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1 **A study of trophic structure, physiological condition and mercury biomagnification in**
2 **swordfish (*Xiphias gladius*): evidence of unfavourable conditions for the swordfish**
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4
5 **Sebastián Biton-Porsmoguer^{a,b,*}, Daniela Bănaru^c, Mireille Harmelin-Vivien^c, Philippe**
6 **Béarez^d, Marc Bouchoucha^e, Françoise Marco-Miralles^e, Montse Marquès^f and Josep**
7 **Lloret^b**

8
9 *^aFrench Biodiversity Agency (OFB), Channel and North Sea Delegation, 4 rue du Colonel*
10 *Fabien, BP 34, 76083, Le Havre, France*

11 *^bUniversity of Girona, Institute of Aquatic Ecology, Faculty of Sciences, C/ Maria Aurèlia*
12 *Capmany 69, E-17003 Girona, Catalonia, Spain*

13 *^cAix-Marseille University, CNRS/INSU, Toulon University, IRD, Mediterranean Institute of*
14 *Oceanography (MIO) UM 110, Campus de Luminy, 13288 Marseille, France*

15 *^dArchéozoologie, archéobotanique : sociétés, pratiques et environnements (AASPE, UMR 7209),*
16 *CNRS/MNHN. Muséum national d'histoire naturelle, 55 rue Buffon, 75005 Paris, France*

17 *^eIfremer, Lab Environm Ressources Provence Azur Corse, CS 20330, F-83507 La Seyne Sur*
18 *Mer, France*

19 *^fLaboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat*
20 *Rovira i Virgili, Reus, 43201 Catalonia, Spain*

21
22 * Corresponding author [sebastien.biton@ofb.gouv.fr]

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31

32 ABSTRACT

33

34 Studies integrating trophic ecology, physiological condition and accumulation of heavy metals in
35 top predators, such as swordfish, are needed to better understand the links between them and the
36 risk to humans associated with consumption of these fish. This research focuses on the swordfish
37 of the Catalan Sea and follows a multi method approach that considers their diet, their liver lipid
38 content, and mercury accumulation in their bodies as well as in their prey. The aim is to highlight
39 the links between trophic ecology, physiology (fish condition), and eco-toxicology. Results
40 indicate that poor condition of swordfish based on size and the levels of lipid in the liver, and the
41 high Hg levels accumulated to the trophic web (particularly from cephalopods) may indicate
42 potential unfavourable feeding and reproduction conditions for swordfish in the NW
43 Mediterranean and that this warrants further investigation.

44

45 **1. Introduction**

46

47 The swordfish (*Xiphias gladius*, Linnaeus, 1758) is one of the most commercially-valuable
48 species in the European Union market, being among the top ten, in monetary value, landed in the
49 EU (EUMOFA, 2018: p 99). It is the target of intensive fishing by pelagic longliners in the
50 north-western Mediterranean due to its high value in Spanish ports (for example, 224 tons landed
51 in 2020 in Catalan ports) (Biton-Porsmoguer, 2017 and GENCAT 2021) and a high consumption
52 rate in Spain (Velez et al., 2010). There is a large body of research, from around the world, on
53 the biology (diet, growth and reproduction), migrations, genetic structure of populations, and
54 heavy metal accumulation of this species (e.g., Viñas et al., 2006; Tserpes et al., 2008; Rosas-
55 Luis et al., 2017; Esposito et al., 2018, and references therein). In the Mediterranean Sea in
56 particular, the diet of swordfish has been studied in the Aegean Sea (Salman, 2004; Ceyhan &
57 Akyol, 2017), the Central Mediterranean Sea (Orsi Relini et al., 1995; Romeo et al., 2009) and
58 more recently in the western Mediterranean Sea (Carmona-Antoñanzas et al., 2016; Navarro et
59 al., 2017). The swordfish is a large migratory top predator and feeds on a broad spectrum of
60 prey, comprising mainly teleost fish, cephalopods and crustaceans, depending on fish size, area
61 and season (Canese et al., 2008; Navarro et al., 2017; Abid et al., 2018). In the North Western
62 Mediterranean Sea swordfish are able to cross long distances in a short time, displaying

63 preferences for different habitats for breeding and feeding (Canese et al., 2008), where they may
64 spend long periods (Orsi Relini et al., 2003). Being one of the larger top predators, the swordfish
65 is susceptible to accumulating high levels of contaminants, particularly mercury (Hg), both by
66 biomagnification up the food web as well as by bioaccumulation during its life span (Storelli &
67 Marcotrigiano, 2001), as has been observed in other top predators (Pethybridge et al., 2012;
68 Matulik et al., 2017; Biton-Porsmoguer et al., 2018). Increasingly high concentrations of Hg in
69 Mediterranean seafood are a worrying seafood safety issue due to the major toxic effects of its
70 organic form, methylmercury (MeHg) (Cossa et al., 2009). Fish consumption is a major source
71 of MeHg exposure among Europeans (Miklavičič Višnjevec et al., 2013), and MeHg generally
72 comprises >90% of total Hg (THg) in fish muscle (Cossa et al., 2012; Chauvelon et al., 2018),
73 including swordfish (Velez et al., 2010, Cinnirella et al., 2019). A number of studies have
74 reported high levels of Hg contamination in swordfish, resulting in high levels of exposure for
75 consumers (Storelli et al., 2010; Damiano et al., 2011; Rodriguez et al., 2013; Esposito et al.,
76 2018; González et al., 2019). Furthermore, heavy metals, such as mercury, have been shown to
77 have serious long-term effects on fish, as potential endocrine disruptors (Kar et al., 2021).

78 Reconstructing food webs and determining biomagnification factors are usually performed by
79 combining analyses of stomach content, and of stable isotopes in prey and predators. Stable
80 isotope ratios of carbon ($\delta^{13}\text{C}$) and of nitrogen ($\delta^{15}\text{N}$) are used to determine, respectively, the
81 sources of the organic matter at the base of the food web and the trophic position of organisms
82 (e.g. Post, 2002; Pethybridge et al., 2012).

83 In contrast to such work on diet and contaminant accumulation in swordfish, research into their
84 physiological condition is sparse, despite indications from studies involving other exploited
85 species which show that condition, particularly during critical periods in their life-cycles (e.g.
86 pre-spawning, migration or early life stages), is an essential factor in the management of
87 sustainable and profitable fisheries (Lloret et al., 2012). Studies from many years ago, such as
88 Raven and LaMonte (1936) and Conrad (1940), did take into consideration the liver's important
89 role as an energy reserve, noting that there was an allometric growth of the liver (the smaller the
90 swordfish, the greater its proportional liver weight), while also highlighting significant seasonal
91 fluctuations in absolute size of the body, and the liver, of swordfish in north-western Atlantic
92 waters. These fluctuations were attributed to environmental conditions, with food supply thought
93 to be the most likely factor. Energy density and lipid content (direct condition indices) are

94 typically measured using bomb calorimetry and proximate composition analysis. From the
95 available biochemical parameters for evaluating the energy reserves of fishes, determination of
96 fat content has been the most widely used. Lipids, together with proteins and carbohydrates, are
97 the principal energy stores in teleosts and are often mobilized during nonfeeding and
98 reproductive periods. Lipid storage and lipid dynamics within the organism are therefore a
99 particularly important aspect of fish health and population success (Lloret et al., 2014).
100 According to Ben Smida et al (2009), who calculated percentage lipid content in swordfish
101 tissue, by far the highest lipid content is found in the liver (26%) followed by the gonads (4.7%)
102 and red muscle (4.5%), which suggests the liver plays an important role as an energy store in
103 swordfish.

