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Mercury levels in Merluccius merluccius muscle tissue in the central Mediterranean Sea: seasonal variation and human health risk

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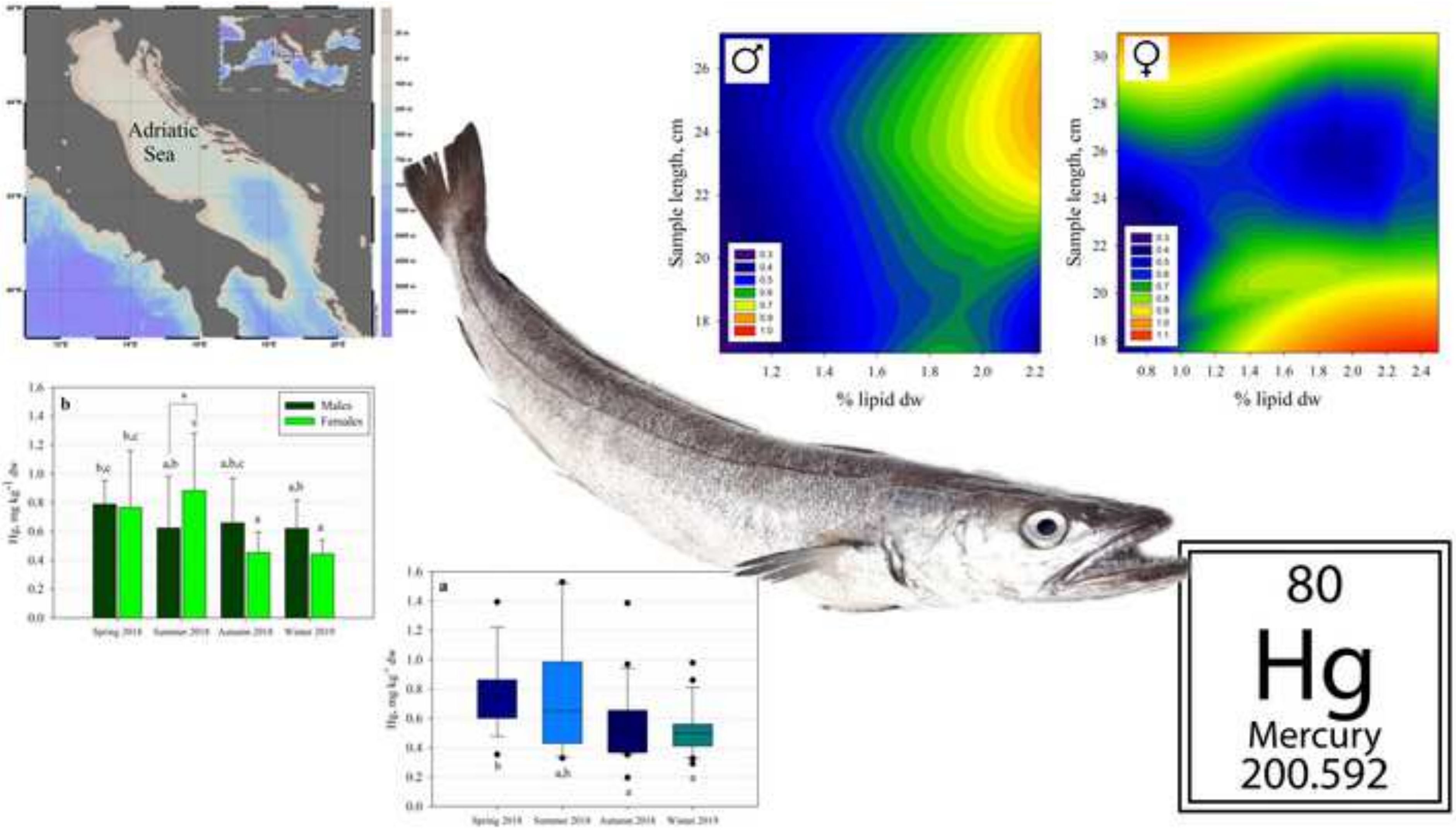
Graphical Abstract



Highlights

- Total mercury (THg) in *Merluccius merluccius* fillets averaged 0.6 (± 0.3) mg/kg, dw
- Significant season-related differences were found in females and not in males
- Only females showed a correlation between THg level and length
- THg was significantly higher in mature compared with immature specimens
- European hake fillets caught in the Adriatic Sea is safe for human consumption

