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A study of trophic structure, physiological condition and mercury biomagnification in swordfish (*Xiphias gladius*): Evidence of unfavourable conditions for the swordfish population in the Western Mediterranean

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Abstract :

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

Keywords : Swordfish, Fisheries, Mercury, Biomagnification, Condition, Catalan Sea

1. Introduction

The swordfish (*Xiphias gladius*, Linnaeus, 1758) is one of the most commercially-valuable species in the European Union market, being among the top ten, in monetary value, landed in the EU (EUMOFA, 2018: p 99). It is the target of intensive fishing by pelagic longliners in the north-western Mediterranean due to its high value in Spanish ports (for example, 224 tons landed in 2020 in Catalan ports) (Biton-Porsmoguer, 2017 and GENCAT 2021) and a high consumption rate in Spain (Velez et al., 2010). There is a large body of research, from around the world, on the biology (diet, growth and reproduction), migrations, genetic structure of populations, and heavy metal accumulation of this species (e.g., Viñas et al., 2006; Tserpes et al., 2008; Rosas-Luis et al., 2017; Esposito et al., 2018, and references therein). In the Mediterranean Sea in particular, the diet of swordfish has been studied in the Aegean Sea (Salman, 2004; Ceyhan & Akyol, 2017), the Central Mediterranean Sea (Orsi Relini et al., 1995; Romeo et al., 2009) and more recently in the western Mediterranean Sea (Carmona-Antoñanzas et al., 2016; Navarro et al., 2017). The swordfish is a large migratory top predator and feeds on a broad spectrum of prey, comprising mainly teleost fish, cephalopods and crustaceans, depending on fish size, area and season (Canese et al., 2008; Navarro et al., 2017; Abid et al., 2018). In the North Western Mediterranean Sea swordfish are able to cross long distances in a short time, displaying preferences for different habitats for breeding and feeding (Canese et al., 2008), where they may spend long periods (Orsi Relini et al., 2003). Being one of the larger top predators, the swordfish is susceptible to accumulating high levels of contaminants, particularly mercury (Hg), both by biomagnification up the food web as well as by bioaccumulation during its life span (Storelli & Marcotrigiano, 2001), as has been observed in other top predators (Pethybridge et al., 2012; Matulik et al., 2017; Biton-Porsmoguer et al., 2018). Increasingly high concentrations of Hg in Mediterranean seafood are a worrying seafood safety issue due to the major toxic effects of its organic form, methylmercury (MeHg) (Cossa et al., 2009). Fish consumption is a major source of MeHg exposure among Europeans (Miklavčič Višnjevec et al., 2013), and MeHg generally comprises >90% of total Hg (THg) in fish muscle (Cossa et al., 2012; Chouvelon et al., 2018), including swordfish (Velez et al., 2010, Cinnirella et al., 2019). A number of studies have reported high levels of Hg contamination in swordfish, resulting in high levels of exposure for consumers (Storelli et al., 2010; Damiano et al., 2011; Rodriguez et al., 2013; Esposito et al., 2018; González et al., 2019). Furthermore, heavy metals, such as mercury, have been shown to have serious long-term effects on fish, as potential endocrine disruptors (Kar et al., 2021).

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

In contrast to such work on diet and contaminant accumulation in swordfish, research into their physiological condition is sparse, despite indications from studies involving other exploited species which show that condition, particularly during critical periods in their life-cycles (e.g. pre-spawning, migration or early life stages), is an essential factor in the management of sustainable and profitable fisheries (Lloret et al., 2012). Studies from many years ago, such as Raven and LaMonte (1936) and Conrad (1940), did take into consideration the liver's important role as an energy reserve, noting that there was an allometric growth of the liver (the smaller the swordfish, the greater its proportional liver weight), while also highlighting significant seasonal fluctuations in absolute size of the body, and the liver, of swordfish in north-western Atlantic waters. These fluctuations were attributed to environmental conditions, with food supply thought to be the most likely factor. Energy density and lipid content (direct condition indices) are typically measured using bomb calorimetry and proximate composition analysis. From the available biochemical parameters for evaluating the energy reserves of fishes, determination of fat content has been the most widely used. Lipids, together with proteins and carbohydrates, are the principal energy stores in teleosts and are often mobilized during nonfeeding and reproductive periods. Lipid storage and lipid dynamics within the organism are therefore a particularly important aspect of fish health and population success (Lloret et al., 2014). According to Ben Smida et al (2009), who calculated percentage lipid content in swordfish tissue, by far the highest lipid content is found in the liver (26%) followed by the gonads (4.7%) and red muscle (4.5%), which suggests the liver plays an important role as an energy store in swordfish.