104 Studies integrating trophic ecology, physiological condition and the accumulation of mercury in
105 top predators such as swordfish are needed to better understand the ecology of top predator
106 fishes, the eco-toxicological aspects of heavy metal accumulation, and the risk associated with
107 human consumption of these fish. The Mediterranean swordfish is affected by a series of
108 unfavourable environmental conditions, such as genetic isolation (Viñas et al., 2006) and the
109 presence of high underlying concentrations of mercury (Cossa et al., 2009), both of which are
110 compounded by fishing pressure (Damiano et al., 2011). In view of the above, proper stock
111 management should not overlook the plurality of causes, and thus this challenge needs to be
112 solved using a multi-methodological approach (Damiano et al., 2011). Atmospheric inputs
113 represent the main source of mercury in the Mediterranean Sea, representing annual fluxes of
114 about 37.7 Mg y^{-1} THg and 0.4 Mg y^{-1} MeHg, with higher THg and MeHg concentrations being
115 recorded in the Western basin compared to the Eastern basin, both in seawater and sediments
116 (Cossa et al., in press). The origin of Hg in the oceans is natural, ancient and anthropogenic
117 (Biton-Porsmoguer et al., 2021).

118
119 In this context, the objectives of the present study are, using a multi-methodological approach: 1)
120 to determine the diet of swordfish in the Catalan Sea by identifying prey in stomach contents; 2)
121 to reconstitute the trophic web of swordfish by stable isotope analyses of its prey; 3) to analyse
122 the condition of swordfish as indicated by their liver lipid content; and 4) to analyse the mercury
123 level in prey and determine the biomagnification factors in the swordfish food web. By analysing
124 these different variables, the paper aims to provide new biological (trophic ecology),

125 physiological (fish condition), and eco-toxicological data that, taken together, can improve our
126 knowledge about the health status of swordfish in the Mediterranean and may be useful for the
127 stock assessment and management of populations.

128

129 **2. Material and Methods**

130

131 *2.1 Sampling*

132

133 The study area was located in the Catalan Sea (north-western Mediterranean Sea (Fig. 1).
134 Swordfishes were caught at depths of 20 m by surface longlines, and stored in the cold-storage
135 chamber of fishing vessels before being landed at the fishing port of Blanes (Fig. 1). A total of
136 26 specimens were sampled between June and August 2018, measured (lower jaw fork length,
137 LJFL in cm), weighed (total mass, Mt, and eviscerated mass, Me, in kg) and sexed (Table S1).
138 Individual specimens, among which were 12 females and 14 males, ranged from 102 cm to 232
139 cm LJFL and from 13.2 kg to 169 kg Mt. Since all individuals had a LJFL of more than 100 cm,
140 they were all deemed to be adults, as the size at sexual maturity for swordfish is 90 cm in males
141 and 110 cm in females in the Mediterranean (ICCAT, 2014-2015). Stomachs and other organs
142 (gonads and livers) were extracted from specimens at the port, immediately frozen and sent to
143 the laboratory for analysis. In the laboratory, stomachs were dissected and all prey extracted,
144 identified, counted and weighed (wet weight, ww, in g). Livers were also weighed and their
145 individual mass recorded (ww in g) before lipid analysis.

146

147 *2.2 Diet*

148

149 The number of empty stomachs was recorded and the index of vacuity (%V) calculated as the
150 percentage of empty stomachs of all stomachs analysed. Prey identification to the lowest taxon
151 possible was carried out by analysing vertebrae and otoliths for teleosts, and beak characteristics
152 for cephalopods (Grassé, 1958; Clarke, 1986). Various dietary indices were calculated to
153 describe swordfish diet: the percentage of occurrence frequency (%O) was the percentage of all
154 non-empty stomachs containing one prey category. Percent number (%N) and percent mass
155 (%M) were the percentages of individual number and mass, respectively, of a given prey

156 category versus the overall number or mass of prey in non-empty stomachs. Prey masses
157 determined in the present study were not those actually recorded in stomach contents, but
158 reconstructed prey mass values that were calculated according to pre-established vertebrae- or
159 beak size-prey mass relationships. Reconstructed prey mass is more appropriate to comprehend
160 the real importance of prey in the diet of large predatory fishes (Biton-Porsmoguer, 2015). The
161 contribution of each prey category to swordfish diet was then estimated using the index of
162 relative importance (IRI), which was calculated as $IRI = (\%N + \%M) \times \%O$, and expressed in %
163 $IRI = (IRI \text{ of one prey category} / \text{sum of \% IRI of all prey categories}) * 100$ (Pinkas et al., 1971;
164 Cortés, 1997).

165

166 2.3 Condition

167

168 To estimate the condition of the sampled swordfish, two different indices were used. The first
169 was the relative condition factor, K_n , proposed by Le Cren (1951), which nullifies the effect of
170 size by taking into account the length of the individuals and their eviscerated mass (M_e). $K_n =$
171 M_e/M_e' , where M_e is the eviscerated mass and M_e' is the eviscerated mass calculated according
172 to the length-mass relationship of all individuals sampled. The second index used was the lipid
173 hepatosomatic index (LHSI), which estimates the lipid reserves stored in liver (LL) (Lloret et al.,
174 2008). LHSI was computed as $ABSL/We * 100$, where ABSL is the absolute lipid content in liver
175 (obtained by multiplying the % LL by the liver mass (LM), and We is the eviscerated mass of the
176 fish in g. Total lipids in the liver were determined gravimetrically with the Soxhlet method
177 according to Shahidi (2001) and following the procedure described in Lloret et al. (2008).

178

179 2.4 Hg content and stable isotope values of prey

180

181 A list of potential swordfish prey was established according to the results of stomach contents in
182 our study and supplemented with data from recent studies on swordfish diet in the Mediterranean
183 Sea (Salman, 2004; Carmona-Antoñanzas et al., 2016; Ceyhan & Akyol, 2017; Navarro et al.,
184 2017; Abid et al., 2018). Samples were taken from stomach contents when their state of digestion
185 permitted (presence of fresh meat) or sampled in the study area and from landings in the fishing
186 port of Palamós, such as the cephalopod, *Illex coindetii*, and the teleosts: *Engraulis encrasicolus*

187 (anchovy), *Micromesistius poutassou* (blue whiting), and *Trachurus trachurus* (Atlantic horse
188 mackerel). Five specimens of each prey species were analysed for Hg content and stable isotope
189 values. Before analyses, the prey were freeze-dried and reduced to a homogenized powder by
190 grinding in an agate mortar.

191 Total mercury concentrations in prey (THg, in $\mu\text{g g}^{-1}$ dry weight, dw) were determined with a
192 semi-automated atomic absorption spectrophotometer, AMA 254 (Altec Ltd., Prague, Czech
193 Republic), with a detection limit of 0.003 ng/mg, following the procedure described in Cossa et
194 al. (2009). Total Hg concentration was determined by the semi-automated atomic absorption
195 spectrophotometer (AMA-254, Altec Ltd., Praha, Czech Republic) in three steps. First, the
196 muscle sample is burnt and mercury is volatilized. Then the evaporated elemental mercury is
197 captured by a gold trap. Finally, the trap is heated (800 C), Hg swept into the flow cell, and Hg
198 content is determined by spectrophotometric atomic absorption.

199 The accuracy of the measurements was assessed every ten samples using certified reference
200 materials from the National Research Council of Canada (fish muscle tissues DORM-4). Wet
201 weight (ww) concentrations were calculated by assuming dry weight (dw) concentration = 5 ww
202 concentration (Cresson et al., 2014).

