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## The round sardinella (*Sardinella aurita* Valenciennes, 1847) from the NW Mediterranean: a healthy and safe choice for human consumption

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### Abstract

The round sardinella (*Sardinella aurita* Valenciennes, 1847) is a common pelagic species in the NW Mediterranean, with a low economic value. To evaluate its potential as a resource, a comprehensive study including the nutritional status (proximate and fatty acid composition), zoonotic parasites and heavy metals of fish captured off the coast of Barcelona (NW Mediterranean) was carried out. Thus, 200 specimens were seasonally caught aboard commercial purse seiner fishing vessels between 2022 and 2023. Present results indicate that round sardinella should be considered a lean fish given its low total lipid content, varying seasonally, being higher in spring (1.69%) and lower in summer (0.30-0.62%). Interestingly, during summer (spawning period) and autumn, a high proportion of omega-3 long-chain polyunsaturated fatty acids (46.7-53.7% of the total fatty acids) was observed whereas palmitic acid (C16:0) (45.8% of total fatty acids) was predominant in winter. These variations, probably linked to diet composition, reproductive period and breeding strategy, are discussed. Consuming a daily meal portion of 100 g of round sardinella in summer and autumn provides 206 to 290 mg of eicosapentaenoic acid (EPA)+DHA, fitting inside the recommended levels. Similarly, the atherogenic and thrombogenic indices from this period remained within the optimal range. Moreover, the absence of zoonotic parasites and low levels of heavy metals detected indicate that its consumption, especially in autumn, is both healthy and safe for human health. Promoting this fish in autumn would also enhance sustainable fishery by distributing fishing pressure across various pelagic fish species, given the current decline in these populations.

**Keywords:** Fatty acids; heavy metals; nutritional status; proximate composition; small pelagic fish; zoonotic parasites.

### Introduction

In the Mediterranean Sea, small pelagic fish species, such as sardines (*Sardina pilchardus* Walbaum, 1792) and European anchovies (*Engraulis encrasicolus* Linnaeus, 1758) represent almost 50% of total fish landings (FAO, 2023) due to their high commercial significance, thus supporting important fisheries and local economies (Palomera *et al.*, 2007). In recent years, a decline in landings as well as a worsening in body condition and growth has been reported in these fish species mainly related to environmental changes such as increasing seawater temperature, reduction of food availability and reduction of lifespan (Queiros *et al.*, 2018; Saraux *et al.*, 2019; Biton-Porsmoguer *et al.*, 2020). In this context, thermophilic fish species gained much attention due to their gradual expansion distribution in colder areas of the Mediterrane-

an, as is the case of the round sardinella (*Sardinella aurita* Valenciennes, 1847) (Sabatés *et al.*, 2006; Tsikliras, 2008). The round sardinella is an important commercial fish species in North African Mediterranean countries but, in contrast, is of very low commercial interest in the rest of the Mediterranean Sea, together with other pelagic fish species such as the horse mackerel (*Trachurus trachurus* Linnaeus, 1758), the Atlantic chub mackerel (*Scombercolias* Gmelin, 1789) and the European sprat (*Sprattus sprattus* Linnaeus, 1758), that are poorly appreciated or scarcely known among consumers (Palomera *et al.*, 2007; ICATMAR, 2020). Although an increase in total landings of round sardinella has been observed in the last eight years (FAO, 2023), it has mostly been used as bait or in fishmeal production (Tsikliras & Antonopoulou, 2006).

The study of lipid content and fatty acid (FA) composition in sardines and European anchovies in the NW

Mediterranean has become crucial to understand marine trophodynamics and evaluate the condition and viability of these small pelagic fish. Findings revealed that the level of total lipids and FA composition are subjected to temporal, seasonal and spatial variations (Rossi *et al.*, 2006; Palomera *et al.*, 2007; Pethybridge *et al.*, 2014; Bilton-Porsmoguer *et al.*, 2020). In the case of *S. aurita*, due to its low economic value, only a few studies focusing on this topic have been carried out to date, most of them focused on a specific period. Among these, Ben Rebah *et al.* (2010) documented annual changes in total lipid content in specimens from off the Tunisian coast and FA composition in individuals collected in February, while Abouel-Yazeed (2013) provided similar data for specimens collected in March off the coast of Egypt. On the Catalan coast (NW Mediterranean Sea), Vila-Belmonte *et al.* (2024) recently reported differences in the FA composition according to the specimen's maturity stage (from May to September). However, no studies regarding the seasonal variation of the FA composition of this fish species have been undertaken to date.

When considering fish as a food product, the nutritional value of fillet composition is a key quality factor. Omega-3 fatty acids (n-3), and specifically long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high proportions in the tissue of marine fish and certain shellfish (Strobel *et al.*, 2012; Amoussou *et al.*, 2022). These n-3 LCPUFA are essential for human beings since they play a key role in human growth and development and are not synthesised sufficiently by the human body (Wiktorowska-Owczarek *et al.*, 2015). Due to their many beneficial properties such as their anti-thrombogenic and anti-atherogenic effect, among others (Calder, 2015; Zárate *et al.*, 2017; Tacon *et al.*, 2020), the European Food Safety Authority (EFSA) established a daily dietary recommendation for EPA and DHA consumption (EFSA, 2012). Moreover, apart from n-3 LCPUFA, it is also important to consider the proportion of other FA to evaluate the nutritional value of these dietary lipids due to their role in human metabolism and their association with the prevention of coronary heart disease. Thus, two nutritional quality indices were proposed, an atherogenic index (AI) and a thrombogenic index (TI) (Ulbricht & Southgate, 1991; Simopoulos, 2002).

Besides their nutritional properties, the potential health risks associated with wild fish consumption such as the presence of pathogens (i.e., parasites) must be considered, as well as the environmental and/or anthropogenic contaminants (i.e., heavy metals) that have been reported in these small pelagic species (Cipriani *et al.*, 2018; Herceg Romanić *et al.*, 2021). Zoonotic parasites are in the spotlight for human health since they can cause a significant food safety problem, mainly when eating infected raw or undercooked fish. One of the most well-known infections is anisakiasis, a fish-borne zoonosis caused by the accidental ingestion of larvae of *Anisakis* spp. (Mattiucci *et al.*, 2022). However, not all fish spe-

cies and not all geographical marine areas are affected in the same way by this parasite (Cipriani *et al.*, 2018). For instance, Ramdani *et al.* (2020) highlighted the absence of zoonotic parasites in round sardinellas on the Algerian coast, whereas those from Sardinian waters were infected in a low prevalence by *Anisakis pegreffii* (Piras *et al.*, 2014), and those from the Adriatic Sea were only infected by *Hysterothylacium* spp. (Goffredo *et al.*, 2019), another nematode genus that, although it has less or negligible zoonotic potential, can occasionally cause allergic reactions (Roca-Geronès *et al.*, 2020). Moreover, other non-zoonotic parasites can also have a significant impact on human consumption since they can modify the quality of the muscle, by altering its aspect and organoleptic properties, such as microparasites (i.e., myxozoans and microsporidians) (Golomazou *et al.*, 2006) and macroparasites (i.e., cestode larvae of the order Trypanorhyncha) (Santoro *et al.*, 2021). Consequently, the visibility of these parasites and/or the damage they can cause to the edible parts of fish significantly impacts consumer perception, leading to rejection and a reduction in the product's marketability. On the other hand, heavy metals are particularly significant for human safety, since there is strong evidence that certain metals can be accumulated in fish tissue and therefore affect human health (Castro-González & Méndez-Armenta, 2008). In particular, lead, cadmium, mercury, arsenic and nickel are considered a risk for food safety and therefore international regulations (European Commission Regulation (EC) No 2023/915) or recommendations that establish safe consumption thresholds for the intake of marine organisms (EFSA, 2014; EFSA, 2020) have been formulated. Other trace metals (i.e., copper and zinc), which are essential for correct function in living organisms (EFSA, 2017), can also cause harmful effects when accumulated in excess amounts (Bosch *et al.*, 2016), therefore, regulatory limits have also been set (Nauen, 1983; BOE, 1991) in this case. To date, high heavy metal levels in the muscles of round sardinella have not yet been reported in the studied area.