Mercury levels in *Merluccius merluccius* muscle tissue in the central Mediterranean Sea: seasonal variation and human health risk

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Abstract

In this study we analysed total mercury (THg) levels in European hake (*Merluccius merluccius*) – an ecologically and commercially important species throughout the Mediterranean – caught in the northern and central Adriatic Sea. To the best of our knowledge, this is the first study evaluating THg levels in hake fillets in relation to ecological (season) and biological (body size, sex, sexual maturity, lipid content) parameters. THg levels in muscle showed no sex-related differences; in contrast, significant season-related differences were found in females, with higher levels in spring-summer compared with autumn-winter. No season-related differences were seen in males. A significant sex effect was found for body size and sexual maturity. Females showed a correlation between THg level and length, THg being significantly higher in mature compared with immature specimens. No significant sex effect was found for muscle lipid content, because a correlation between THg concentration and tissue lipids was found in both sexes.

Since the mean THg concentration found in *M. merluccius* fillets (0.64 ± 0.29 mg kg^{-1} dry weight; range, 0.20-1.53) was consistently under the level set by EU regulations, this study demonstrates that European hake caught in the northern and central Adriatic is safe for human consumption.

Keywords: Total mercury, *Merluccius merluccius*, seasonal variability, biological parameters, lipid content

1. Introduction

Mercury (Hg) contamination in marine organisms is of great concern due to its effects on ecosystem and human health. Mercury is a highly toxic element naturally present in the Earth's crust, where its average abundance by mass is 0.08 mg kg^{-1} (Ehrlich *et al*., 2008). When inorganic mercury reaches seawater, it is microbiologically transformed into methylmercury (MeHg), the organic and more toxic form, by abiotic and biotic reactions which can affect its solubility, volatility, bioavailability and toxicity.

Since its lipophilic nature facilitates MeHg accumulation in aquatic organisms, including fish tissues, and its biomagnification in food chains (Baeyens *et al*., 2003; Celo *et al*., 2006), human populations with a traditionally high dietary intake of food of freshwater or marine origin may be at risk of MeHg poisoning. Exposure to mercury has toxic effects on several organs and is the cause of neurological damage and a variety of diseases (Guallar *et al*., 2002; Jaishankar *et al*., 2014). As a neurotoxic agent, mercury affects nervous system development, inducing psychological disturbances, impaired hearing, loss of sight and motor control, ataxia and general debilitation (Storelli *et al*., 2005). It also has mutagenic and teratogenic effects, since it can pass through the placental and blood-brain barriers, inducing chromosomal aberrations (Bernhoft, 2012; Satoh, 2000; Zahir *et al*., 2005).

Since the diet seems to be the chief route of human MeHg exposure (Kim *et al*., 2016; McClain *et al*., 2006; Storelli *et al*., 2005; Zhang and Wong, 2007), it is essential to improve seafood security by monitoring its concentrations in aquatic organisms. The EU has set a maximum MeHg level of 0.50 mg kg^{-1} in fishery products and fish muscle (Directive 1881/2006/EU and amending regulation 420/2011/EU), whereas the EFSA Panel on Contaminants in the Food Chain has set a tolerable weekly intake (TWI) of inorganic mercury of 4 µg kg^{-1} body weight (bw) (Panel E. C., 2012).

The Mediterranean is very rich in Hg from both natural and anthropogenic sources (Cossa *et al*., 2005; Rajar *et al*., 2007) and although it accounts for only about 1% of the world's oceans, its deposits of cinnabar (HgS) are about 65% of the world's Hg reserves. Moreover, this semi-closed basin is surrounded by some of the most heavily populated and industrialised countries in the world, whose strong pressure on the sea makes it particularly prone to pollution (Andersen *et al*., 2019).

Although several studies have assessed the temporal and spatial distribution of mercury species and levels in the water column and sediments in the Mediterranean Sea (Kotnik *et al*., 2014; Rajar *et al*., 2007), data on their accumulation in aquatic organisms were investigated in relation to biological factor, such as body size, sex, sexual maturity and muscle lipid content (Bonsignore *et al*., 2013; Ghosn *et al*., 2020; Mille *et al*., 2021; Storelli *et al*., 2005); nevertheless, information on mercury seasonal changes in edible fish are limited.

