Interestingly, depth was also proposed as an explanation of the difference in parasites – among which
341 *Anisakis* – in four flatfish species differing in their trophic and vertical behavior. Halibut *Hippoglossus*
342 *hippoglossus*, a pelagic species, have markedly higher levels of *Anisakis* (prevalence of 100% and mean
343 abundance of 291 *Anisakis* per fish) than benthos-feeding species (prevalence between 0 and 8%,
344 abundance between 0 and 1.6). The depth effect is then also observed for the three benthic species.
345 Witch flounder *Glyptocephalus cynoglossus* and American plaice *Hippoglossoides platessoides* caught
346 in shallow areas (0-60 m) exhibited low but non-null values while *Anisakis* was not observed in the gut
347 of the deep (300-350 m) flounder *Platichthys flesus* (Lile 1998). In both cases, depth was a driver of
348 dietary variability, and thus explained *Anisakis* loads.

349 **Length drives interindividual variation in *Anisakis* levels**

350 Length is potentially the parameter mostly used to describe and summarize ecosystem functioning, at
351 all levels of organization, notably as length controls interaction between predators and preys (Scharf
352 et al. 2000; Trebilco et al. 2013; Travers-Trolet et al. 2019). Thus, observing a significant effect of length
353 on the levels of a parasite transmitted through predator-prey interactions is somehow expected.
354 Indeed, many studies observed correlations between anisakids abundance and fish size: *e.g.* for
355 European hake (Mille et al. 2020), cod (Marcogliese 2001; Münster et al. 2015; Zuo et al. 2016;
356 Horbowy et al. 2016; Gay et al. 2018; Levsen et al. 2022), herring (Horbowy and Podolska 2001),
357 seabass (Morsy et al. 2012), sardine (Pulleiro-Potel et al. 2015) or whiting and haddock (Pierce et al.
358 2018). The size effect is also conserved when patterns are observed at larger spatial (*e.g.* in *T. trachurus*
359 all over Europe; Abaunza et al. 2008; MacKenzie et al. 2008) or taxonomic scale, *i.e.* analyzing the
360 global levels of *Anisakis* in fish (Fiorenza et al. 2020). Nonetheless, none of these works specifically
361 investigated the underlying drivers, even if three major mechanisms are usually proposed. In an
362 analogy with the bioaccumulation of chemicals, higher levels of parasitism in larger fish may result
363 from chronic and constant exposure to parasites, and long-time survival in their hosts' tissues, after
364 migrating from stomach (Morsy et al. 2012 and references therein; Pulleiro-Potel et al. 2015; Gay et al.
365 2018; Pierce et al. 2018). Larger tissues and higher energetic reserves of larger individuals are also
366 considered as an explanation, as larger tissues would represent more available niches for parasites
367 (Sasal et al. 1997; Morsy et al. 2012; Mille et al. 2020). This mechanism may be somehow consistent
368 with the relative proportion of *Anisakis* in each tissue and the contamination kinetics it suggests (see
369 the next section). Finally, the effect of ontogenic dietary switch is somehow suggested as a potential
370 driver of *Anisakis* variation (Horbowy and Podolska 2001; Gay et al. 2018; Pierce et al. 2018).

371 Combined analysis of stomach content and *Anisakis* abundance was able to provide a link between
372 diet and parasitism in squid *Illex coindetii* (Petrić et al. 2011) and in cod (Mouritsen et al. 2010; Münster
373 et al. 2015). Squid, cod, or whiting (Pierce et al. 2018) are piscivorous species, precluding thus from a