Hence, considering the fact that *S. aurita* is currently among the most abundant catches in purse seiner fishing, following sardines and European anchovies on the Catalan coast (ICATMAR, 2020), and that the port of Barcelona has the largest fleet of purse seine vessels, accounting for the highest volume of pelagic fish catches in this area (2699.99 tonnes in 2022) (ICATMAR, 2023), this study aims to assess its seasonal nutritional status (proximate and fatty acid composition) in order to understand the condition of this potential pelagic fish resource, which is crucial in terms of assessing its commercial viability alongside its nutritional value for human consumption. Moreover, due to the lack of available information on the presence of zoonotic pathogens and heavy metals in this species in the Catalan coast, particularly in the highly populated and industrially active area off the coast of Barcelona, a special focus on the assessment of these two hazards is also included in this study.

## Materials and Methods

### Study area and sample collection

A total of 200 *S. aurita* specimens were collected on the continental shelf of the Catalan coast off Barcelona (NW Mediterranean Sea) (Fig. 1). Fish were seasonally caught aboard commercial purse seiner fishing vessels in summer (July) and autumn (November) 2022 and winter (March), spring (May) and summer (July) 2023. In each season, 20 specimens were immediately fixed *in toto* in 10% buffered formalin for parasitological analysis and 20 additional individuals were weighted, eviscerated and stored in ice before further preservation in laboratory conditions. Moreover, ten specimens from winter (February) 2022 were also collected and frozen at -20 °C for subsequent metal analyses. Selected individuals were of similar size whenever possible to enable data comparison and were representative of the average size of fish caught in each season.

### Analysis of fillet proximate composition and fatty acids

Once in the laboratory, standard length (SL), eviscerated weight (EW) and fillet weight (from both the right and left sides) was recorded in each individual. Fillets were then frozen and stored at -20 °C until lyophilisation (for 72 hours). After lyophilisation, dried fillets were weighed again for moisture determination. For each season, skinless fillets were grouped into five groups of four individuals each and were homogenised with a grinder. Analytical determinations of chemical composition were performed according to AOAC (Association of Official Agricultural Chemists) International methods. Crude protein (method 954.01) was determined according to the Kjeldahl method using a Kjeltec™ 8400 Analyzer

Unit (Foss España S.A.) after acid digestion. Total lipid was determined as ether extract through Soxhlet analysis (920.39) with a SoxTec™ 2055 auto-extraction unit after hydrolysis with a SoxCap™ 2047. Fatty acid composition was determined by direct transesterification following the methodology used by Carrapiso *et al.* (2000). Nonadecanoic acid (C19:0; Sigma-Aldrich Chemical Co.; St. Louis, MO, USA) was added as an internal standard. The methyl esters obtained were injected into a gas chromatographer coupled with a flame ionization detector (HP6890, Agilent Technologies). Chromatographic fatty acid peaks were identified using standard samples (Supelco 37 Component FAME Mix, Sigma Aldrich). The content of each fatty acid was expressed as the percentage of the total fatty acid content.

To estimate the nutritional quality of the lipids, atherogenic (AI) and thrombogenic (TI) indices were calculated according to Ulbricht & Southgate (1991) using the following formulas. AI and TI values lower than 1 were considered beneficial to human health.

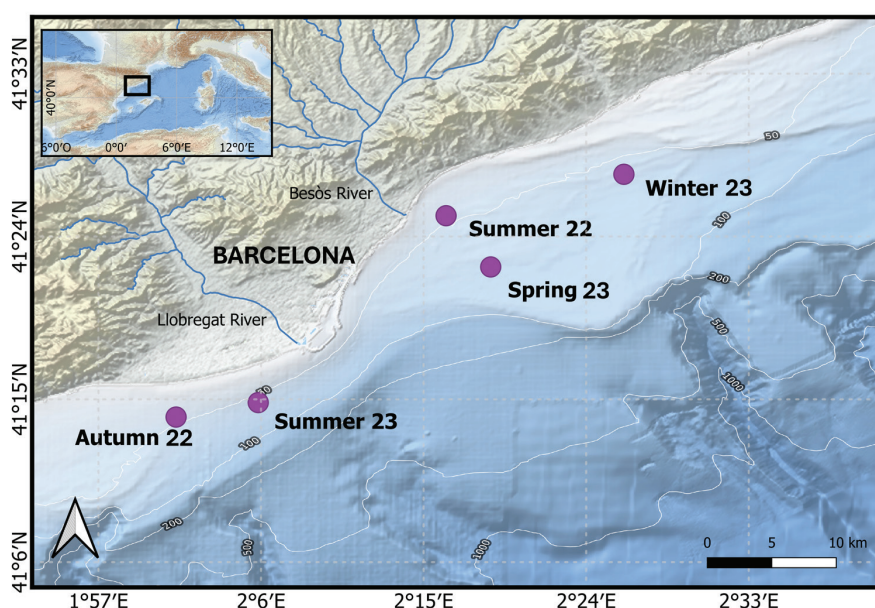
$$AI = \frac{[C12:0 + (4 * C14:0) + C16:0]}{(\sum MUFA + \sum n-6 + \sum n-3)}$$

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{\left[ (0.5 * \sum MUFA) + \left( 0.5 * \sum n-6 + (3 * \sum n-3) + \left( \frac{\sum n-3}{\sum n-6} \right) \right) \right]}$$

Moreover, the nutritional intake contribution of EPA+DHA from round sardinella consumption was estimated according to Costa *et al.* (2013) using the following formula:

$$\text{Nutritional contribution (\%)} = [(C \times M) / (DRI)] \times 100$$

where C is the mean concentration of EPA+DHA (mg kg<sup>-1</sup>), M is the meal portion consumed (kg) and DRI is the dietary reference intake value (mg) established by the Europe-



**Fig. 1:** Map of the study area. Purple circles show the location of the different seasonal sampling points on the central Catalan coast (NW Mediterranean).



an Food Safety Authority (EFSA, 2012). The DRI of EPA and DHA for preventing cardiovascular diseases ranges between 250 and 500 mg/day. Hence, in the present study the minimum DRI of 250 mg was used for the calculations. Moreover, taking into account the fact that the annual consumption of fish and seafood in Spain was around 42.98 kg per capita in 2023, representing 117g daily per capita (European Commission, 2023), and that the mean fillet weight of one round sardinella specimen is approximately 25 g, a meal portion of four individuals representing 100 g/day of fillet was used for the calculation.

### **Parasitological examination**

Standard length (SL) and total weight (TW) were recorded for each fish specimen fixed in buffered formalin. Fish were first externally examined for the presence of ectoparasites. Then they were dissected, and their organs excised (stomach, pyloric caeca, intestine, liver, gonads, spleen, heart, kidney, brain, gills and fillet muscle) then examined under a stereomicroscope for the presence of endoparasites. All parasites found were collected, counted, stored in 70% ethanol, and identified to the lowest taxonomic level using standard and specific parasitological techniques (Gibson *et al.*, 2002).