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

In this context, the objectives of the present study are, using a multi-methodological approach: 1) to determine the diet of swordfish in the Catalan Sea by identifying prey in stomach contents; 2) to reconstitute the trophic web of swordfish by stable isotope analyses of its prey; 3) to analyse the condition of swordfish as indicated by their liver lipid content; and 4) to analyse the mercury level in prey and determine the biomagnification factors in the swordfish food web. By analysing these different variables, the paper aims to provide new biological (trophic ecology), physiological (fish condition), and eco-toxicological data that, taken together, can improve our knowledge about the health status of swordfish in the Mediterranean and may be useful for the stock assessment and management of populations.

2. Material and Methods

2.1 Sampling

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

2.2 Diet

The number of empty stomachs was recorded and the index of vacuity (%V) calculated as the percentage of empty stomachs of all stomachs analysed. Prey identification to the lowest taxon possible was carried out by analysing vertebrae and otoliths for teleosts, and beak characteristics for cephalopods (Grassé, 1958; Clarke, 1986). Various dietary indices were calculated to describe swordfish diet: the percentage of occurrence frequency (%O) was the percentage of all non-empty stomachs containing one prey category. Percent number (%N) and percent mass (%M) were the percentages of individual number and mass, respectively, of a given prey category versus the overall number or mass of prey in non-empty stomachs. Prey masses determined in the present study were not those actually recorded in stomach contents, but reconstructed prey mass values that were calculated according to pre-established vertebrae- or beak size-prey mass relationships. Reconstructed prey mass is more appropriate to comprehend the real importance of prey in the diet of large predatory fishes (Biton-Porsmoguer, 2015). The contribution of each prey category to swordfish diet was then estimated using the index of relative importance (IRI), which was calculated as IRI = (%N + %M) × %O, and expressed in % IRI = (IRI of one prey category / sum of % IRI of all prey categories) * 100 (Pinkas et al., 1971; Cortés, 1997).

2.3 Condition

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

2.4 Hg content and stable isotope values of prey

A list of potential swordfish prey was established according to the results of stomach contents in our study and supplemented with data from recent studies on swordfish diet in the Mediterranean Sea (Salman, 2004; Carmona-Antoñanzas et al., 2016; Ceyhan & Akyol, 2017; Navarro et al., 2017; Abid et al., 2018). Samples were taken from stomach contents when their state of digestion permitted (presence of fresh meat) or sampled in the study area and from landings in the fishing port of Palamós, such as the cephalopod, *Illex coindetii*, and the teleosts: *Engraulis encrasicolus* (anchovy), *Micromesistius poutassou* (blue whiting), and *Trachurus trachurus* (Atlantic horse mackerel). Five specimens of each prey species were analysed for Hg content and stable isotope values. Before analyses, the prey were freeze-dried and reduced to a homogenized powder by grinding in an agate mortar.

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

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

Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) within prey tissue (from the natural environment and stomach contents) were analysed with a continuous flow isotope-ratio mass spectrometer (Flash HT Elemental Analyzer, coupled to a Finnigan ConFlo III interface) at the Stable Isotope Analysis Laboratory (IRMS), Autonomous University of Barcelona (Spain). Results were expressed in the conventional form, as per mil (‰) relative to international standard materials: Vienna PeeDee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, according to the equation: $\delta X = [(R_{sample}/R_{standard})-1]*10^3$, where X is ^{13}C or ^{15}N and R is the heavy to light isotope ratio for either $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The international standard materials used were Vienna Pee Dee B for C, and atmospheric N2 for N, with an experimental precision of <0.1 ‰ for both elements, checked with international standard laboratory IAEA 600 (caffeine).

2.5. Trophic level and biomagnification factors

Trophic levels (TL) of prey and swordfish were estimated from their $\delta^{15}N$ values based on the equation given by Badalamenti et al. (2002): $TL = 1 + (\delta^{15}N_{Predator} - \delta^{15}N_{PP}) / 3.4$, with a mean value of 1.3 ‰ for nanophytoplankton (primary production, PP) in the NW Mediterranean (Rau et al., 1990), as it was the main source of primary production at the base of food webs in this region (Cresson et al., 2014).