203 Stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) within prey tissue (from the natural environment and
204 stomach contents) were analysed with a continuous flow isotope-ratio mass spectrometer (Flash
205 HT Elemental Analyzer, coupled to a Finnigan ConFlo III interface) at the Stable Isotope
206 Analysis Laboratory (IRMS), Autonomous University of Barcelona (Spain). Results were
207 expressed in the conventional form, as per mil (‰) relative to international standard materials:
208 Vienna PeeDee Belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$, according to the equation: δX
209 = $[(R_{\text{sample}}/R_{\text{standard}})-1]*10^3$, where X is ^{13}C or ^{15}N and R is the heavy to light isotope ratio for
210 either $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The international standard materials used were Vienna Pee Dee B for
211 C, and atmospheric N_2 for N, with an experimental precision of <0.1 ‰ for both elements,
212 checked with international standard laboratory IAEA 600 (caffeine).

213

214 2.5. Trophic level and biomagnification factors

215

216 Trophic levels (TL) of prey and swordfish were estimated from their $\delta^{15}\text{N}$ values based on the
217 equation given by Badalamenti et al. (2002): $\text{TL} = 1 + (\delta^{15}\text{N}_{\text{Predator}} - \delta^{15}\text{N}_{\text{PP}}) / 3.4$, with a mean

218 value of 1.3 ‰ for nanophytoplankton (primary production, PP) in the NW Mediterranean (Rau
219 et al., 1990), as it was the main source of primary production at the base of food webs in this
220 region (Cresson et al., 2014).

221 In order to reconstruct swordfish food webs in the Catalan Sea and determine the THg
222 biomagnification factors for the species in this area, we used data from studies conducted in the
223 NW Mediterranean for zooplankton (Espinasse et al., 2014, regarding stable isotopes and
224 Chouvelon et al., 2019, regarding THg concentrations), and for swordfish (Navarro et al. 2017
225 and Barone et al. 2018 for $\delta^{15}\text{N}$ and THg, respectively). A THg-weight relationship in swordfish
226 muscle was established from data supplied by Barone et al. (2018), as the linear correlation was
227 better with weight than with length ($\text{THg} = 0.009 \cdot \text{weight} + 0.304$, $r = 0.63$, $p < 0.01$). Similarly,
228 a linear correlation between $\delta^{15}\text{N}$ and swordfish length was established from data in Navarro et
229 al. (2017) ($\delta^{15}\text{N} = 0.033 \cdot \text{length} + 7.124$, $r = 0.49$, $p=0.01$). THg content and $\delta^{15}\text{N}$ values of
230 swordfish were estimated according to these correlations (Table S1). Mean values of THg and
231 TL of the swordfish diet as a whole (bulk diet) were calculated following Harmelin-Vivien et al.
232 (2012): $\text{THg}_{\text{Diet}} = \sum[\text{THg}_{\text{Prey}(i)} \cdot \%M_{\text{Prey}(i)}]$, using the mean values of THg and M of each of the
233 three main prey groups, (i), recorded in swordfish stomach contents (crustaceans, cephalopods
234 and teleosts). Similarly, mean values of TL and M were used as follows: $\text{TL}_{\text{Diet}} =$
235 $\sum[\text{TL}_{\text{Prey}(i)} \cdot \%M_{\text{Prey}(i)}]$.

236 Various types of biomagnification factors were calculated. The simplest measure is the
237 biomagnification factors (BMF) at species level, which corresponds to the ratio of the chemical
238 concentrations of the predator relative to its prey: $\text{BMF} = \text{THg}_{\text{Predator}} / \text{THg}_{\text{Prey}}$.
239 Gobas et al. (2009) calculated the BMF_{TL} when normalizing the BMF factor to trophic level (TL)
240 of predator and prey, as follows:

$$\text{BMF}_{\text{TL}} = \frac{[\text{THg}]_{\text{Predator}} / [\text{THg}]_{\text{Prey}}}{\text{TL}_{\text{Predator}} / \text{TL}_{\text{Prey}}}$$

241
242 Mean BMF values were also calculated for bulk diet, using THg_{Diet} and TL_{Diet} values.

243 The food web magnification factor (FWMF) is an estimation at the system level. FWMF is
244 calculated as the antilog of the regression slope of $\log\text{THg}$ concentrations in food web organisms
245 in relation to their trophic level (Fisk et al., 2001; Borgå et al., 2011). To calculate FWMF in the
246 swordfish food web we used, as the base of the food web, the mean values obtained for
247 zooplankton by Chouvelon et al. (2019) for THg (0.008 mg kg^{-1} wet weight) and by Espinasse et

248 al. (2014) for trophic level ($\delta^{15}\text{N} = 3.5\text{‰}$; $\text{TL} = 2$) in the nearby Gulf of Lion. BMF and FWMF
249 values that are statistically greater than 1.0 (t-test, $p < 0.05$) indicate a significant THg
250 biomagnification from predator to prey (in the case of BMF) or along the food web (in the case
251 of FWMF), whereas values statistically lower than 1.0 indicate bio-dilution or bio-reduction
252 processes, suggesting active elimination or interrupted trophic transfer (Dehn et al., 2006).

253

254 *2.6 Statistical analysis*

255

256 Differences in parameter values between sexes were tested by Student *t*-tests, since data were
257 normally distributed. Differences in diet composition between female and male swordfish were
258 tested by Spearman rank correlation analysis, based on prey abundance (%N). Pearson linear
259 correlations were used to investigate the influence of size on some parameters. Differences in
260 mean THg, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ between swordfish and different prey were analysed by ANOVA with
261 Tukey HSD post-hoc tests when data were normally distributed, or else by non-parametric
262 Kruskal-Wallis analyses. A hierarchical clustering on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values based on normalized
263 Euclidean distances and Ward's criterion was applied to individualized prey groups with similar
264 stable isotope ratios.

265

266

267 **3. Results**

268

269 No significant differences in length, mass, liver parameters and condition factors were observed
270 between sexes (Table 1). Similarly, no difference in prey composition and abundance was
271 observed between male and female swordfish diets (Spearman's rank order coefficient, $\text{Rho} =$
272 0.689 , $p < 0.01$). Thus, all individuals (male and female) were subsequently grouped together for
273 further analyses.

274

275 *3.1. Swordfish diet and condition*

276

277 Only four of the 26 stomachs analysed (15%) were empty. The ingested prey belonged to three
278 taxonomic groups: crustaceans (2 species), cephalopods (6 species) and teleosts (3 species)

279 (Table 2). The indices of relative importance (IRI) show that the most frequent and abundant
280 prey of these swordfish were cephalopods (%IRI >87), which comprised >90% of the
281 reconstituted wet mass of ingested prey. Three cephalopod species dominated, *Todarodes*
282 *sagittatus* (%IRI >43), *Todaropsis eblanae* (%IRI >25) and *Ancistroteuthis lichtensteinii* (%IRI
283 >22). Teleosts comprised the second largest group of prey (%IRI >12) dominated numerically by
284 *Arctozenus risso* while there was also a relatively high frequency of *Sardina pilchardus* and
285 *Lestidiops jayakari*. Crustaceans, mainly shrimps, were very infrequent (%IRI = 0.1). No
286 significant difference in the relative importance of the three prey groups in relation to swordfish
287 length was observed ($p > 0.05$ for all correlations) for the size range of individuals analysed (102-
288 232 cm LJFL). However, a significant positive linear correlation was observed between prey
289 mass and swordfish length ($r = 0.17$, $p = 0.007$) and swordfish mass ($r = 0.19$, $p = 0.003$). In
290 swordfish stomachs, the mean mass of cephalopods (146.6 ± 179.6 g ww) was significantly
291 higher than the mean teleost mass (32.2 ± 57.1 g ww), and there was a large variability of mass
292 among species and individuals (from 2.2 to 1479.3 g ww in cephalopods, and from 5.7 to 280.0 g
293 ww in teleosts). The few shrimps observed weighed less than 10 g ww each.