### **Analysis of heavy metals**

Considering the fact that the reported heavy metals in *S. aurita* fillets from the Mediterranean Sea were below the limit values established by the European Community (Commission Regulation No 2023/915) for safe consumption, and that no seasonal changes were detected in terms of heavy metals levels in previous studies (Lo Turco *et al.*, 2013; Copat *et al.*, 2015; Monier *et al.*, 2023), a single batch of ten frozen fish from winter 2022 was used for these analyses. For each fish, the SL was recorded, the skin was removed, and the fillet samples (~5 g of mean weight) were lyophilised at 60 °C for 72 hours. Samples were weighed prior to and after lyophilization to determine their moisture content. Briefly, 300 mg of homogenised samples were subjected to an acid digestion using concentrated HNO<sub>3</sub> (PlasmaPURE, SCP Sciences) on Teflon reactors in a microwave digestion system (MARS6, CEM) according to Besada *et al.* (2014). Given the fact that some metals were near the limit of quantification (LOQ), the analyses were repeated using a hotplate digestion method as described in Sánchez-Marín *et al.* (2023). The results obtained from both digestion methods were very similar, so results are provided as the average of both methodological replicates where possible.

Concentrations of cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), and arsenic (As) was determined by inductively coupled plasma-mass spectrometry (Agilent 8900 ICP-MS) as outlined by Sánchez-Marín *et al.* (2023). Total mercury (Hg) concentration was determined in the solid samples by pyrolysis atomic absorption spectrometry with gold amalgamation (employ-

ing an AMA254 Advanced Mercury Analyser (LECO Instruments)), as described in Belmonte *et al.* (2021). This method determines total Hg but not methylmercury (MeHg) content. However, considering that MeHg represents 70 to 100% of Hg in teleosts, total Hg is a good indicator of MeHg (Cresson *et al.*, 2014). Certified reference materials (ERM®-BB422 Fish Muscle, Joint Research Centre; DORM-2 from the National Research Council of Canada) and laboratory blanks were included. Quality assurance was also endorsed through participation in QUASIMEME (Quality Assurance of Information in Marine Environmental Monitoring in Europe) with satisfactory Z-scores for each metal reported. No corrections were applied as the percentage recovery from certified reference materials was acceptable (ranging between 88 and 114%).

Limits of detection (LOD) were 0.003, 0.0005, 0.005, 0.0003, 0.0003, 0.0008 and 0.0008 µg/g dry weight (µg/g dw) for Ni, Cu, Zn, As, Cd, Pb and Hg, respectively. LOQ were 0.011, 0.0018, 0.018, 0.0011, 0.0009, 0.0028 and 0.0026 µg/g dw for Ni, Cu, Zn, As, Cd, Pb and Hg, respectively.

To calculate the metal intake contribution of a meal portion of 100 g/day of fillet of round sardinella in an adult person (70 kg of assumed weight), Costa *et al.* (2013) the following formula was applied:

$$\text{Contribution (\%)} = [(C \times M) / (BW \times \text{PTDI, PTWI or PTMI})] \times 100$$

where C is the mean concentration of heavy metal (mg kg<sup>-1</sup>) in the meal, M is the meal portion consumed (kg), BW is the body weight (kg), PTDI, PTWI or PTMI is the provisional tolerable daily, weekly or monthly intake (mg kg<sup>-1</sup>), respectively, established by the Expert Committee on Food Additives (JECFA) of the FAO/WHO (2010a,b) and by the European Food Safety Authority (EFSA, 2020).

### **Data analysis**

All variables used for analyses were tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene test. Parasite prevalence (P) and mean intensity (MI) for each season were calculated according to Bush *et al.* (1997) for each parasite taxon. Parasite taxa with a prevalence >15 % in at least one season were considered common and therefore were used for the following statistical calculations.

Fish size effect was first tested for P and MI of common parasites and for fillet moisture, crude protein and total lipid content and fatty acid composition, using General Linear Models (GLM) (logistic model for P and negative binomial model for the rest of the variables) for parametric data or Generalised Linear Models (GZM) for non-parametric data.

To test differences between seasons for fish biometric data (SL and EW) and for all the variables mentioned above, GLM or GZM were applied using fish size as a covariable. When no interactions with fish size were de-

tected, ANOVA and Tukey's HSD post hoc tests were used for parametric data or Kruskal-Wallis and Wilcoxon post-hoc pairwise comparisons were carried out for non-parametric data.

Data analysis was performed using RStudio software, version 2023.09.1+494. Statistical significance was set at 0.05.

## Results

### Proximate and fatty acid fillet composition

The size and weight (EW) of round sardinella used in this study as well as the proximal and fatty acid composition of its fillets are present in Table 1. Fish used in this study ranged from 13.39 to 15.59 cm of SL and from

**Table 1.** Number (n) and mean with the standard deviation (SD) of the standard length (SL), eviscerated weight (EW), edible fillet weight (FW), fillet yield (FY in %) in relation to EW, moisture crude protein, and total lipid content (in % wet weight), fatty acid profiles (% of total fatty acids) of the muscle of specimens of *Sardinella aurita* sampled in each season in the central Catalan coast (NW Mediterranean) are given. Mean with the standard deviation for the ratios calculated between omega-6 fatty acids (n-6) and omega-3 fatty acids (n-3), between C16:1 (PO) and C16:0 (P) as well as for the atherogenic index (AI), thrombogenic index (TI) for each season are provided. The nutritional intake contribution (%) of EPA+DHA (NC) for the consumption of *S. aurita* for each season was also calculated. Different superscript letters shows significant differences ( $p < 0.05$ ) among seasons. Dashes indicate absence of the fatty acid.