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

Various types of biomagnification factors were calculated. The simplest measure is the biomagnification factors (BMF) at species level, which corresponds to the ratio of the chemical concentrations of the predator relative to its prey: $BMF = THg_{Predator} / THg_{Prey}$.

Gobas et al. (2009) calculated the BMF_{TL} when normalizing the BMF factor to trophic level (TL) of predator and prey, as follows:

$$BMF_{TL} = \frac{[THg]_{Predator}/[THg]_{Prey}}{TL_{Predator}/TL_{Prey}}$$

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

The food web magnification factor (FWMF) is an estimation at the system level. FWMF is calculated as the antilog of the regression slope of logTHg concentrations in food web organisms in relation to their trophic level (Fisk et al., 2001; Borgå et al., 2011. To calculate FWMF in the swordfish food web we used, as the base of the food web, the mean values obtained for zooplankton by Chouvelon et al. (2019) for THg (0.008 mg kg^{-1} wet weight) and by Espinasse et al. (2014) for trophic level ($\delta^{15}N$ = 3.5‰; TL = 2) in the nearby Gulf of Lion. BMF and FWMF values that are statistically greater than 1.0 (t-test, $p<0.05$) indicate a significant THg biomagnification from predator to prey (in the case of BMF) or along the food web (in the case of FWMF), whereas values statistically lower than 1.0 indicate bio-dilution or bio-reduction processes, suggesting active elimination or interrupted trophic transfer (Dehn et al., 2006).

2.6 Statistical analysis

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

3. Results

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

3.1. Swordfish diet and condition

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

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

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

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

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

3.3. Hg biomagnification in the swordfish food web

THg biomagnification factors in swordfish differed significantly among prey species, with BMF being always higher than BMF_{TL}, which took into account the trophic levels of prey and predator (Table 4). BMF factors were significantly >1 for *A. antennatus*, three of the four cephalopods and the two small pelagic fish, *E. encrasicolus* and *S. pilchardus*, indicating a biomagnification in swordfish for these prey, while a significantly <1 BMF was observed for *A. risso*, indicating a bioreduction in swordfish. Both mean BMF and BMF_{TL} were significantly higher in cephalopods than in teleosts and crustaceans, between which no significant difference was found ($F = 3.79$, $p = 0.030$ for BMF, $F = 3.77$, $p = 0.030$ for BMF_{TL}). The BMF values calculated for the bulk diet of swordfish were close – albeit slightly higher – to the mean BMF values obtained for all prey species (1.82 *vs* 1.65 for BMF, and 1.28 *vs* 1.23 for BMF_{TL}).

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

4. Discussion

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

4.1. Swordfish condition

New data on liver size and liver lipid content in swordfish from the Catalan Sea are provided, which highlight the relevance of this organ for energy storage and, furthermore, suggest that current environmental conditions in this Mediterranean region are unfavourable for swordfish. Total liver mass increased with increasing body length and weight in swordfish; however, liver mass relative to body weight, and to lipid percentage, both decreased in relation to increasing fish size. Correspondingly, the LHSI index decreased with increasing swordfish size. A similar decrease versus increasing weight was found in the hepatosomatic index (which only takes into account the liver size but not liver lipid content) by Conrad (1940) in swordfish in Nova Scotia in the 1930s. Our results support Conrad's observations by incorporating the lipid content. Of further interest is the fact that our Mediterranean specimens captured in 2018 had smaller relative liver weights (between 0.6% and 1.2% of total body weight) than Conrad's NW Atlantic specimens captured in the 1930s (between 1.6% to 2.8% of total body weight) (Conrad, 1940). This apparent reduction in the relative liver weights of the Mediterranean specimens merits further investigation as it may indicate unfavourable food supplies for the Mediterranean swordfish at present.

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

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

4.2. Swordfish diet

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

4.3 Food web and Hg biomagnification

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

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

Our study has established the biomagnification of mercury along the food web from zooplankton to swordfish in the Catalan Sea, as has previously been demonstrated for swordfish in the northwest Atlantic (Harding et al., 2018) and for other top predators such as sharks (Kiszla et al., 2015; Biton-Porsmoguer et al., 2018). BMF values indicated a significant biomagnification in swordfish in relation to most of its prey, but also one case of bioreduction from the highly contaminated bathypelagic teleost, *A. risso*. However, biomagnification factors vary with the type of calculation employed (Borga et al., 2011). We observed that simple BMF values were systematically higher than they were when the trophic level of prey was taken into account (BMF> BMF_{TL}), which was also observed by Murillo-Cisneros et al. (2019) in Pacific rays. Similarly, FWMF was lower when calculated with $\delta^{15}N$ rather than with the TL of organisms (1.10 vs 1.38, respectively). However, all these values are within the range of those recorded for Hg biomagnification in other food webs (Harding et al., 2018).