374 direct comparison of their parasitic pattern with the pattern observed in a zooplankton-feeder here.
375 In piscivorous species, fish become an important prey after reaching a sufficient size, *e.g.* around 20-
376 30 cm for both cod and whiting (Mouritsen et al. 2010; Zuo et al. 2016; Timmerman et al. 2020). As
377 fish consumed (mostly herring, sprat or capelin) are zooplankton-feeders, they occur one step above
378 zooplankton in the parasitic cycle and thus have higher levels than zooplankton (Horbowy and
379 Podolska 2001). Inclusion of fish in the diet of piscivores leads to a steep increase of *Anisakis* burdens,
380 concomitantly with an increase of the trophic level. This pattern may be amplified by the fact that
381 previous preys are mostly benthic invertebrates, *i.e.* free from *Anisakis* (Busch et al., 2012; cf. previous
382 section). Dietary switch observed in *T. trachurus* stomach differs from the switch observed in
383 piscivorous species: it does not result in a change in $\delta^{15}\text{N}$ nor trophic level, as euphausiids and
384 copepods occupy a similar position in the food webs and exhibit similar $\delta^{15}\text{N}$ values (P. Cresson, C.
385 Giraldo, unpubl. results). But it dramatically change from a parasitic point of view: euphausiids are the
386 mostly reported hosts of *Anisakis* while copepods are not (Busch et al. 2012; Gregori et al. 2015). The
387 crucial role of euphausiids as a infective vector for mackerel is consistent with previous results,
388 explaining the high abundance of *Anisakis* in mackerels from New Zealand waters by the important
389 consumption of euphausiids (Smith 1983a).

390 In addition, the non-linear size-parasitism pattern observed here is consistent with the pattern
391 observed in the few studies that accurately examined it (Horbowy and Podolska 2001; Mouritsen et al.
392 2010; Zuo et al. 2016). In Baltic herring, the shape of the relationship between length and *Anisakis* is
393 by example very similar with the shape of the loess regression observed for mackerel here (Fig. 5). This
394 nonlinear pattern was explained for herring by the consumption of euphausiids only during the
395 migration between Baltic and North Sea (Horbowy and Podolska 2001). Similarly, several papers
396 demonstrated that the inclusion of fish in the diet of cod generates a steep increase of the parasitic
397 burden (Mouritsen et al. 2010; Zuo et al. 2016). Mouritsen et al. (2010) demonstrated that *Anisakis*
398 burden increases faster in 30-55 cm cod than in 20-30 cm ones, *i.e.* after/before the inclusion of fish in
399 the diet. Consistent results observed here seem to confirm that *Anisakis* abundance in diet and
400 inclusion of preys with higher burdens through dietary shifts is more important than chronic exposure
401 to explain size-effect on burdens.

402 **Tissue specificity, as a tracer of micro niches availability within the host**

403 Understanding the drivers of the site of infestation by *Anisakis* is informative about parasite behavior,
404 source of energy and ultimately by the drivers of fish – parasite interactions. *Anisakis* can occur in
405 several tissues of fish, but with preferences for liver and muscle. Liver is the tissue of main abundance,
406 notably for lipid-poor species like seabass (Morsy et al. 2012), cod (Strømnes and Andersen 1998; Gay
407 et al. 2018; Levsen et al. 2022), saithe and/or redfish (Strømnes and Andersen 1998; Levsen et al.

408 2022), haddock and whiting (Pierce et al. 2018). Nonetheless, high burdens are observed in muscle of
409 starving cod and saithe, *i.e.* individuals which consumed all fat reserves in liver (Berland 2006; Levsen
410 et al. 2022). Strømnes and Andersen (1998) demonstrated that tissue colonization by *Anisakis* larvae
411 is neither random nor driven by host size, but responded to biological drivers. If migration was random
412 and constrained by movement radius only, proportion in muscle should be higher in small fish, as the
413 distance between entry point in stomach and muscle is lower. On the contrary, their work and present
414 results (Fig. S3) demonstrate that *Anisakis* migration is limited in distance, but results from an active
415 selection, with lipid content of the tissue being a potential driver of the selection. Even if lipid content
416 was not measured here, several previous works highlighted the importance of these macromolecules
417 in the interaction between *Anisakis* and its hosts. Experiments in controlled conditions demonstrated
418 the ability of *Anisakis* to migrate in the richer medium (Strømnes 2014) while larvae naturally occurring
419 in lipid-rich tissues grow faster (Strømnes and Andersen 2003), potentially as a result of the uptake of
420 lipids from their hosts, indirectly demonstrated by the accumulation of lipid-bound contaminants
421 (Mille et al. 2020). In lean species, liver concentrates most of the lipid, explaining the predominance of
422 this tissue in the total abdominal burden. The pattern observed here could be viewed as specific of
423 lipid-rich species. Predominant encapsulation of *Anisakis* individuals in the abdominal cavity of
424 *T. trachurus* is consistent with the observation of fat masses directly surrounding the stomach and of
425 *Anisakis* individuals in it (Fig.6). Abdominal cavity can thus provide sufficient resources for the *Anisakis*
426 individuals and allow their principal encapsulation off any organs. Similarly, the high lipid content in
427 muscle could also provide sufficient resources, allowing muscle to be a reliable microhabitat for
428 *Anisakis* (Pierce et al. 2018). Distance and accessibility of tissue was also proposed as an important
429 factor determining the colonization (Berland 2006; Levsen et al. 2022). Here, as illustrated by Fig. 6,
430 liver is located in the upper part of the abdominal cavity, *i.e.* far from the place where digestion occurs.
431 Reaching the liver can be harder than the muscle. It could be hypothesized that, as nutritional
432 conditions are favorable in muscle or abdominal cavity, larvae do not migrate up to the liver. More
433 largely, recent results demonstrated that parasites are able to select the macromolecule of interest
434 they use from their host, sometimes at a larval stage to store nutrients for future reproduction
435 (Sabadel et al. 2022; Hesse et al. 2023).