	SUMMER 22	AUTUMN 22	WINTER 23	SPRING 23	SUMMER 23
n	20	20	20	20	20
SL (cm)	15.59 <sup>a</sup> (0.69)	13.39 <sup>b</sup> (0.65)	15.07 <sup>a</sup> (0.51)	15.35 <sup>a</sup> (2.63)	15.51 <sup>a</sup> (0.69)
EW (g)	42.44 <sup>a</sup> (5.37)	26.16 <sup>b</sup> (4.53)	38.59 <sup>a</sup> (4.60)	39.52 <sup>a</sup> (11.67)	39.36 <sup>a</sup> (5.04)
FW (g)	20.15 <sup>a</sup> (3.01)	15.94 <sup>b</sup> (3.02)	22.60 <sup>a</sup> (2.85)	22.45 <sup>a</sup> (7.11)	21.15 <sup>a</sup> (3.32)
FY (%)	47.47	60.93	58.56	56.80	53.73
Moisture (%)	76.84 <sup>a</sup> (0.61)	76.56 <sup>a</sup> (0.65)	76.56 <sup>a</sup> (0.65)	73.35 <sup>b</sup> (0.98)	76.59 <sup>a</sup> (0.87)
Crude protein (%)	19.69 <sup>a</sup> (0.75)	20.30 <sup>a</sup> (0.82)	16.70 <sup>b</sup> (0.45)	18.78 <sup>a</sup> (0.91)	20.32 <sup>a</sup> (0.40)
Total lipid (%)	0.30 <sup>d</sup> (0.06)	0.67 <sup>bc</sup> (0.22)	0.92 <sup>b</sup> (0.04)	1.69 <sup>a</sup> (0.36)	0.62 <sup>c</sup> (0.02)
C13:0	–	–	–	0.29 (0.08)	–
C14:0	2.16 <sup>b</sup> (0.59)	2.20 <sup>b</sup> (0.68)	9.32 <sup>a</sup> (0.53)	8.99 <sup>a</sup> (2.04)	1.81 <sup>b</sup> (0.36)
C15:0	0.86 (0.11)	0.89 (0.13)	1.77 (0.18)	1.88 (0.56)	0.64 (0.04)
C16:0 (P)	26.81 <sup>c</sup> (1.71)	22.64 <sup>c</sup> (0.80)	45.82 <sup>a</sup> (0.34)	35.69 <sup>b</sup> (7.17)	22.21 <sup>c</sup> (1.00)
C16:1 (PO)	1.25 <sup>c</sup> (0.34)	1.31 <sup>c</sup> (0.39)	4.93 <sup>b</sup> (0.40)	6.56 <sup>a</sup> (0.60)	1.12 <sup>c</sup> (0.21)
C17:0	1.60 <sup>bc</sup> (0.14)	1.43 <sup>c</sup> (0.09)	2.24 <sup>a</sup> (0.23)	1.89 <sup>ab</sup> (0.48)	1.23 <sup>c</sup> (0.05)
C18:0	10.03 <sup>b</sup> (0.35)	7.35 <sup>c</sup> (0.18)	12.04 <sup>a</sup> (0.81)	8.79 <sup>bc</sup> (1.91)	8.66 <sup>bc</sup> (0.36)
C18:1n9c	4.65 <sup>b</sup> (0.40)	4.36 <sup>b</sup> (0.39)	7.03 <sup>a</sup> (0.22)	7.24 <sup>a</sup> (1.23)	4.05 <sup>b</sup> (0.30)
C18:1n7	2.42 <sup>b</sup> (0.26)	2.09 <sup>b</sup> (0.20)	3.87 <sup>a</sup> (0.19)	4.43 <sup>a</sup> (0.37)	2.43 <sup>b</sup> (0.17)
C18:2n6c	1.58 <sup>ab</sup> (0.12)	1.93 <sup>a</sup> (0.35)	0.48 <sup>c</sup> (0.08)	1.23 <sup>b</sup> (0.62)	1.75 <sup>ab</sup> (0.14)
C18:3n3	–	0.72 (0.10)	–	0.85 (0.28)	0.54 (0.08)
C20:0	–	0.37 <sup>b</sup> (0.01)	0.76 <sup>a</sup> (0.15)	0.47 <sup>b</sup> (0.14)	–
C20:1n9	–	0.39 <sup>c</sup> (0.05)	4.65 <sup>a</sup> (0.71)	2.07 <sup>b</sup> (0.45)	0.52 <sup>c</sup> (0.02)
C20:2	–	0.39 (0.02)	–	0.39 (0.03)	–
C20:4n6	1.86 <sup>a</sup> (0.19)	1.93 <sup>a</sup> (0.08)	–	0.93 <sup>b</sup> (0.26)	2.14 <sup>a</sup> (0.13)
C20:5n3 (EPA)	3.89 <sup>a</sup> (0.28)	6.12 <sup>a</sup> (0.32)	0.99 <sup>b</sup> (0.13)	4.28 <sup>a</sup> (3.35)	4.89 <sup>a</sup> (0.41)
C22:6n3 (DHA)	42.88 <sup>a</sup> (3.17)	46.41 <sup>a</sup> (3.18)	4.69 <sup>c</sup> (0.69)	14.09 <sup>b</sup> (9.14)	48.26 <sup>a</sup> (2.27)
C24:1n9	–	–	1.42 <sup>a</sup> (0.10)	0.98 <sup>b</sup> (0.24)	–
Total SFA	40.61 <sup>c</sup> (2.79)	34.00 <sup>c</sup> (1.75)	70.18 <sup>a</sup> (2.06)	55.84 <sup>b</sup> (11.74)	33.92 <sup>c</sup> (1.78)
Total MUFA	5.90 <sup>b</sup> (0.74)	6.06 <sup>b</sup> (0.84)	18.03 <sup>a</sup> (1.43)	16.85 <sup>a</sup> (2.53)	5.69 <sup>b</sup> (0.53)
Total n-3 PUFA	46.77 <sup>a</sup> (3.46)	53.25 <sup>a</sup> (3.59)	5.67 <sup>c</sup> (0.82)	19.23 <sup>b</sup> (12.78)	53.69 <sup>a</sup> (2.76)
Total n-6 PUFA	3.44 <sup>a</sup> (0.32)	3.86 <sup>a</sup> (0.43)	0.48 <sup>c</sup> (0.08)	2.16 <sup>b</sup> (0.87)	3.89 <sup>a</sup> (0.28)
Total PUFA	50.21 <sup>a</sup> (3.77)	57.11 <sup>a</sup> (4.02)	6.15 <sup>c</sup> (0.90)	21.39 <sup>b</sup> (13.65)	57.58 <sup>a</sup> (3.04)
EPA/DHA	0.09 <sup>b</sup> (0.004)	0.13 <sup>b</sup> (0.015)	0.21 <sup>a</sup> (0.05)	0.30 <sup>a</sup> (0.09)	0.10 <sup>b</sup> (0.01)
n-6/n-3	0.07 <sup>b</sup> (0.002)	0.07 <sup>b</sup> (0.007)	0.08 <sup>ab</sup> (0.009)	0.11 <sup>a</sup> (0.04)	0.07 <sup>b</sup> (0.004)
PO/P	0.05 <sup>c</sup> (0.01)	0.06 <sup>c</sup> (0.017)	0.11 <sup>b</sup> (0.01)	0.18 <sup>a</sup> (0.04)	0.05 <sup>c</sup> (0.008)
AI	0.63 <sup>c</sup>	0.50 <sup>c</sup>	3.44 <sup>a</sup>	1.87 <sup>b</sup>	0.47 <sup>c</sup>
TI	0.70 <sup>b</sup>	0.57 <sup>b</sup>	1.00 <sup>a</sup>	1.34 <sup>a</sup>	0.58 <sup>b</sup>
NC (%)	28.26	116.08	14.20	199.11	82.21

26.16 to 42.44 g of EW, being those from autumn significantly smaller and lighter than those from the other seasons (ANOVA,  $F_{(19,4)} = 8.32, p < 0.001$  and  $F_{(20,4)} = 5.23, p = 0.004$ , respectively) (Table 1). In contrast, fish fillet yield (the proportion of the edible fillet weight with respect to the EW) was highest (60.97%) in autumn (Table 1). No fish size interaction was observed in terms of crude protein and total lipid content (% wet weight) in *S. aurita* fillets. Significant differences were observed regarding proximal composition between seasons but were not linked in any case to fish size. Crude protein ranged from 16.70 to 20.32% of wet weight, with winter-23 being the season in which individuals displayed significantly lower values compared to other seasons (K-W,  $\chi^2 = 17.34, p = 0.001$ ). Moisture (ranging from 73.35 to 76.84%) was found to be inversely proportionate to the total lipid content, with spring-23 being the season with significantly lower values of moisture (K-W,  $\chi^2 = 13.37, p = 0.009$ ) and higher values of lipid content compared to the other seasons (ANOVA,  $F_{(20,4)} = 46.66, p < 0.001$ ). In general, total lipid content in all seasons was below 2% (between 0.30 and 1.69%). Differences were also found between years in the same season, such as the fact that significantly lower levels of lipid were found in summer-22 compared to summer-23 (Wilcoxon,  $W = 13, p < 0.001$ ) (Table 1).