4.4. Environmental context and health risks

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

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

5. Conclusions

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

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Tables

Table 1. Morphometric parameters, hepatic parameters, lipid hepatosomatic index (LHSI) and condition index (Kn) of 26 specimens of *Xiphias gladius* from the Catalan Sea grouped by sex. Differences between sexes were tested by Student t-tests (t) and the probability (p) is indicated. N = number of individuals

	N	Size (cm) LJFL	Total mass (kg ww) Mt	Eviscerated mass (kg ww) Me	Liver mass (g ww) LM	Total Liver lipids (g ww) ABSL	Lipid hepato - somatic index LHSI	Condition index 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*

Table 2. Swordfish diet based on stomach content analysis. For each prey category, the table shows number of prey (N), % of occurrence (%O), % by number (%N), % by reconstituted wet mass (%M) and % index of relative importance (%IRI). NI = not identified. The asterix (*) indicates a value of <0.1%

Prey	N	%O	%N	%M	%IRI
Crustaceans	**3**	**13.0**	**1.1**	**0.1**	**0.1**
Aristeus antennatus	1	4.4	0.4	*	*
Parapenaeus longirostris	1	4.4	0.4	*	*
Decapods NI	1	4.4	0.4	*	*
CEPHALOPODS	**195**	**87.0**	**72.0**	**93.1**	**87.2**
Abralia veranyi	5	17.4	1.8	0.4	0.4
Ancistroteuthis lichtensteinii	48	52.2	17.7	22.5	22.6
Histioteuthis bonnellii	2	8.7	0.7	0.2	0.1
Histioteuthis reversa	2	4.4	0.7	0.6	0.1
Todarodes sagittatus	71	52.2	26.2	51.4	43.6
Todaropsis eblanae	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**
Arctozenus risso	43	13.0	15.9	2.6	2.6
Lestidiops jayakari	7	17.4	2.6	0.2	0.6
Sardina pilchardus	8	34.8	3.0	3.4	2.1
Teleosts NI	15	26.1	5.5	0.6	2.1

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

Table 4. Biomagnification factors, BMF, in the food web components of the swordfish, *Xiphias gladius*, alongside BMF_{TL} values (which take into account the trophic level (TL) of prey and predator) with mean values given for individual prey species, for all prey species (Mean prey BMF), and for the prey consumed by swordfish calculated with the mean percentage mass of each prey group (Diet BMF). The asterix (*) indicates a significant difference from 1 (t-test, $p<0.05$).

Prey species	**BMF**	**BMF_{TL}**
Aristeus antennatus	1.74*	1.21*
Parapenaeus longirostris	0.71	0.51
Ancistroteuthis lichtensteinii	1.53*	1.16*
Illex coindetii	1.74*	1.27*
Todaropsis eblanae	1.74*	1.23*
Todarodes sagittatus	3.88	3.01
Arctozenus risso	0.59*	0.46*
Engraulis encrasicolus	1.78*	1.26*
Micromesistius poutassou	0.83	0.66
Sardina pilchardus	2.87*	2.16*
Trachurus trachurus	0.79	0.61
Mean prey BMF ± SD	1.65 ± 0.99*	1.23 ± 0.77*
Diet BMF	1.82*	1.28*