294 The condition index Kn varied from 0.57 to 1.50, with a mean value of 1.03 ± 0.24 (Table S1).
295 Swordfish liver mass was significantly and positively correlated with both fish length and total
296 body mass ($r = 0.75$ and 0.90 , respectively; $p < 0.001$ in both cases; Fig. 2). In contrast, liver
297 weight relative to body weight decreased as fish size increased: i.e., the percentage of body mass
298 represented by the liver (versus the total body weight) decreased from 1.2% in fish measuring
299 <110 cm LJFL to 0.6% in fish measuring >200 cm LJFL – excluding the largest male caught
300 (LJFL = 232 cm, liver mass = 1.7% body mass). Correspondingly, a decreasing trend in LHSI
301 with length and total mass was observed, which was statistically significant when the largest
302 specimen was excluded as an outlier ($r = -0.48$, $p = 0.023$ with length, and $r = -0.48$, $p = 0.025$
303 with mass) (Fig. 2). The mean percentage of lipids in the liver was $10.2 \pm 5.4\%$ ww, with the
304 percentage varying from 4.0% to 22.0% and there was a significant decrease in relation to
305 increasing fish size ($r = -0.41$, $p = 0.05$).

306

307 *3.2. Stable isotope values, trophic level and Hg content of swordfish and prey*

308

309 Significant differences of stable isotope value, trophic level and THg concentration were
310 observed among prey species and swordfish (Table 3). Swordfish presented significantly higher
311 mean $\delta^{15}\text{N}$ (11.70 ‰) than any of its prey (7.56 - 8.87 ‰) ($p < 0.001$), while its mean $\delta^{13}\text{C}$ (-18.9
312 ‰) did not differ significantly from those of shrimps, *M. poutassou*, *T. trachurus* and *Arctozenus*
313 *risso*. The hierarchical clustering based on stable isotope values individualized two groups of
314 prey (Fig. 3). One group, with lower $\delta^{13}\text{C}$ values, consisted of the two small zooplanktivorous
315 pelagic teleosts, *S. pilchardus* and *E. encrasicolus*, while the second group, with $\delta^{13}\text{C} > -21\text{‰}$,
316 included shrimps, cephalopods and the other teleost prey, the bathypelagic *A. risso* and *M.*
317 *poutassou*, and the pelago-demersal *T. trachurus*. Swordfish presented the highest TL (4.06),
318 which was significantly higher than those of its prey (2.84 – 3.23). Among prey, crustaceans
319 were positioned at a significantly lower mean TL (2.89 ± 0.15) than cephalopods (3.02 ± 0.16)
320 and teleosts (3.08 ± 0.20) ($F = 3.31$, $p = 0.045$). THg concentrations highly varied among
321 species, the highest value being recorded in the bathypelagic teleost, *A. risso* (Table 3).
322 However, THg concentrations were significantly positively correlated to both $\delta^{15}\text{N}$ ($r = 0.30$, $p =$
323 0.007) and $\delta^{13}\text{C}$ ($r = 0.33$, $p = 0.004$) of the organisms. The mean THg concentration was
324 significantly lower in the cephalopods ($0.34 \pm 0.29 \text{ mg kg}^{-1} \text{ ww}$, $N = 20$) than in the teleost fish
325 ($0.65 \pm 0.50 \text{ mg kg}^{-1} \text{ ww}$, $N = 25$) ($t = 2.432$, $p = 0.019$). Among teleosts, *X. gladius* presented a
326 rather high THg concentration ($0.66 \pm 0.29 \text{ mg kg}^{-1} \text{ ww}$). THg largely differed between the two
327 crustacean species, while the difference was not significant due to the high variance of data and
328 the low number of specimen analysed. When related to their respective biomasses in stomach
329 contents, cephalopods accounted for 87.1% of the THg load of Catalan swordfish diet in
330 summer, teleosts 12.7% and crustaceans only 0.2%.

331

332 3.3. Hg biomagnification in the swordfish food web

333

334 THg biomagnification factors in swordfish differed significantly among prey species, with BMF
335 being always higher than BMF_{TL} , which took into account the trophic levels of prey and predator
336 (Table 4). BMF factors were significantly >1 for *A. antennatus*, three of the four cephalopods
337 and the two small pelagic fish, *E. encrasicolus* and *S. pilchardus*, indicating a biomagnification
338 in swordfish for these prey, while a significantly <1 BMF was observed for *A. risso*, indicating a
339 bio-reduction in swordfish. Both mean BMF and BMF_{TL} were significantly higher in cephalopods

340 than in teleosts and crustaceans, between which no significant difference was found ($F = 3.79$, p
341 $= 0.030$ for BMF, $F = 3.77$, $p = 0.030$ for BMF_{TL}). The BMF values calculated for the bulk diet
342 of swordfish were close – albeit slightly higher – to the mean BMF values obtained for all prey
343 species (1.82 vs 1.65 for BMF, and 1.28 vs 1.23 for BMF_{TL}).

344 A significant linear regression ($r = 0.47$, $p < 0.0001$) was observed in the food web of swordfish
345 between log THg and trophic level of organisms (Fig. 4). The food web biomagnification factor
346 calculated for swordfish food web in the Catalan Sea was found to be significantly higher than 1
347 ($FWMF = 1.383$ when calculated with TL, and $FWMF = 1.103$ when calculated with $\delta^{15}N$),
348 which is a clear indication that THg biomagnification is taking place in the swordfish food web.

349

350

351 **4. Discussion**

352

353 By bringing together different biological and toxicological data, this paper presents an
354 integrative study that sheds light on the trophic ecology, physiological condition, and toxicology
355 of a top predator, the swordfish, which is an important commercial species. New information is
356 provided on 1) the condition of swordfish based on liver lipid levels in relation to size and
357 weight, and 2) the biomagnification of mercury in its trophic web in the Catalan Sea, based on
358 the stable isotope values and mercury levels found in the main prey consumed by swordfish in
359 summer. These results contribute towards a better understanding of the trophic ecology,
360 physiological condition, and mercury accumulation from the prey to the predator (swordfish)
361 through the trophic chain, in the context of unfavourable environmental conditions affecting
362 swordfish (Damiano et al., 2011).

363

364 *4.1. Swordfish condition*

365

366 New data on liver size and liver lipid content in swordfish from the Catalan Sea are provided,
367 which highlight the relevance of this organ for energy storage and, furthermore, suggest that
368 current environmental conditions in this Mediterranean region are unfavourable for swordfish.
369 Total liver mass increased with increasing body length and weight in swordfish; however, liver
370 mass relative to body weight, and to lipid percentage, both decreased in relation to increasing

371 fish size. Correspondingly, the LHSI index decreased with increasing swordfish size. A similar
372 decrease versus increasing weight was found in the hepatosomatic index (which only takes into
373 account the liver size but not liver lipid content) by Conrad (1940) in swordfish in Nova Scotia
374 in the 1930s. Our results support Conrad's observations by incorporating the lipid content. Of
375 further interest is the fact that our Mediterranean specimens captured in 2018 had smaller relative
376 liver weights (between 0.6% and 1.2% of total body weight) than Conrad's NW Atlantic
377 specimens captured in the 1930s (between 1.6% to 2.8% of total body weight) (Conrad, 1940).
378 This apparent reduction in the relative liver weights of the Mediterranean specimens merits
379 further investigation as it may indicate unfavourable food supplies for the Mediterranean
380 swordfish at present.