436 Results of the present study demonstrated that interactions between the parasitic nematode *Anisakis*
437 and one of its hosts the Atlantic horse mackerel *Trachurus trachurus* are directly affected by biological
438 and environmental drivers. As depth and fish length drive the diet of fish in this ecosystem, they control
439 the potential encounters between host and parasite. In addition, abdominal condition and internal
440 structure - apprehended here by the level and position of lipids reserves – is a proxy of the niches
441 available for this parasite. Results also highlighted the current lacks of knowledge, mostly regarding

442 the non-harvested component of the parasitic cycle. The role of first and final hosts is as paramount
443 as the one of fish in the completion of the cycle and as drivers of the variation of the parasite levels.
444 But the lack of knowledge, due to both technical issues and to a lower interest for non-health adverse
445 groups, precludes from an accurate understanding of the exact role of these components.
446 Consequently, it highlights the importance of integrating parasite in our perception of ecosystem
447 functioning, whether if parasites are observed with applied or theoretical aims.

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456 **Data Availability statement**

457 All data used for this paper are freely available in the SEANOE repository
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460 The authors declare that they have no known competing financial interests or personal relationships
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763 Figures captions

764 Fig.1: Time series of Atlantic horse mackerel *Trachurus trachurus* importance in the Eastern English
 765 Channel fish assemblage. (a) Relative biomass of mackerel (in blue) in the whole fish assemblage; (b)
 766 Estimated biomass (in 10^3 tons) in the ecosystem (in purple) and in the catches and landings (in red).
 767 Relative biomass of all fish species in the ecosystem are from Ifremer's Fisheries Information System
 768 (<http://www.ifremer.fr/SIH-indices-campagnes/index>), and are corrected to take into account (i) the use
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 771 allow estimating directly the absolute biomass, but should be rather considered as a relative
 772 indicator of biomass changes. Catches data are from ICES (2019) statistics for the VIId (Eastern
 773 English Channel) division. Mackerel picture courtesy of Pierre Porché.

774 Fig. 2: Stations sampled (a) depth – (b) average abundance of parasites per individual fish. Maps is
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777 Fig. 3: Trophic ecology of Atlantic horse mackerel *Trachurus trachurus* in the English Channel. (a) Preys
 778 abundance in individuals smaller or larger than 200 mm. The cutoff is a rounded value of the inflexion
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 781 preys. (c) Effect of depth on the pelagic contribution, as calculated by the isotopic mixing model.

782 **Fig. 4:** *Anisakis* sp. burden in horse mackerel. (a) Average parasite abundance. (b) Relative
 783 importance of the different tissues considered in the total intensity. Proportions are displayed when
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 786 stations highlighted in the text are surrounded by squares and numbered. All fish were considered
 787 for the calculation of average abundance, while individuals with no parasites were excluded for the
 788 calculation of tissues' relative importance

789 Fig. 5: Combined effect of fish length and depth on total number of parasites. (a) Effect of length on
 790 total number of parasites. Regression lines represent the loess relationship fitted to the entire
 791 dataset and the linear segmented relationship for the two subsets (0-193 mm and >193mm). (b):
 792 Effect of depth on the residuals of the linear relationship between size and total number of parasites.
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794 Fig.6: Picture of the internal cavity of a horse mackerel. Liver and free fat are highlighted, as well as an
 795 *Anisakis* individual observed in the free fat of the abdominal cavity.