Figures

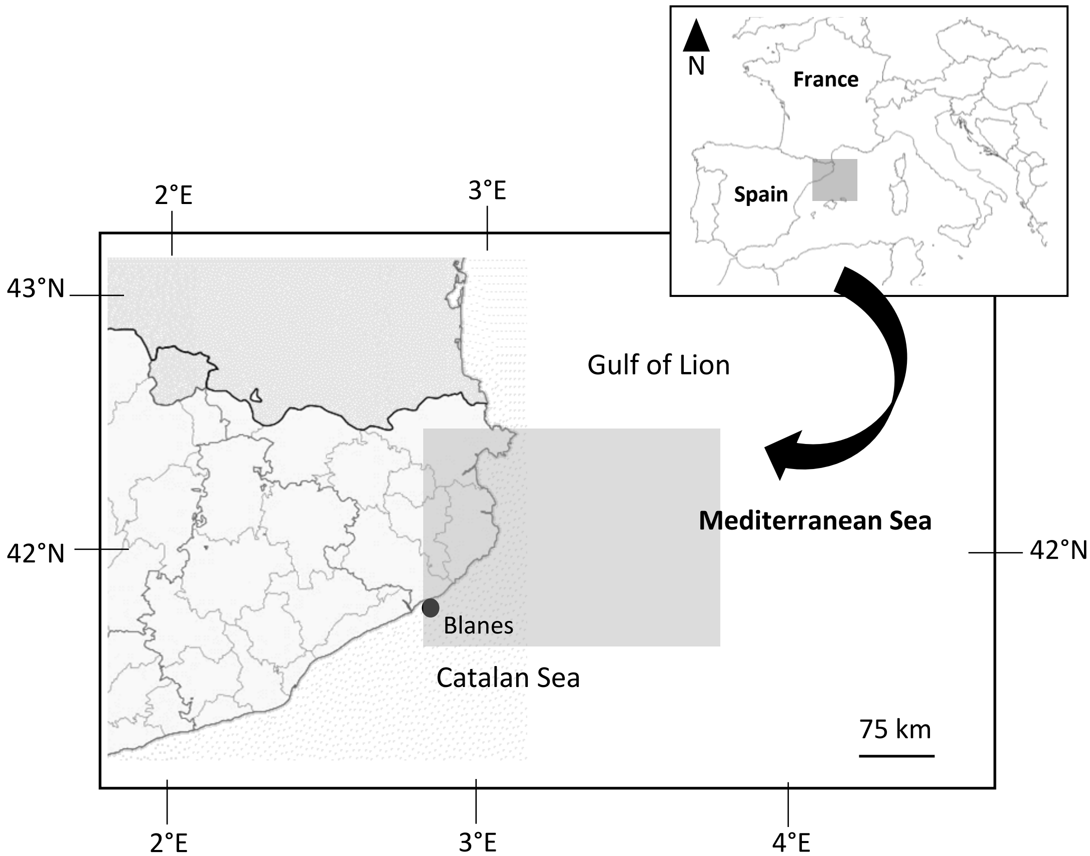

Figure 1. Map of the fishing port (Blanes) in the northern Catalan Sea (NW Mediterranean) where swordfish were sampled

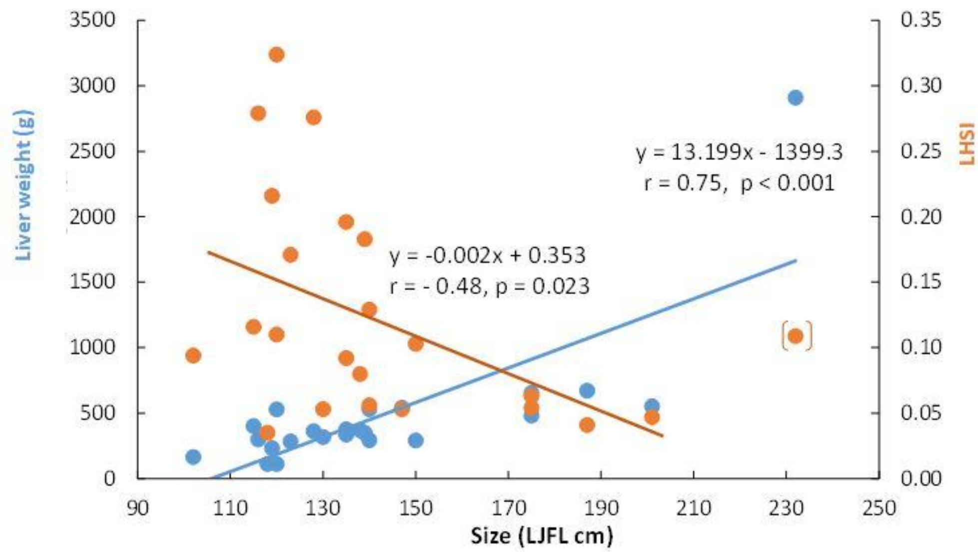

Figure 2. Linear relationships between liver weight (g) and liver hepatosomatic index (LHSI) with fish length (LJFL, cm) of swordfish in the Catalan Sea. The correlation LHSI-Fish length was calculated with the longest individual (indicated in brackets) excluded.