381 Furthermore, the liver lipid content found in swordfish in 2018 (10% ww, LJFL = 139 cm) was
382 lower than that which was reported for this species in the southern Tyrrhenian Sea in 2005 (16%
383 ww, LJFL = 140 cm) (Corsolini et al., 2008). Again, such a difference could be due to
384 differences in regional food resources or else it may indicate a temporary decrease in swordfish
385 condition in the Mediterranean. Either way, this requires further investigation to confirm any
386 unfavourable conditions in food supplies that swordfish may be experiencing in the
387 Mediterranean (Damiano et al., 2011).

388 Although our study provides evidence of the importance of the liver as an energy (lipid) store for
389 swordfish, muscle lipid should also be investigated as a pool of energy. Taking into
390 consideration the relative mass of muscle (75%, according to Conrad, 1940) and of liver, and that
391 Corsolini et al. (2008) reported a mean lipid content of 9% in swordfish muscle, we estimated
392 that, in absolute values, swordfish muscle should contain nearly 40 times more lipid than the
393 liver. The liver is usually the first site for lipid (energy) storage in a number of benthic and
394 demersal species such as gadoids and sharks, as well as deep-sea fish such as macrourids (Lloret
395 et al., 2014). For swordfish, lipids may be stored in the liver during feeding periods but are
396 mobilised quickly towards the muscle when energy is needed, for example, for swimming and
397 during non-feeding periods. This may explain the decline of LHSI with size, because larger,
398 adult fish need to divert more energy from the liver to the muscle for migratory (reproduction)
399 purposes. Further studies on changes in liver lipid reserves in this top predator, which has a
400 particularly high metabolism (Brill, 1996), are needed to help understand how climate change in

401 the Mediterranean Sea might affect the condition and physiology of swordfish and, in turn, the
402 status of swordfish populations.

403

404 4.2. Swordfish diet

405

406 No difference in swordfish diet with sex or size was recorded in the Catalan Sea, although there
407 was an increase in prey size with increasing predator size, as was also the case in the Strait of
408 Gibraltar (Abid et al., 2017). The stomach content analysis showed that cephalopods were the
409 main prey of swordfish in the Catalan Sea during summer. These results are in line with previous
410 studies in the central Mediterranean (Bello, 1991; Orsi Rellini et al., 1995). In contrast, other
411 studies in other regions, such as in the Aegean Sea (Salman, 2004), the strait of Messine (Romeo
412 et al., 2009), but also in the NW Mediterranean (Carmona-Antoñanza et al., 2016; Navarro et al.,
413 2017), have indicated that teleosts dominate the swordfish diet. However, regional comparisons
414 are hampered by the diversity of metrics used to analyze stomach contents, such as percentage of
415 occurrence, frequency, number, or weight of prey, and whether prey weight is reconstructed or
416 not. Teleosts are often more numerous than cephalopods in swordfish stomach contents, but their
417 mean weight is generally lower than that of cephalopods, as observed in the present study. Thus,
418 cephalopods most likely represent the largest biomass of prey ingested by swordfish in the
419 Mediterranean Sea. Although all authors recognized a high trophic plasticity for this top
420 predator, the dominant prey recorded in stomach contents reflect a clear preference for a small
421 number of species in the NW Mediterranean, such as *Todarodes sagittatus*, *Illex coindetii* and
422 *Ancistroteuthis lichtensteinii* among the cephalopods, and some Clupeidae (*Sardinella aurita* and
423 *Sardina pilchardus*), Gadidae (*Micromesistius poutassou*), Trichiuridae (*Lepidopus caudatus*)
424 and Paralepididae (*Arctozenus risso*) among the teleosts. Peristeraki et al. (2005) suggested that
425 *T. sagittatus* is a very abundant cephalopod in the Aegean Sea, particularly in summer and
426 autumn, and hence its importance in the swordfish diet, an observation confirmed in other
427 Mediterranean regions (Bello, 1991; Romeo et al., 2009; Carmona-Antoñanza et al., 2016; and
428 this study). As expected, the prey of swordfish are season-dependent, with the epipelagic species,
429 including engraulids and clupeids preferentially eaten by the eastern Mediterranean swordfish
430 population in winter and spring (Salman et al., 2004). In addition to seasonal variation in food
431 availability, swordfish diet also varies with sampling habitat (coastal or oceanic) and depth of

432 catches (Carmona-Antoñanza et al., 2016; Abid et al. 2017). In the present study, swordfish
433 ingested both shallow coastal small pelagic fish (mainly sardine) and deep small mesopelagic
434 fish (mainly barracudina).

435

436 4.3 Food web and Hg biomagnification

437

438 This study of the swordfish food web in the Catalan Sea based on the trophic level of its prey
439 (estimated from $\delta^{15}\text{N}$) has produced results consistent with other studies carried out in the same
440 region (Navarro et al., 2017) and elsewhere, or with different top predators (*Thunnus alalunga*,
441 *Prionace glauca* and *Isurus oxyrinchus*) (Biton-Porsmoguer, 2015).

442 Mercury contamination was found throughout all of the Catalan swordfish prey groups:
443 crustaceans, cephalopods, epi- and bathypelagic teleosts. The THg concentrations found in these
444 organisms were within the range of other studies (Cardoso et al., 2019; Morrison et al., 2015;
445 Minet et al., 2021; Di Benedetto et al., 2021) and were significantly positively correlated to both
446 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Correlations have also been found by McCormack et al. (2020) between THg and
447 $\delta^{13}\text{C}$, and by Seco et al. (2021) between THg and $\delta^{15}\text{N}$ in other top predators. Their importance
448 as a source of food means that cephalopods contribute significantly to the swordfish's mercury
449 intake, not only because of the frequency of consumption and the quantity ingested (individuals
450 are often swallowed whole), but also because of the fairly high levels of mercury recorded in
451 some species of cephalopods (Storelli et al., 2006). In the Catalan Sea, cephalopods presented
452 similar Hg concentrations to those of the small epipelagic sardine and anchovy, although it must
453 be said that much higher Hg concentrations were recorded in bathypelagic and bentho-demersal
454 teleosts. Such results indicate that higher Hg contents would be found in swordfish whose main
455 prey was fish, as has also been suggested by other authors (Esposito et al., 2018). The
456 importance of diet composition on Hg accumulation in swordfish, in addition to their size, has
457 also been pointed out (Mendez et al., 2001; Branco et al., 2007; Damiano et al., 2011; Esposito et
458 al., 2018).

459 Our study has established the biomagnification of mercury along the food web from zooplankton
460 to swordfish in the Catalan Sea, as has previously been demonstrated for swordfish in the
461 northwest Atlantic (Harding et al., 2018) and for other top predators such as sharks (Kiszla et al.,
462 2015; Biton-Porsmoguer et al., 2018). BMF values indicated a significant biomagnification in

463 swordfish in relation to most of its prey, but also one case of bioreduction from the highly
464 contaminated bathypelagic teleost, *A. risso*. However, biomagnification factors vary with the
465 type of calculation employed (Borga et al., 2011). We observed that simple BMF values were
466 systematically higher than they were when the trophic level of prey was taken into account
467 ($\text{BMF} > \text{BMF}_{\text{TL}}$), which was also observed by Murillo-Cisneros et al. (2019) in Pacific rays.
468 Similarly, FWMF was lower when calculated with $\delta^{15}\text{N}$ rather than with the TL of organisms
469 (1.10 vs 1.38, respectively). However, all these values are within the range of those recorded for
470 Hg biomagnification in other food webs (Harding et al., 2018).