- 1 Tables
- 2 Table 1: Sampling resolution, isotopic ratios and trophic levels. N: number of fishes analyzed at each
- 3 station

Station	Depth (m)	N	Standard length (mm) mean \pm sd	$\delta^{13}\text{C}$ (‰) mean \pm sd	$\delta^{15}\text{N}$ (‰) mean \pm sd	Trophic level Mode [95% interval]
S0575	96.84	10	250 \pm 6	-18.95 \pm 0.43	12.33 \pm 0.65	3.28 [2.82 - 3.72]
S0577	94.02	10	250 \pm 11	-19.01 \pm 0.26	12.32 \pm 0.60	2.88 [2.49 - 3.29]
S0601	74.88	10	153 \pm 5	-18.40 \pm 0.41	12.71 \pm 0.33	3.12 [2.81 - 3.48]
S0613	77.61	20	131 \pm 24	-19.12 \pm 0.71	13.04 \pm 0.61	2.98 [2.42 - 3.49]
S0639	73.12	10	154 \pm 7	-18.35 \pm 0.46	12.85 \pm 0.25	3.52 [3.18 - 3.88]
S0648	65.63	10	151 \pm 5	-18.10 \pm 0.38	12.85 \pm 0.41	3.42 [3.03 - 3.79]
S0670	66.38	10	238 \pm 19	-18.75 \pm 0.39	12.94 \pm 0.81	3.20 [2.81 - 3.61]
S0705	59.36	10	235 \pm 19	-18.61 \pm 0.56	13.75 \pm 0.92	3.41 [2.97 - 3.85]
S0718	55.61	11	212 \pm 8	-18.60 \pm 0.41	13.43 \pm 0.60	3.50 [3.13 - 3.81]
S0754	37.30	10	170 \pm 9	-18.13 \pm 0.35	13.06 \pm 0.28	3.17 [2.88 - 3.49]

4

5 **Table 2:** *Anisakis* prevalence, per tissue and considering all tissues jointly and per station or considering all
 6 stations jointly. Stations and tissues are sorted by increasing order of total prevalence.

Station	Gonads	Liver	Muscle	Stomach	Abdominal cavity	Total
S0613	0%	0%	0%	15%	15%	30%
S0648	10%	0%	10%	10%	20%	50%
S0639	0%	10%	0%	10%	40%	50%
S0601	0%	0%	0%	40%	40%	60%
S0754	0%	10%	10%	20%	60%	60%
S0718	0%	18%	27%	9%	73%	73%
S0705	40%	60%	60%	60%	70%	90%
S0577	10%	20%	80%	60%	100%	100%
S0670	50%	70%	70%	60%	100%	100%
S0575	40%	90%	100%	80%	100%	100%
All stations	14%	25%	32%	34%	58%	68%