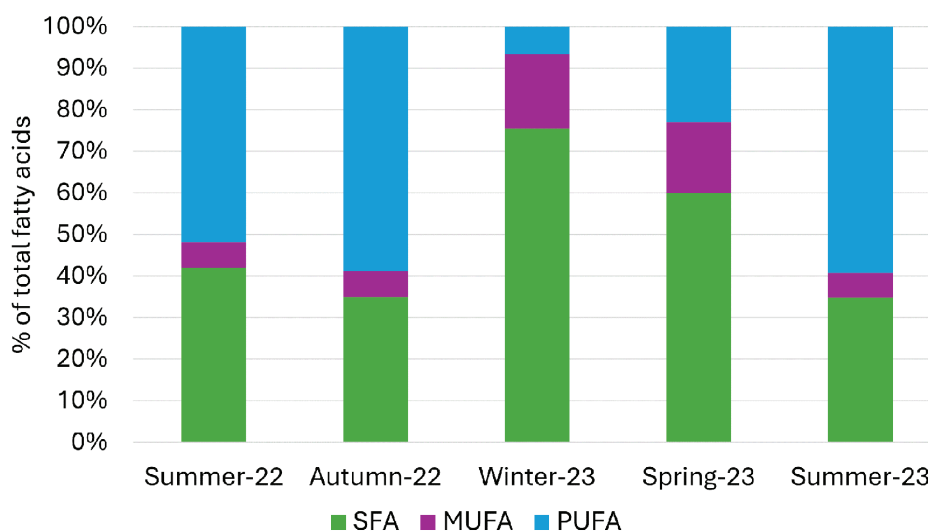
In relation to fatty acid composition in round sardinella fillets, significant differences were observed in different seasons, but no fish size effect was observed (Table 1). Winter-23 and spring-23 were the seasons with the highest proportions of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). On the other hand, summer-22, autumn-22 and summer-23 were the seasons with the highest concentration of polyunsaturated fatty acids (PUFA) (Fig. 2).

The percentage of SFA ranged between 33.92% and 70.18% of total fatty acids. The highest content (70.18%) was observed in winter-23 (K-W,  $\chi^2 = 21.60, p < 0.001$ ), followed by spring (55.84%). In both seasons, C16:0

(palmitic acid) was the most abundant (K-W,  $\chi^2 = 21.45, p < 0.001$ ), representing 63.91% and 66.58% of the total SFA, respectively (Table 1, Fig. 2). Higher proportions of both C18:0 (stearic acid) and C14:0 (miristic acid) were also obtained in winter-23 (ANOVA,  $F_{(20,4)} = 15.65, p < 0.001$ ; K-W,  $\chi^2 = 17.71, p = 0.001$ , respectively), as well as in spring-23 for C14:0 (K-W,  $\chi^2 = 17.71, p = 0.001$ ).

As for SFA, significant differences were obtained in relation to MUFA content, which ranged between 5.69% and 18.03% of the total fatty acids. Winter-23 and spring-23 were the seasons with a significantly higher percentage of MUFA (K-W,  $\chi^2 = 21.54, p < 0.001$ ) (Table 1, Fig. 2) and C18:1n9c (oleic acid) (K-W,  $\chi^2 = 19.13, p < 0.001$ ), the most abundant MUFA in each season (between 38.99 and 78.81% of total MUFA). Spring-23 presented a higher proportion of C16:1 (palmitoleic acid) (38.93% of total MUFA) (K-W,  $\chi^2 = 18.58, p < 0.001$ ) but a lower content of C20:1n9 (eicosanoic acid) than in winter-23 (ANOVA,  $F_{(11,3)} = 49.55, p < 0.001$ ). The PO/P ratio (C16:1/C16:0) displayed low values in all seasons but was significantly higher in spring-23 (K-W,  $\chi^2 = 19.28, p < 0.001$ ) (Table 1).

In the case of PUFA content, significant variations were also found between seasons. The percentage of PUFA ranged between 6.15% to 57.58% of total fatty acids. The most abundant PUFA were n-3 PUFA, specifically C22:6n3 (DHA) representing between 65.87 and 85.4% of the total PUFA (Table 1), which is reflected in the n-6/n-3 ratio. Total PUFA and DHA were significantly higher in summer-22, summer-23 and autumn-22 compared to winter-23 (K-W,  $\chi^2 = 14.91, p = 0.001$  and  $\chi^2 = 13.53, p = 0.003$ , respectively) and spring-23 (K-W,  $\chi^2 = 14.91, p = 0.002$  and  $\chi^2 = 13.53, p = 0.003$ , respectively). The EPA content was lower but followed a similar pattern to DHA, being significantly higher during summer-22, summer-23 and autumn-22 and also in spring (K-W,  $\chi^2 = 13.57, p = 0.008$ ) (Table 1, Fig. 2). Regarding the EPA/DHA ratio, higher values was obtained for winter-23 and spring-23 (K-W,  $\chi^2 = 21.12, p < 0.001$ ), due to a notice-



**Fig. 2:** Seasonal variation in the percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids with respect to the total fatty acid composition in the flesh of *Sardinella aurita* captured in the central Catalan coast (NW Mediterranean).

able decrease of DHA in this period. Other PUFA such as C18:2n6, C18:3n3 and C20:4n6 were also present in almost all seasons but in a low proportion, representing less than 10% of the total PUFA.

The lowest AI and TI values were obtained for summer-22, summer-23 and autumn-22 (K-W,  $\chi^2 = 24.00$ ,  $p < 0.001$  and  $\chi^2 = 24.00$ ,  $p < 0.001$ , respectively), ranging from 0.47 to 0.70; whereas in winter-23 and spring-23 these ranged from 1.00 to 3.44. The estimated nutritional intake contribution of EPA+DHA, assuming a consumption of 100 g of round sardinella per day, ranged from 14.20 to 199.11%, being higher in spring 23 and autumn 22 (Table 1).

### Parasitological results

Fish size ranged from 12.66 to 17.22 cm of SL, with autumn fish being significantly smaller than those from other seasons (K-W,  $\chi^2 = 53.76$ ,  $p < 0.001$ ). All specimens examined in all seasons were infected by at least one par-

asite. A total of 11468 parasites belonging to six different taxa were identified, and all of them were located in the digestive tract, but the copepod was located in gills (Table 2). No zoonotic parasites were detected in any organ or fillet in any individual in each season. Among the recovered taxa, digeneans were the most prevalent group, reaching 100% of prevalence, and the main contributors to MI values in each season. Among them, *Aphanurus* sp. was the most prevalent and presented the highest MI values followed by *Bacciger bacciger*, *Parahemiurus* sp., and *Lecithaster* sp.

Larger fish presented higher MI values of total digeneans (GLM,  $F_{(98,1)} = 28.70$ ,  $p < 0.001$ ), specifically *Aphanurus* sp. (GZM,  $z = 5.09$ ,  $p < 0.001$ ), *Parahemiurus* sp. (GLM,  $F_{(98,1)} = 42.91$ ,  $p < 0.001$ ) and *Lecithaster* sp. (GZM,  $z = 2.15$ ,  $p = 0.03$ ). In terms of P, only *Lecithaster* sp. was found to present significantly higher values in larger fish (GZM,  $z = 2.53$ ,  $p = 0.011$ ). The cestode larvae of the order Tetraphyllidea was also more prevalent (GZM,  $z = 2.57$ ,  $p = 0.01$ ) and displayed higher MI values (GZM,  $z = 2.71$ ,  $p = 0.006$ ) in larger fish (Table 2).

**Table 2.** Number (n) and mean followed by the standard deviation (SD) of the standard length (SL) of the specimens of *Sardinella aurita* sampled in each season on the central Catalan coast (NW Mediterranean). Prevalence (%) and mean intensity (MI) values, followed by the range, of the parasites found are shown. Keys for locations within host: G, gills; I, intestine; S, stomach; PC, pyloric caeca. Different superscript letters and numbers show significant differences ( $p < 0.05$ ) among seasons in the prevalence and mean intensity of parasites, respectively. Dashes indicate absence of the parasite.