471

472 *4.4. Environmental context and health risks*

473

474 Changing environmental conditions in the Mediterranean may favour certain species over others,
475 depending on their levels of adaptability and dependency (Marbà et al., 2015). Environmental
476 changes, such as sea warming, are occurring and having an impact on the phytoplankton and
477 zooplankton (Marbà et al., 2015) at the basis of marine food webs (Bănaru et al., 2019),
478 providing evidence that sustains the hypothesis of a bottom-up mechanism affecting
479 planktivorous species (Diaz et al., 2019). In the NW Mediterranean, the decrease in mean size
480 and condition of small pelagic fish is probably related to changes in the quantity and/or quality of
481 zooplankton (Chen et al., 2019; Biton-Porsmoguer et al., 2020) and this may impact the
482 condition of their predators. Cephalopods (squids), the main prey of swordfish in the
483 Mediterranean, present better adaptive trophic capacities than other groups and are generally
484 proliferating (Doubleday et al., 2016). However, in the NW Mediterranean Sea, as squids
485 generally feed on small pelagic fish (Bănaru, pers. com.), it would not be unreasonable to assume
486 that their condition may also be affected by the decreasing mean size and condition of their prey.
487 In this study, the fact that mean mercury levels in the main swordfish prey (cephalopods and
488 epipelagic teleost) were lower than those in bathypelagic and bentho-demersal teleosts may
489 explain why the mean mercury levels in swordfish fall below the UE maximum regulatory levels
490 (European Commission, 2006). Any impoverishment of the pelagic community that may lead to
491 a change in swordfish diet toward more benthic prey is undesirable as this could amplify the
492 observed biomagnification of Hg in the food web, since benthic organisms contain generally
493 higher Hg concentrations than pelagic ones (Cresson et al. 2014).

494 Schartup et al. (2019) describe how climate change is likely to exacerbate human exposure to
495 methylmercury (MeHg) through marine fish, and suggest that stronger regulations are needed to
496 protect ecosystems and human health. According to the literature, up to 90% of the THg in
497 marine species examined thus far is probably MeHg (Cossa et al., 2012; Polak-Juszczak, 2018).
498 MeHg is an organic form of mercury, which is prone to bioaccumulation, but also
499 biomagnification (Ilmiwati et al., 2015). Between 80% and 90% of the organic mercury in
500 human bodies comes from fish intake (Hong et al., 2012). Exposure to MeHg is an issue of great
501 concern, as it is neurotoxic, mutagenic and causes disruptions in the circulatory, nervous and
502 reproductive systems (Hammerschmidt et al., 2002; Kwaśniak et al., 2005). Hence, excessive
503 consumption of some marine top predator species might represent a risk for human health (Velez
504 et al., 2010; Barone et al., 2018). Moreover, high concentrations of contaminants can alter the
505 reproductive processes of fish by interfering with endocrine functions (De Metrio et al., 2003;
506 Kar et al., 2021). The concentrations of mercury in swordfish may represent not only a potential
507 problem for human consumption, but also for the sustainability of the swordfish stocks and
508 fisheries in the Mediterranean (Damiano et al., 2011).

509

510 **5. Conclusions**

511

512 These results indicate that swordfish condition (in terms of liver size and liver lipid content)
513 seems to be impaired in the Catalan Sea compared to other areas of the Mediterranean Sea,
514 indicating a potentially unfavourable food supply that should be explored in the future.
515 Cephalopods are the main prey of swordfish in summer in the Catalan Sea and the main vector of
516 Hg accumulation in swordfish. Despite the biomagnification of mercury in their food web, the
517 mean mercury levels of swordfish remain below the EU maximum regulatory levels. However,
518 these levels may well be high enough to act as endocrine disruptors which may cause
519 dysfunctions in the swordfish reproductive system, altering their ability to adjust to outside
520 agents, which can weaken the populations. Future management of swordfish stocks in the
521 Mediterranean should consider integrating the monitoring of physiological, feeding and eco-
522 toxicological data in the light of these potential unfavourable trophic conditions (low food supply
523 and high input of Hg from prey) that swordfish may be experiencing in the Mediterranean Sea.

524

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526

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529

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886 **Tables**

887

888 Table 1. Morphometric parameters, hepatic parameters, lipid hepatosomatic index (LHSI) and
889 condition index (Kn) of 26 specimens of *Xiphias gladius* from the Catalan Sea grouped by sex.

890 Differences between sexes were tested by Student t-tests (t) and the probability (p) is indicated.

891 N = number of individuals

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			Eviscerated		Total Liver	Lipid		
	N	Size (cm)	Total mass	mass	Liver mass	lipids	hepato	Condition
		LJFL	(kg ww)	(kg ww)	(g ww)	ww)	index	index
			Mt	Me	LM	ABSL	LHSI	Kn
Total	26	139.1 (31.3)	40.6 (33.0)	37.0 (30.1)	457.0 (536.7)	42.0 (34.0)	0.13 (0.08)	1.03 (0.24)
Males	14	142.9 (34.6)	45.1 (40.6)	41.1 (37.0)	548.1 (702.9)	46.4 (42.2)	0.12 (0.08)	1.02 (0.20)
Females	12	134.8 (27.7)	35.3 (21.7)	32.1 (19.8)	340.9 (150.2)	36.4 (19.8)	0.13 (0.09)	1.04 (0.28)
t-test (t)		0.64	0.75	0.76	0.96	0.70	-0.18	-0.24
p		0.525	0.459	0.457	0.349	0.494	0.861	0.811

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894

895 Table 2. Swordfish diet based on stomach content analysis. For each prey category, the table
 896 shows number of prey (N), % of occurrence (%O), % by number (%N), % by reconstituted wet
 897 mass (%M) and % index of relative importance (%IRI). NI = not identified. The asterix (*)
 898 indicates a value of <0.1%
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Prey	N	%O	%N	%M	%IRI
Crustaceans	3	13.0	1.1	0.1	0.1
<i>Aristeus antennatus</i>	1	4.4	0.4	*	*
<i>Parapenaeus longirostris</i>	1	4.4	0.4	*	*
Decapods NI	1	4.4	0.4	*	*
CEPHALOPODS	195	87.0	72.0	93.1	87.2
<i>Abralia veranyi</i>	5	17.4	1.8	0.4	0.4
<i>Ancistroteuthis lichtensteinii</i>	48	52.2	17.7	22.5	22.6
<i>Histioteuthis bonnellii</i>	2	8.7	0.7	0.2	0.1
<i>Histioteuthis reversa</i>	2	4.4	0.7	0.6	0.1
<i>Todarodes sagittatus</i>	71	52.2	26.2	51.4	43.6
<i>Todaropsis eblanae</i>	65	56.5	24.0	18.4	25.8
Cephalopods NI	2	4.4	0.7	0.2	*
TELEOSTS	73	60.9	26.9	6.9	12.8
<i>Arctozenus risso</i>	43	13.0	15.9	2.6	2.6
<i>Lestidiops jayakari</i>	7	17.4	2.6	0.2	0.6
<i>Sardina pilchardus</i>	8	34.8	3.0	3.4	2.1
Teleosts NI	15	26.1	5.5	0.6	2.1

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Table 3. Mean \pm standard deviation of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, trophic level (TL) and THg concentration of *Xiphias gladius* and its prey in the Catalan Sea (W Mediterranean). N = number of samples. * = estimated from Navarro et al. (2017), ** = estimated from Barone et al. (2018). Differences among species were tested by ANOVA (F) or Kruskal-Wallis (H) analyses. p = probability. The letters a, b and ab indicate values that are significantly different from each other.