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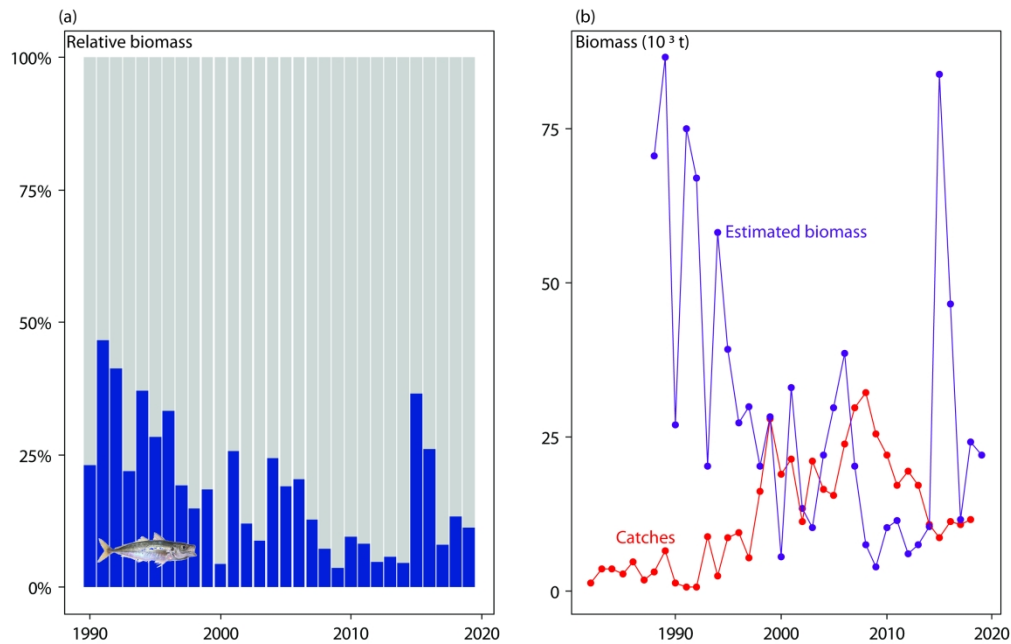


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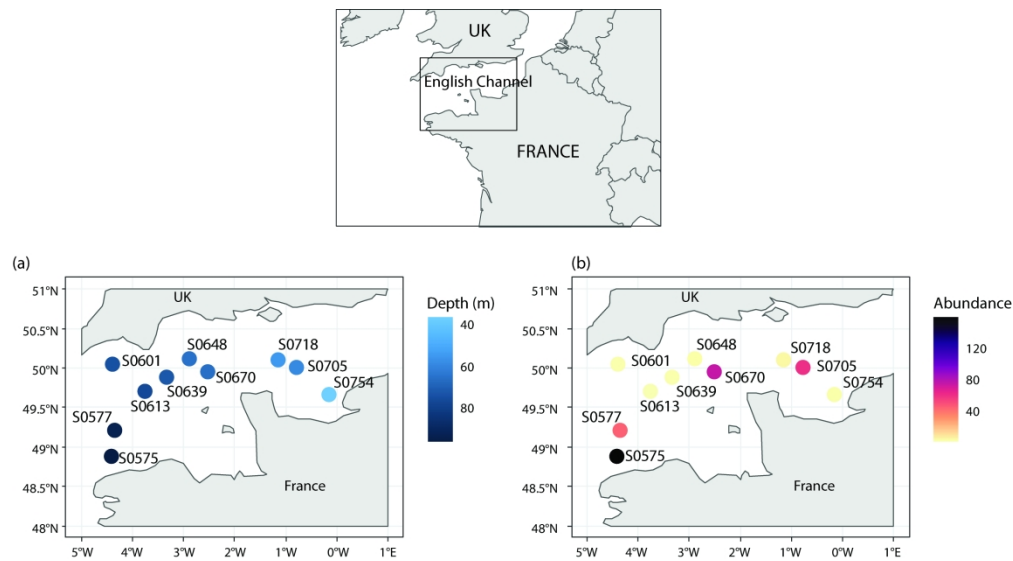