		SUMMER 22		AUTUMN 22		WINTER 23		SPRING 23		SUMMER 23	
n		20		20		20		20		20	
SL		15.59 <sup>a</sup> (0.69)		12.66 <sup>b</sup> (0.48)		15.42 <sup>a</sup> (0.59)		16.13 <sup>a</sup> (1.33)		17.22 <sup>a</sup> (2.10)	
PARASITE TAXA	Location	P%	MI (min–max)	P%	MI (min–max)	P%	MI (min–max)	P%	MI (min–max)	P%	MI (min–max)
<b>MICROSPORIDIA</b>	PC, I	–	–	15.00	2.33 (1–4)	–	–	–	–	5.00	3.00 (3–3)
<b>PLATYHELMINTHES</b>											
<b>Digenea</b>											
<i>Parahemiurus</i> sp.	S	95.00	19.95 <sup>a</sup> (6–53)	90.00	7.44 <sup>b</sup> (1–22)	75.00	2.87 <sup>c</sup> (1–13)	85.00	4.76 <sup>bc</sup> (1–9)	100.00	31.25 <sup>a</sup> (4–70)
<i>Aphanurus</i> sp.	S, I	100.00	143.15 <sup>a</sup> (37–325)	100.00	22.00 <sup>c</sup> (5–53)	100.00	16.60 <sup>c</sup> (3–29)	100.00	70.55 <sup>b</sup> (31–170)	100.00	135.95 <sup>ab</sup> (9–420)
<i>Lecithaster</i> sp.	PC, I	85.00 <sup>1</sup>	11.00 <sup>a</sup> (1–36)	25.00 <sup>2</sup>	3.00 <sup>b</sup> (1–8)	60.00 <sup>12</sup>	2.58 <sup>b</sup> (1–7)	30.00 <sup>2</sup>	3.33 <sup>b</sup> (1–9)	75.00 <sup>1</sup>	12.53 <sup>a</sup> (1–31)
<i>Bacciger bacciger</i>	PC	95.00	12.74 <sup>a</sup> (1–46)	85.00	20.88 <sup>a</sup> (1–97)	80.00	7.63 <sup>b</sup> (3–15)	100.00	7.40 <sup>b</sup> (3–19)	95.00	37.68 <sup>a</sup> (1–361)
<b>Cestoda</b>											
Tetraphyllidea larvae sp.	PC, I	70.00 <sup>2</sup>	11.57 <sup>a</sup> (1–42)	35.00 <sup>3</sup>	2.14 <sup>b</sup> (1–4)	70.00 <sup>2</sup>	3.36 <sup>b</sup> (1–8)	90.00 <sup>1</sup>	4.61 <sup>b</sup> (1–22)	50.00 <sup>2</sup>	10.80 <sup>a</sup> (1–44)
<b>ARTHROPODA</b>											
<b>Copepoda</b>											
<i>Clavellisa</i> sp.	G	10.00	2.00 (2–2)	–	–	–	–	–	–	–	–



Regarding seasonality, significant higher MI values were detected in summer for all digeneans, except for *Bacciger bacciger*, which also had higher values in autumn (*Parahemiurus* sp.: GZM,  $z = 2.02$ ,  $p = 0.04$ ; *Aphanurus* sp.: GLM,  $F_{(95,4)} = 41.42$ ,  $p < 0.001$ ; *B. bacciger*: GLM,  $F_{(95,4)} = 41.42$ ,  $p < 0.001$ ; *Lecithaster* sp.: GZM,  $z = 2.81$ ,  $p = 0.004$ ). The same trend was also observed in Tetracystid cestode larvae (GZM,  $z = 3.67$ ,  $p < 0.001$ ). In no case did fish size interact with seasonal differences, except in the case of *Parahemiurus* sp. In contrast, only *Lecithaster* sp. showed significantly higher P in both summer-22 and summer-23 (GZM,  $z = 2.26$ ,  $p = 0.02$ ), and higher P of Tetracystid cestode larvae was detected in spring-23 (GZM,  $z = 3.35$ ,  $p < 0.001$ ), but these differences were associated with fish size.

In addition, microsporidian xenomas were detected in low prevalence in the intestine and pyloric caeca only in specimens from autumn-22 and summer-23 (Table 2).

### Heavy metals

Heavy metals values, maximum permissible concentration and the PTDI, PTWI or PTMI for each quantified heavy metal are shown in Table 3. In general, low values were detected in the muscle samples of analysed fish. Zn and As displayed the highest concentrations compared to the other heavy metals. Metal intake contribution by consuming a meal portion of 100 g /day of round sardinella from the present study ranged between 0.004 and 3.05 % of the estab-

lished PTDI, PTWI or PTMI for each metal, with Hg being the one with the highest contribution (Table 3).

### Discussion

This study offers new insights into the seasonal variations of proximate and fatty acid composition, zoonotic parasites, and heavy metal levels in round sardinella from the central Catalan coast (NW Mediterranean), which is valuable in terms of highlighting this species as a potential commercial fish resource in this region.

Depending on the lipid content of their flesh, fish can be classified into three categories: lean fish (<5 % fat), mid-fat fish (5-10 % fat), and fatty fish (10-25 % fat) (Taşbozan & Gökçe, 2017). Given that round sardinella is a small pelagic fish species, to the expectation was that it would be a fatty fish, as reported for example in sardines (7.79–28.51 % flesh lipidic content) in the Gulf of Lion (NW Mediterranean) (Pethybridge *et al.*, 2014). In contrast, the results obtained in this study indicate that *S. aurita* could not be considered a fatty fish, due to the low lipid levels in the fillet observed in all seasons (<2%). Hence, it should be classified as a lean fish, with a similar flesh lipidic content to that of Atlantic cod (*Gadus morhua* Linnaeus, 1758) from the Atlantic Ocean (0.8 % fat content; Zeng *et al.*, 2010) and European hake (*Merluccius merluccius* Linnaeus, 1758) from the Central Mediterranean Sea (0.90-1.38 % fat; Roncarati *et al.*, 2012). This outcome differs from the mid-fat values that Ben Rebah

**Table 3.** Number (n) and mean with the standard deviation (SD) of the standard length (SL) of the specimens of *Sardinella aurita* sampled in winter 2022. Mean and standard deviation (SD) values of heavy metals (Ni, Cu, Zn, As, Cd, Pb, Hg) and their maximum permissible concentration (MPC) are given in mg/kg wet weight. Heavy metals intake contribution (%) by consuming raw flesh of *S. aurita* is calculated, taking into account a meal of 100 g of fish fillet and a consumer with a body weight (bw) of 70 kg. PTDI – provisional tolerable daily intake (mg/kg bw); PTWI – provisional tolerable weekly intake (mg/kg bw); PTMI – provisional tolerable monthly intake (mg/kg bw). Dashes indicate absence of data.