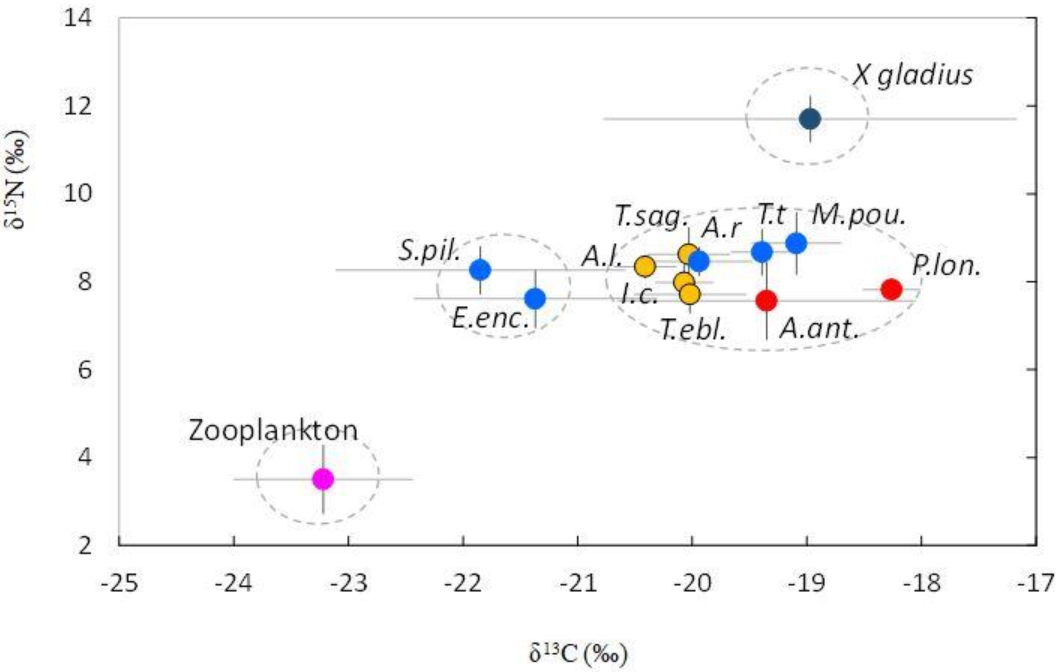

Figure 3. Biplot of mean (± SD) C and N stable isotope values of the food web components of swordfish, *Xiphias gladius*, in the Catalan Sea. Swordfish prey are marked as follows: red for crustaceans, orange for cephalopods and blue for teleosts. *A.ant.* = *Aristeus antennatus*, *A.l.* = *Ancistroteuthis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

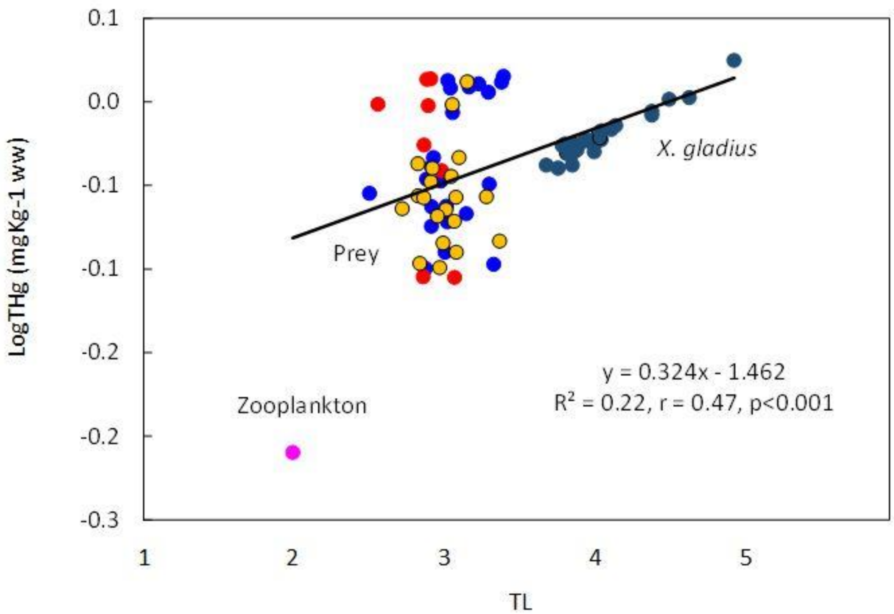

Figure 4. Biomagnification of THg in the food web of swordfish, *X. gladius*, shown by linear correlation between logTHg and the trophic level (TL) of organisms. Swordfish prey are marked as follows: red for crustaceans, orange for cephalopods and blue for teleosts.