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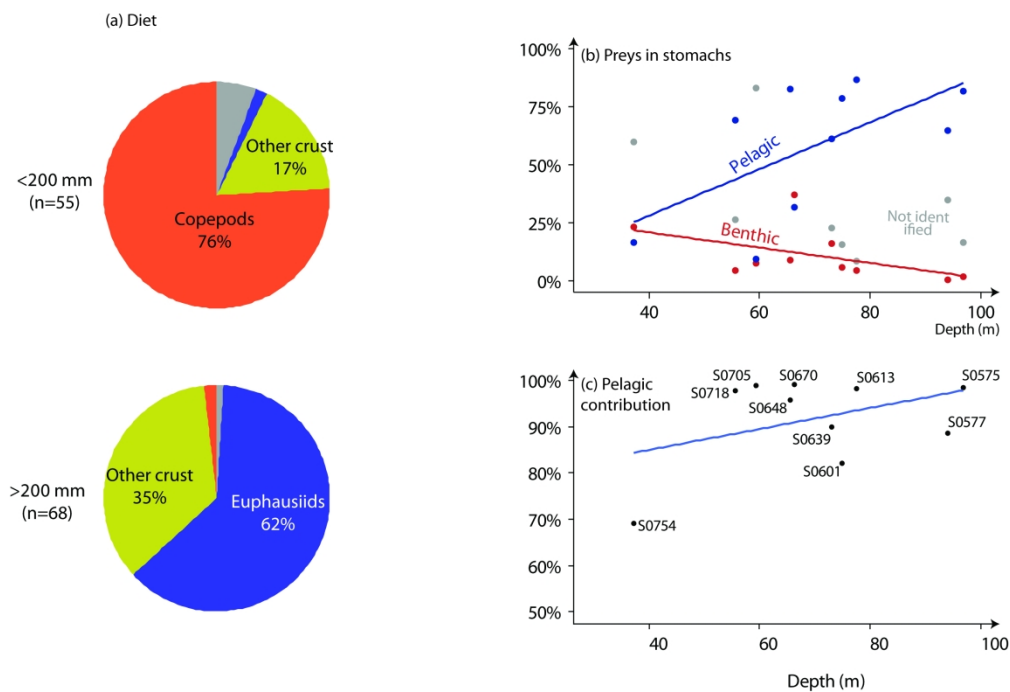


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245x166mm (300 x 300 DPI)