Heavy metal	Mean (SD)	MPC	Regulatory body	PTDI	PTWI	PTMI	Contribution (%)
n	10						
SL(cm)	15.0 (0.84)						
Ni	0.009 (0.004)	–	–	0.013	–	–	0.10
Cu	1.173 (0.224)	20.0	BOE (1991)	0.5	–	–	0.34
Zn	5.261 (0.562)	40.0	FAO/WHO (1989)	0.65	–	–	1.16
As	6.141 (2.215)	–	–	–	–	–	–
Cd	0.001 (0.0001)	0.05	Commission Regulation (EC) No. 2023/915	–	–	0.025	0.004
Pb	0.042 (0.027)	0.3	Commission Regulation (EC) No. 2023/915	–	0.025	–	0.24
Hg	0.085 (0.023)	0.3	Commission Regulation (EC) No. 2023/915	–	0.004	–	3.05

*et al.* (2010) previously reported for *S. aurita* in the Gulf of Gables (Southern Central Mediterranean) more than 10 years ago (2.50–10.25 % fat content). This difference could be attributed to geographic and temporal variations mainly associated with differences in the abundance and composition of plankton communities, as was also noted in sprat from the Baltic Sea (Usydus *et al.*, 2012). In fact, inter-annual variations in oceanographic conditions (i.e., variability in primary production) were reported to influence the level of lipid reserves that fish accumulate until the beginning of their spawning period, as was noticed in the Pacific sardine (*Sardinops sagax* Jenyns, 1842) (Litz *et al.*, 2010). Thus, the herein observed lower level of lipid content from summer 2022 compared to that of summer 2023 (spawning period) could indicate a worse ecosystem condition in the previous year (from autumn 2021 to spring 2022) in terms of food supply and availability of primary production, affecting the condition of individuals. Indeed, a decrease in primary production has been related to a decline in the lipid content in sardines (1.78–5.86% fat) and European anchovies (0.89–2.46% fat) from the Catalan coast in recent years (Nunes *et al.*, 2018), resulting in a reduction in the stock size and body condition of these populations (Biton-Porsmoguer *et al.*, 2020), and consequently affecting the sustainability of fishing (ICATMAR, 2020). In fact, this decreasing trend and/or a shift in the abundance and composition of phytoplankton communities in the Mediterranean have also been pointed out in other studies, probably as a response to global warming (Fullgrabe *et al.*, 2020; Feuilloley *et al.*, 2022). According to these findings, the sardine and the European anchovy should also be considered a mid-fat and lean fish, respectively, rather than fatty fish. Moreover, the reproductive stage and breeding strategy of these fish are also significant factors linked to the seasonal variations observed herein regarding the lipid content of round sardinella fillet, as was commonly reported in other fish species (Pethybridge *et al.*, 2014; Røjbek *et al.*, 2014; Biton-Porsmoguer *et al.*, 2020). On the one hand, the highest lipid values observed in spring indicate that maximum energy resources are accumulated during the autumn-winter period before reproduction, thus supporting a capital breeding strategy (Sainmont *et al.*, 2014; Albo-Puigserver *et al.*, 2017). In contrast, the lowest values were noted in summer, coinciding with the spawning stage, when fat reserves were previously mobilised for the maturation of gonads (Lloret *et al.*, 2014). This is in line with what has recently been stated about round sardinella from the Catalan coast (Vila-Belmonte *et al.*, 2024), from the Gulf of Gables (Ben Rebah *et al.*, 2010) and from the Adriatic Sea (Mustać & Sinovčić, 2012), but also about the Indian oil sardine (*Sardinella longiceps* Valenciennes, 1847) and the fringescale sardinella (*Sardinella fimbriata* Valenciennes, 1847) from the Cochin coast (Indian Ocean) (Som & Radhakrishnan, 2013), and other pelagic fish species such as the sardine in the NW Mediterranean (Pethybridge *et al.*, 2014; Biton-Porsmoguer *et al.*, 2020).

Not only lipid content, but also the fatty acid composition of fish fillet differs considerably, depending on a

variety of factors including the species under study, diet, feeding behaviour, reproductive status, sexual maturity, seasonality and environmental conditions such as temperature (Taşbozan & Gökçe, 2017). Variations in diet are reported to contribute largely to the seasonal differences observed in the fatty acid composition in fish flesh (Pethybridge *et al.*, 2014; Biton-Porsmoguer *et al.*, 2020; Vila-Belmonte *et al.*, 2024). Focusing on this, the round sardinella displays a generalist and opportunistic feeding behaviour mainly based on zooplankton, but seasonal variations in diet composition are also reported, suggesting this is related to their strong adaptability in terms of variation in food availability (Lomiri *et al.*, 2008; Albo-Puigserver *et al.*, 2019). This notwithstanding, dietary input might not always reflect flesh FA composition when diet changes have occurred recently, as it takes a certain time for muscle tissue to reflect new FA levels (Xu *et al.*, 2020). To link the diet of round sardinella with the observed seasonal differences in FA composition, data from Albo-Puigserver *et al.* (2019) has been used, as this study described the diet of this fish species from the same area (Catalan coast) ten years ago. In winter, microplankton such as tintinnids (rich in n-3 LCPUFA; Desvillettes *et al.*, 1997) and diatoms (rich in C16:1 and EPA) and in a lower proportion copepod (i.e., *Acartia* spp. and *Euterpina acutifrons*, rich in C16:1 and n-3 LCPUFA) (Dalsgaard *et al.*, 2003; Merquirol, 2022), were reported to be prevalent in the diet of round sardinella (Albo-Puigserver *et al.*, 2019). The observed high proportion of SFA, particularly C16:0, in fish fillets from this season and the low ratios of PO/P (C16:1/C16:0) and EPA/DHA (<0.5), suggest that this fish species primarily consumed phytoplankton, rather than zooplankton, and that their diet was not predominantly based on diatoms or tintinnids (Dalsgaard *et al.*, 2003), but probably on other microplankton groups. This finding aligns with observations made by Biton-Porsmoguer *et al.* (2020) in sardines from the Catalan Sea. In fact, these authors also revealed a shift and/or reduction in the proportion of diatoms in sardine's diet compared to that of ten years ago in the Gulf of Lion (Pethybridge *et al.*, 2014). Thus, correlating the FA results herein with existing literature on fish diet might not always be accurate as changes in plankton composition and abundance within a ten-year period have occurred in this region (Nunes *et al.*, 2018). In spring, the predominant ingestion of salps (Albo-Puigserver *et al.*, 2019), which are particularly rich in SFA (C16:0 and C14:0), MUFA (C16:1 and C18:1n9) and in a lower proportion DHA (Merquirol, 2022), is in accordance with the high levels of SFA and MUFA found in fish fillets from this period. In contrast, in summer (in both 2022 and 2023) and autumn (2022), a sharp decrease in SFA and MUFA alongside an increase in PUFA was detected in round sardinella fillets. In this case, the predominance of n-3 LCPUFA, and mainly DHA found in their flesh might be directly influenced by diet, dominated by large zooplankton (0.5–0.9 mm) such as decapod larvae, amphipoda and copepoda (Albo-Puigserver *et al.*, 2019), particularly rich in n-3 LCPUFA (Merquirol, 2022). Simultaneously, a reduction in total lipid content was observed during this period, indicating the mobilisation of

these stored lipids for meeting the increased energy demands associated with reproduction. Although PUFA are also crucial for gonadal and egg development, they can be incorporated from the diet to maintain the fluidity of cell membranes and their structure (Garrido *et al.*, 2008). These results were similar to what was reported for spawning European anchovies from the same area during the same period (spring/summer) despite having different reproductive strategies (capital vs income breeding strategies) (Biton-Porsmoguer *et al.*, 2020). Therefore, although round sardinella primarily uses the energy accumulated throughout the year for reproduction, it may also depend on the energy obtained from its summer dietary input to meet the high demands of reproduction (Albo-Puigserver *et al.*, 2019).