The Adriatic Sea is a highly productive fishing ground for several commercial species and a basin among the most vulnerable to environmental pollution. Its unique oceanographic and geographical characteristics are believed to enhance contaminant biomagnification (Artegiani *et al*., 1997a, 1997b; Rovere *et al*., 2021; Russo and Artegiani, 1996). Here, the mean mercury concentration in marine surface sediments is 0.0526 mg kg^{-1} (range, 0.0106-0.123 mg kg^{-1}) (Droghini *et al*., 2019; Spagnoli *et al*., 2021) and its mean concentration in seawater is 3.3 pM (range, 0.78 - 6.97 pM) (Kotnik *et al*., 2015).

In this study we determined the Hg levels in edible tissue of European hake (*Merluccius merluccius*, Linnaeus, 1758), a major species throughout the Atlantic and the Mediterranean that lives in close contact with the bottom and is widely distributed between 70 and 370 m. It is one of the most important commercial demersal species in the Adriatic Sea and in the past few years has been subject to a particularly high fishing pressure (FAO, 2020). In 2018, total hake landings in the Mediterranean Sea were 20,170 tons (FAO-GFCM, 2020); of these, 15% were in the northern and central Adriatic.

M. merluccius is considered as a batch spawner with asynchronous ovary development and a protracted reproductive period encompassing the whole year, although the main spawning peak is from spring to summer (Candelma *et al*., 2021). Its meat, fresh or dried, is highly prized by consumers because it is lean and digestible and is indicated in the Mediterranean diet for its protein, phosphorus and potassium content (INRAN; National Research Institute for Food and Nutrition, Italy). Its ability to bioaccumulate and biomagnify along the trophic chain makes *M. merluccius* a highly suitable bioindicator of marine environmental pollution. For these reasons this species was chosen in the present study.

Several studies have demonstrated Hg in *M. merluccius* muscle (Acosta-Lizárraga *et al.*, 2020; Storelli, 2008). However, data on its concentration in specimens caught in the Adriatic Sea are available for limited areas such as the Croatian coast (Grgec *et al*., 2020; Jureša and Blanuša, 2003) and the southern Adriatic (Perugini *et al*., 2014; Storelli *et al*., 2005). To the best of our knowledge, there is very little information on its bioaccumulation in relation to seasonality and to key biological factors such as specimen body size, lipid content, reproduction cycle and sex.

This study was conducted to determine total mercury (THg) concentrations in the muscle of *M. merluccius* caught in the Adriatic Sea, to *i)* assess the level of contamination of the species in the northern and central Adriatic Sea; *ii)* evaluate for the first time the seasonal variation of Hg levels in muscle; *iii)* investigate the relationship between Hg and some key biological parameters (fish length, weight, sex, reproduction stage) and lipid content; and *iv)* estimate the risk of Hg exposure associated with human consumption.

2. Materials and methods

2.1 Specimen collection

We analysed 74 specimens, (36 males and 38 females) of *M. merluccius* caught by a commercial bottom trawler in a rich offshore fishing ground spanning the Northern and Central Adriatic Sea (FAO-GFCM geographical sub-area 17; Figure S1, Supplementary Material) from May 2018 to January 2019. In the laboratory, specimens were measured for total length (TL) and total weight (TW) and sorted by sex. The gonad maturity stage was established macroscopically based on the classification proposed by Candelma *et al*. (2021); accordingly, specimens were divided into immature (virgin) or mature (actively spawning or capable of spawning).

2.2 Laboratory analyses and mercury determination

All analytical steps were carried out in a clean room laboratory ISO 14644-1 Class 6, with areas in ISO Class 5 under a laminar flow. All laboratory materials were subjected to a specific acid-cleaning procedure (Illuminati *et al.*, 2014, 2016). Specimens were weighed using an AT261 Mettler Toledo analytical balance (Greifensee, Switzerland, readability, 0.01 mg; repeatability standard deviation, 0.015 mg). The variable volume micropipettes and neutral tips were from Brand (Transferpette, Wertheim, Germany) and the scalpels with sterile stainless-steel blades from Granton (Mod. 91021, Sheffield, UK). Ultrapure water was obtained from a Milli-Q water system (Merck Millipore, Darmstadt, Germany). Acetone and petroleum ether, used for lipid extraction, were RS grade for pesticide analysis (Carlo Erba, Milano, Italy). Dogfish muscle DORM-2 (NRCC, Ottawa, ON, Canada) was used as certified reference material for Hg content.