Species	N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	TL	THg (mg kg ⁻¹ ww)
Deep-sea shrimp (<i>A. antennatus</i>)	3	-19.35 \pm 1.30 ab	7.56 \pm 0.88 b	2.84 \pm 0.26 b	0.38 \pm 0.51 ab
Deep-water rose shrimp (<i>P. longirostris</i>)	5	-18.26 \pm 0.25 a	7.82 \pm 0.16 b	2.92 \pm 0.05 b	0.93 \pm 0.45 ab
Angel clubhook squid (<i>A. lichtensteinii</i>)	5	-20.41 \pm 0.27 b	8.34 \pm 0.26 b	3.07 \pm 0.08 b	0.43 \pm 0.50 ab
Broadtail shortfin squid (<i>I. coindetii</i>)	5	-20.07 \pm 0.25 b	7.98 \pm 0.40 b	2.97 \pm 0.12 b	0.38 \pm 0.08 ab
Striped squid (<i>T. eblanae</i>)	5	-20.02 \pm 0.49 b	7.71 \pm 0.42 b	2.88 \pm 0.12 b	0.38 \pm 0.34 ab
European flying squid (<i>T. sagittatus</i>)	5	-20.03 \pm 0.36 b	8.62 \pm 0.61 b	3.15 \pm 0.18 b	0.17 \pm 0.06 b
Spotted barracudina (<i>A. risso</i>)	4	-19.94 \pm 0.46 ab	8.46 \pm 0.32 b	3.11 \pm 0.09 b	1.11 \pm 0.24 a
Anchovy (<i>E. encrasicolus</i>)	5	-21.37 \pm 1.06 b	7.61 \pm 0.66 b	2.86 \pm 0.19 b	0.37 \pm 0.07 ab
Blue whiting (<i>M. poutassou</i>)	5	-19.09 \pm 0.39 ab	8.87 \pm 0.70 b	3.23 \pm 0.20 b	0.80 \pm 0.63 ab
Sardine (<i>S. pilchardus</i>)	5	-21.85 \pm 1.26 b	8.26 \pm 0.54 b	3.05 \pm 0.16 b	0.23 \pm 0.06 ab
Atlantic horse mackerel (<i>T. trachurus</i>)	5	-19.39 \pm 0.27 ab	8.67 \pm 0.52 b	3.17 \pm 0.15 b	0.84 \pm 0.61 ab
Swordfish (<i>X. gladius</i>)	26	-18.97 \pm 1.80* a	11.70 \pm 0.52* a	4.06 \pm 0.30 a	0.66 \pm 0.29** a
F (ANOVA) or H (Kruskal-Wallis)		H = 60.43	F = 37.75	F = 37.74	H = 33.62
values p		<0.0001	<0.0001	<0.0001	P = 0.0004

911 Table 4. Biomagnification factors, BMF, in the food web components of the swordfish, *Xiphias*
 912 *gladius*, alongside BMF_{TL} values (which take into account the trophic level (TL) of prey and
 913 predator) with mean values given for individual prey species, for all prey species (Mean prey
 914 BMF), and for the prey consumed by swordfish calculated with the mean percentage mass of
 915 each prey group (Diet BMF). The asterix (*) indicates a significant difference from 1 (t-test,
 916 p<0.05).
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Prey species	BMF	BMF_{TL}
<i>Aristeus antennatus</i>	1.74*	1.21*
<i>Parapenaeus longirostris</i>	0.71	0.51
<i>Ancistroteuthis lichtensteinii</i>	1.53*	1.16*
<i>Illex coindetii</i>	1.74*	1.27*
<i>Todaropsis eblanae</i>	1.74*	1.23*
<i>Todarodes sagittatus</i>	3.88	3.01
<i>Arctozenus risso</i>	0.59*	0.46*
<i>Engraulis encrasicolus</i>	1.78*	1.26*
<i>Micromesistius poutassou</i>	0.83	0.66
<i>Sardina pilchardus</i>	2.87*	2.16*
<i>Trachurus trachurus</i>	0.79	0.61
Mean prey BMF ± SD	1.65 ± 0.99*	1.23 ± 0.77*
Diet BMF	1.82*	1.28*

918

919 **Figures**

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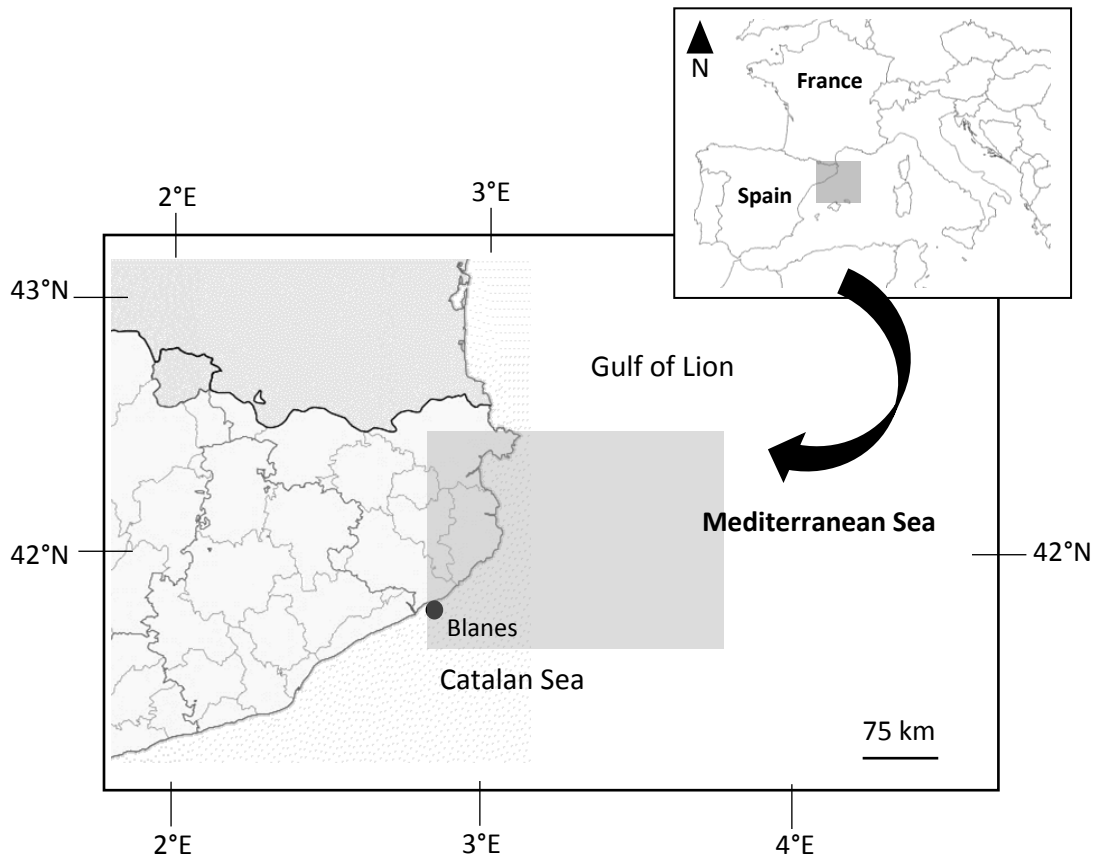
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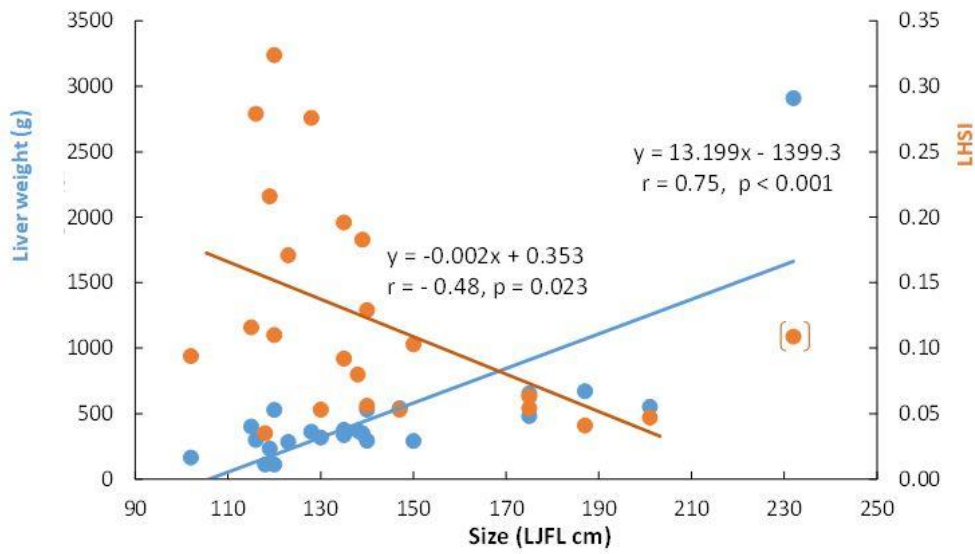
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942 Figure 1. Map of the fishing port (Blanes) in the northern Catalan Sea (NW Mediterranean)
943 where swordfish were sampled