Regarding implications for human health, global recommendations issued by WHO (WHO, 2023) and previous studies have demonstrated that the dietary replacement of SFA with unsaturated fat, including MUFA and PUFA, decreased the risk of suffering from cardiovascular diseases (Siri-Tarino *et al.*, 2015; Kris-Etherton & Krauss, 2020). Also, an excessive intake of n-6 PUFA and a very high n-6/n-3 ratio promotes the development of many chronic diseases that are highly prevalent in Western societies such as cardiovascular disease, cancer, diabetes, arthritis and inflammatory and autoimmune diseases (Simopoulos, 2002; Simopoulos, 2008; EFSA, 2012). The n-6/n-3 ratio in Western diets is considered 15/1 to 16.7/1, far above the 5/1 optimal ratio that is beneficial for human health (Simopoulos, 2008; Molvermyr *et al.*, 2022) but even lower ratios (<1) have been proven to significantly reduce blood triglyceride concentration and increase high-density lipoprotein cholesterol concentration (Li *et al.*, 2021). In the current study, the calculated ratios of n-6/n-3 in round sardinellas from each season lies within the recommended levels and are similar to those reported for other fish species of commercial interest such as wild and farmed seabass (*Dicentrarchus labrax* Linnaeus, 1758), and seabream (*Sparus aurata* Linnaeus, 1758) (Amoussou *et al.*, 2022) and wild Atlantic salmon (*Salmo salar* Linnaeus, 1758) (Molvermyr *et al.*, 2022). Hence, the consumption of round sardinella would contribute to reducing the n-6/n-3 ratio in the human diet. Likewise, the AI and TI values of round sardinella from summer 2022, 2023 and autumn 2022 were lower than 1, indicating that their consumption would be nutritionally beneficial for human health and provide a cardio-protective effect as was reported for Atlantic salmon (Molvermyr *et al.*, 2022) and for Blue Fin Tuna (*Thunnus thynnus* Linnaeus, 1758) (Garaffo *et al.*, 2011). Moreover, according to the DRI of EPA+DHA recommended by EFSA to prevent chronic disease (EFSA, 2012), the estimated nutritional contribution of 100g of sardinella per day (~4 individuals) from summer 2023 and autumn 2022 appeared to be the best (82.21-116.08% of DRI), providing an intake of 206 and 290 mg of EPA+DHA per day, respectively. Hence, round sardinella would be a good candidate for consumption during autumn, when n-3 LCPUFA levels are highest, and AI and TI values are optimal. Additionally, it would allow this fish species to

spawn undisturbed by fishing pressure during summer, which is crucial to ensure their viability. Moreover, although fish catches in the studied area mostly focus on sardines and the European anchovy (with spring being the peak catch period), a decline in the abundance of both species has been observed during autumn (ICATMAR, 2020). For this reason, focusing most catches on round sardinella during this period for human consumption would support sustainable fishing by distributing fishing pressure across different species and seasons and simultaneously enhance the seasonal diversification of these fish species in the market.

Apart from nutritional benefits, food security is a fundamental aspect to consider when dealing with fishery food resources. In this case, current results have provided evidence of the absence of zoonotic parasites in round sardinella from the central Catalan coast, supporting previous outcomes of the low prevalence of these parasites detected in geographical areas close to the Mediterranean Sea (Piras *et al.*, 2014; Goffredo *et al.*, 2019; Ramdani *et al.*, 2020). In fact, the absence or very low prevalence of *Anisakis* obtained is in line with other studies performed in the same area with other fish species such as the European anchovy (Rodríguez-Romeu *et al.*, 2022; Biton-Porsmoguer *et al.*, 2020) and the European hake (Muns-Pujadas *et al.*, 2024). The differences observed between seasons for digeneans and Tetracyllidean cestode larvae could be related to the seasonal dietary shift discussed above, as these parasites are trophically transmitted. The detected increase in prevalence and mean intensity of digeneans found in summer is in accordance with the predominance of zooplankton species (i.e., amphipoda and copepoda) in the diet of round sardinella (Albo-Puigserver *et al.*, 2019), which are intermediate hosts of many digenean species (Gibson *et al.*, 2002). This observation aligns with the results reported by Ramdani *et al.* (2020), as they also noted an increase in the prevalence of digenean species during this period. In any case, the presence of digeneans and Tetracyllidean cestode larvae do not pose a risk to human consumption nor to the fish meat quality, since these parasites are not zoonotic, were observed only in the digestive tract and this fish species is usually eaten eviscerated. No parasites were found in the flesh.

As mentioned above, another aspect to take into consideration related to fish as a food product is the potential presence of contaminants of anthropogenic origin. The study area (the coast off Barcelona) has been reported to display high levels of contamination in shallow coastal sediments related to the proximity of urban cities, port activities and river mouths (e.g. the Besòs River), that increase the transport and accumulation of metals (Palanques *et al.*, 2022). Once in the marine environment, the accumulation of metals in fish flesh depends on a variety of factors (i.e., concentration, environmental conditions, exposure duration, bioavailability, fish size, age and feeding habits, amongst others) (Perugini *et al.*, 2014; Sauliute & Svecevičius, 2015). Although the herein round sardinella proceed from the vicinity of Barcelona, the heavy metal levels detected in their flesh were much lower than those reported in other regions of the Mediterranean. Levels



of Hg from the herein study were four times lower than those found in round sardinella from the Adriatic Sea (0.3 mg/kg w.w.) (Storelli *et al.*, 2003). Concentrations found in present results of Pb and Cd ranged between five to 17 times and ten to 300 times lower, respectively, compared to round sardinella from the Strait of Messina (Lo Turco *et al.*, 2013) and from the Eastern Mediterranean (Tepe, 2009). Regarding As and Ni levels, our results appeared to be twice and 18 times lower, respectively, than those reported in the Strait of Messina (Lo Turco *et al.*, 2013). In terms of food safety, Cd, Pb and Hg content from the present study fall within the limits set by European legislation (Commission Regulation No 2023/915). Regarding As, although the inorganic form is considered toxic for human health and therefore limits have been established (EFSA, 2014), its presence in fish is usually very low, so its consumption would not represent a significant health risk (Copat, 2015). Similarly, while the European Food Safety Authority established a tolerable daily intake for Ni, its absorption from food is lower than that from drinking water, making fish an unlikely significant route of Ni intake (EFSA, 2020). In addition, the low levels of other trace metals (Cu and Zn) detected in this study, far below the maximum permissible limits, indicate that consuming round sardinella does not pose a risk for human health. The calculated contribution of heavy metal intake for a meal of 100 g of round sardinella per week is very low (<3.05%), without exceeding the PTDI, PTWI or PTMI established for each heavy metal. In the case of Hg, the metal with the highest detected concentration, even in the case of increasing the frequency of consumption of round sardinella to three times per week, the contribution of this element would represent <10% of the established PTWI (0.004 mg/kg body weight) and would not pose any risk to human health.

## Conclusions

The present study provides novel seasonal information on the nutritional status of round sardinella from off the central Catalan coast in the NW Mediterranean Sea. Seasonal differences have been observed in fillet lipid content and fatty acid composition, probably related to the reproductive stage, breeding strategy and diet composition of the analysed fish. According to the observed low lipid content, this species represents a lean fish in contrast to other pelagic related species, such as sardines. Despite its low commercial value, it has a high proportion of omega-3 LCPUFA, and in particular DHA (about 42-48 % of the total fatty acids) during summer and autumn, in line with the recommended levels of EPA+DHA for a meal portion of 100 g of round sardinella/day. Additionally, the absence of zoonotic parasites across all seasons of the year together with the low levels of heavy metals found in the fillet, indicates that it is safe for human consumption. Thus, taking into account all the results together, autumn-caught round sardinella seems to be a nutritionally beneficial choice for consumers. Indeed, encouraging the consumption of round sardinella in autumn, knowing that

the spawning period occurs in summer, would represent a strategy to support sustainable fishing by spreading fishing pressure across various fish species and times of the year, a crucial strategy for the current context of the decline in important pelagic fish species. Looking towards the future, the positive outcomes in terms of nutritional properties and food safety of round sardinella highlight its potential as an important food resource. This includes not only direct consumption but also its use as raw material for the food industry and derived products such as canned goods. Exploring these possibilities further is worthwhile, in order to enhance both the nutritional and sustainability benefits related to this species.

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