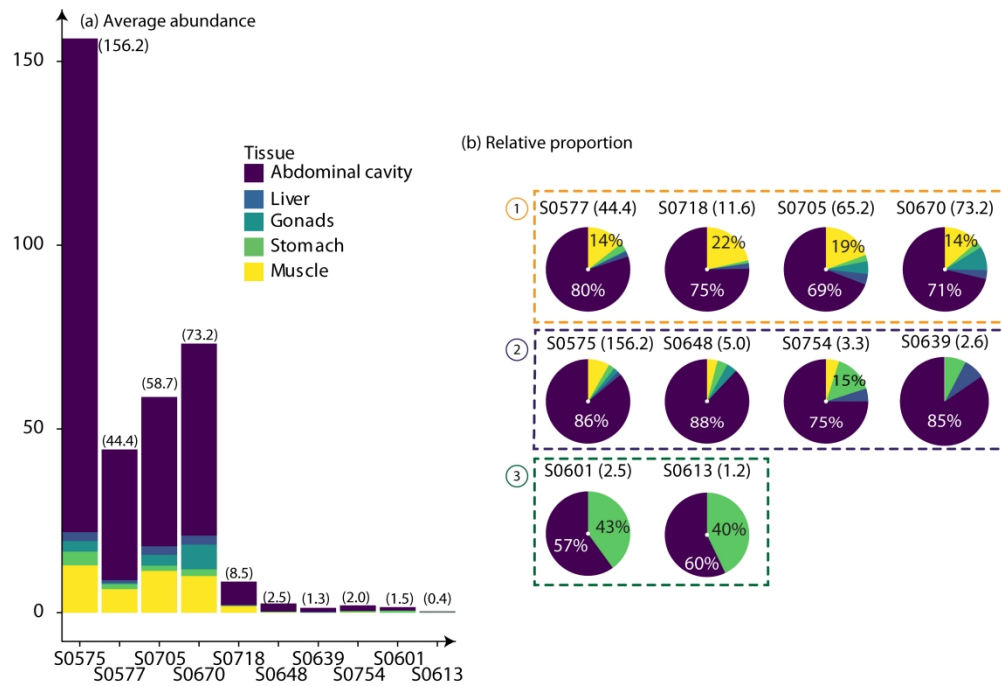


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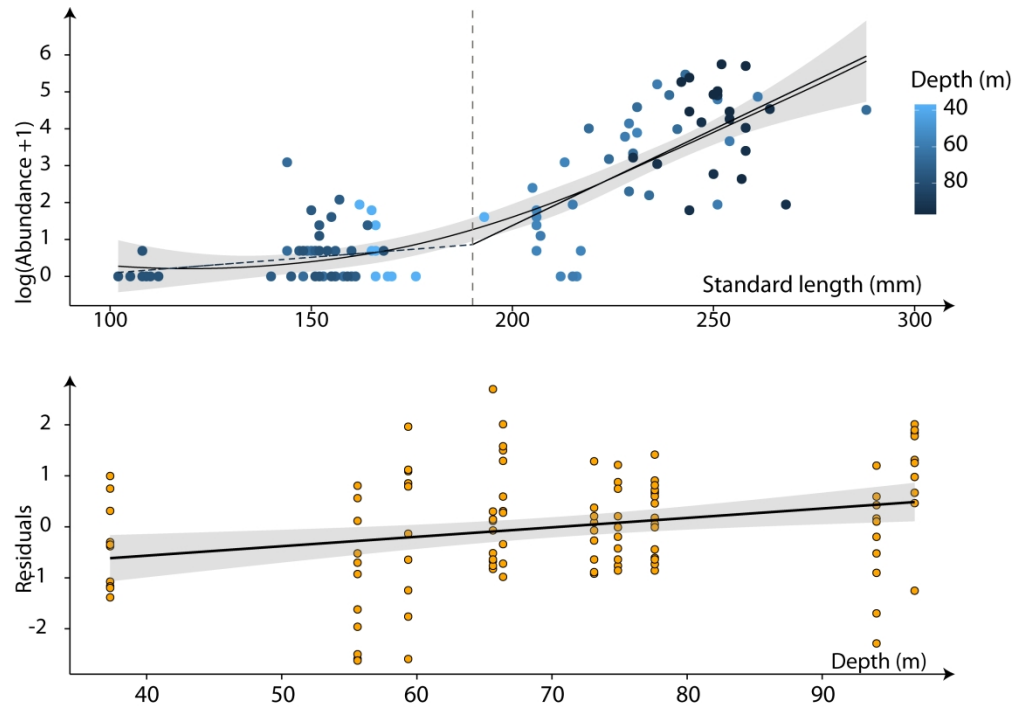


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280x196mm (300 x 300 DPI)

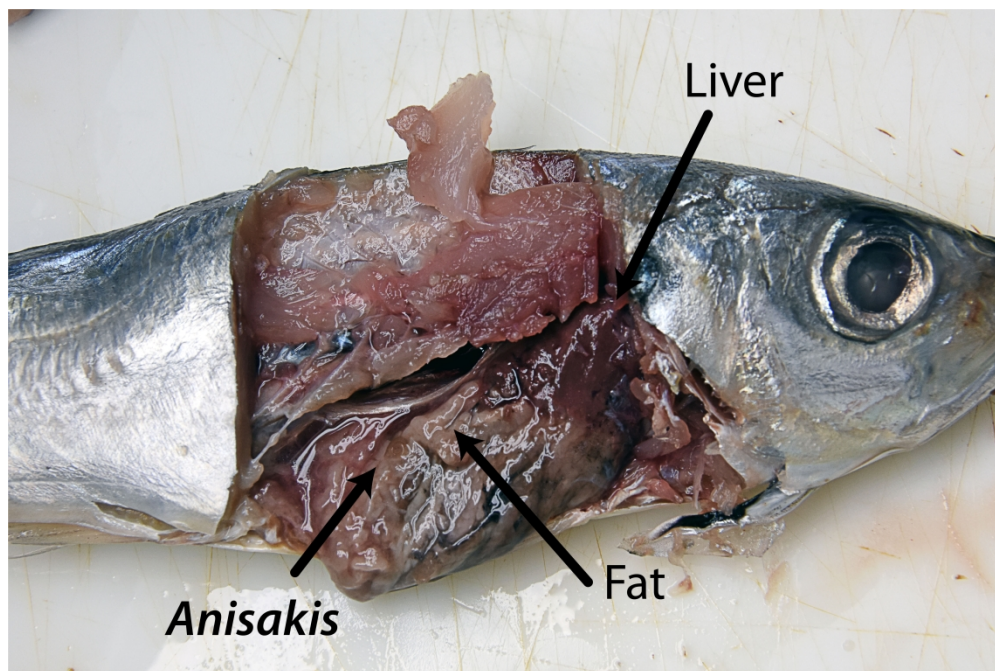


Fig.6: Picture of the internal cavity of a horse mackerel. Liver and free fat are highlighted, as well as an *Anisakis* individual observed in the free fat of the abdominal cavity.

508x338mm (300 x 300 DPI)