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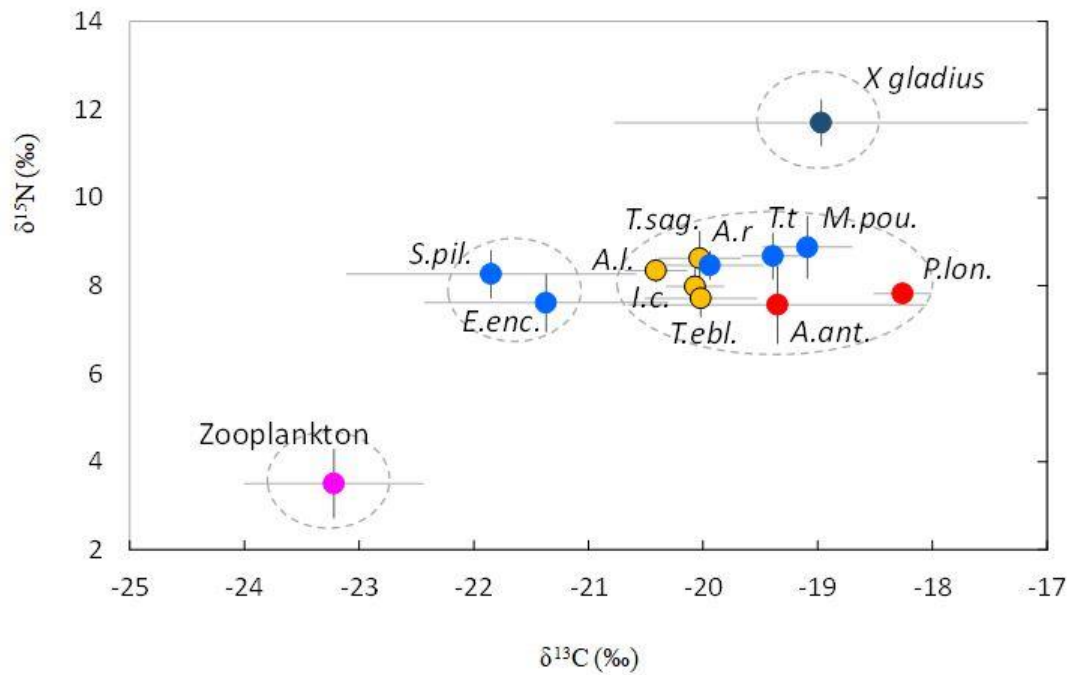
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955 Figure 2. Linear relationships between liver weight (g) and liver hepatosomatic index
956 (LHSI) with fish length (LJFL, cm) of swordfish in the Catalan Sea. The correlation
957 LHSI-Fish length was calculated with the longest individual (indicated in brackets)
958 excluded.

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963 Figure 3. Biplot of mean (\pm SD) C and N stable isotope values of the food web components of
964 swordfish, *Xiphias gladius*, in the Catalan Sea. Swordfish prey are marked as follows: red for
965 crustaceans, orange for cephalopods and blue for teleosts. *A.ant.* = *Aristeus antennatus*, *A.l.* =
966 *Ancistroteuthis lichtensteinii*, *A.r.* = *Arctozenus risso*, *E.enc.* = *Engraulis encrasicolus*, *I.C.* =
967 *Illex coindetii*, *M.pou.* = *Micromesistius poutassou*, *P.lon.* = *Parapenaeus longirostris*, *S.pil.* =
968 *Sardina pilchardus*, *T.ebl.* = *Todaropsis eblanae*, *T.sag.* = *Todarodes sagittatus*.

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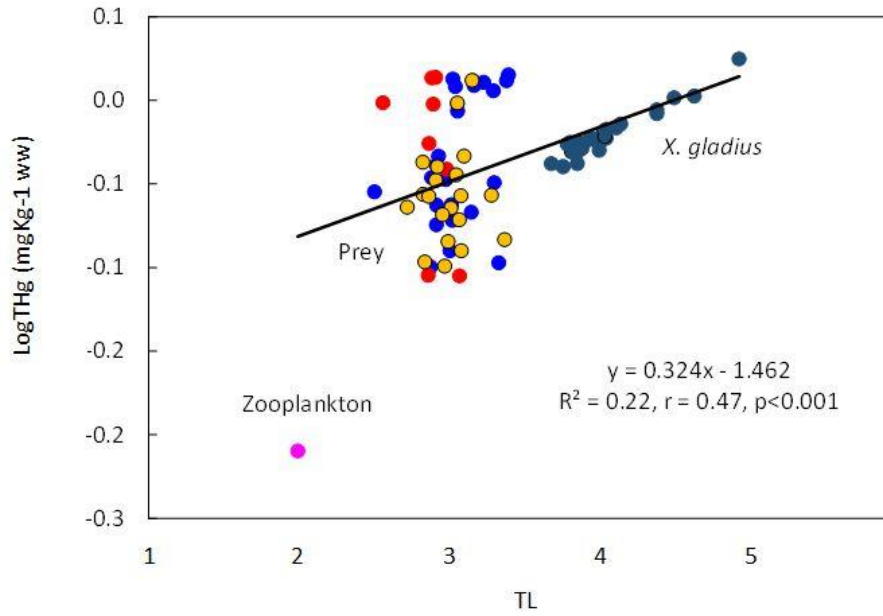
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Figure 4. Biomagnification of THg in the food web of swordfish, *X. gladius*, shown by linear correlation between logTHg and the trophic level (TL) of organisms. Swordfish prey are marked as follows: red for crustaceans, orange for cephalopods and blue for teleosts.