The THg muscle content was determined by thermal decomposition amalgamation atomic absorption spectrometry (TDA AAS) using a Direct Mercury Analyzer (DMA-1, FKV, Milestone, Sorisole, Italy) (Illuminati *et al*., 2020; Roveta *et al*., 2020; Truzzi *et al*., 2019, 2020). Muscle from each specimen was minced and homogenised (homogenizer MZ 4110, DCG Eltronic). About 0.05 g of each sample was weighed directly into quartz tubes and heated using compressed air (purity 99.998%) as the oxidant gas. The Hg vapours passed through a catalyst, then the products of combustion were removed and trapped in a gold amalgamator. High temperatures (850 °C) were applied for desorption and the Hg content was quantified by determining absorption at 253.7 nm. TDA AAS involved the following steps (Annibaldi *et al*., 2019): drying at 250 °C for 60 s, decomposition at 650 °C for 120 s and desorption at 650 °C for 60 s. The detection cell ranges were 0.03-200 ng and 200-1500 ng. Hg was quantified using the calibration curve technique. To correct for possible Hg contamination during the analysis, the THg concentration of a blank was subtracted from the concentration measured in samples. At least 3 aliquots *per* specimen were analysed.

Use of DORM-2 certified reference material ensured analytical quality control. The mean experimental Hg value of 4.47 ± 0.10 mg kg^{-1} dry weight (dw) was in line ($p > 0.05$) with the mean value of the certified material (4.43 ± 0.05 mg kg^{-1} dw), demonstrating method accuracy and repeatability.

2.3 Lipid content

Muscle tissue from each specimen was minced, homogenised, accurately weighed and freeze-dried (Edwards EF4 Modulyo, Crawley, Sussex, UK) to constant weight (± 0.2 mg). Total lipid content was determined in 3 aliquots *per* specimen. The average percent moisture was calculated in each sample. For Microwave-Assisted Extraction (MAE), about 0.5 g of each aliquot was placed in the Teflon extraction vessel of a Microwave Accelerated Reaction System (MARS-5, 1500 W; CEM, Mathews, NC, USA) with 10 mL petroleum ether and 5 mL acetone (Truzzi *et al.*, 2017, 2018a, 2018b). The extract was filtered through Whatman GF/C filter papers (Ø 90 mm, GE Healthcare Life Sciences, Buckinghamshire, UK) filled with anhydrous sodium sulphate (Carlo Erba) and rinsed twice with a further 2 mL of a petroleum ether:acetone mixture (2:1 v/v). The filtrate was evaporated to constant weight under laminar flow inert gas (N_2), then the mass of extracted lipids was determined.

2.4 Risk assessment

The risk assessment was based on the level of 0.5 mg kg^{-1} wet weight (ww) set by Commission Regulation (EU) No 420/2011. Since in the EU the 4 µg kg^{-1} bw TWI is based on a weekly average consumption of 18.03 g hake (0.94 kg/inhabitant/year) (EUMOFA, 2019), we evaluated the safety of its consumption by a person weighing on average 70 kg.

2.5 Statistical analysis

Data are expressed as mean ± standard deviation of the replications. We applied analysis of variance (one-way ANOVA) followed by the multiple range test after testing the homogeneity of the variance with Levene’s test (Wayne 2005). If the data variance was not homogeneous, we applied the non-parametric Kolmogorov-Smirnov test for between group comparisons or the Kruskal-Wallis test for comparisons among 3 or more groups. Significant differences were evaluated at the 95% confidence level. We used STATGRAPHICS software (STATGRAPHICS Centurion 2019, Statgraphics Technologies Inc., The Plains, VA, USA) for the analyses and Systat SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA) to create the graphs.

3. Results

3.1 Mercury concentrations in fish muscle

The muscle tissue of all 74 individuals of European hake contained Hg. The average moisture was 79 ± 1% (range, 77.1-80.1) and was similar to the values reported in previous studies of specimens caught in the Adriatic and Mediterranean Seas (80.4 ± 0.5%, Acosta-Lizarraga *et al.*, 2020; 79-82 %, Aubourg *et al.*, 1999; 79.5 ± 2%, Méndez and Gonzalez, 1997; 79 ± 1.0 %, Pérez-Villareal and Howgate, 1987).

Table 1 shows the THg concentrations (ww and dw) measured in males and females. Detailed data on Hg levels and body size are listed in Table S1 (Supplementary Material). Total mercury levels determined in hake caught in other areas of the Mediterranean show wide variation (Table S2, Supplementary Material). In this study, the mean THg concentration was 0.64 ± 0.29 mg kg^{-1} dw (range, 0.20-1.53 mg kg^{-1} dw). Although values were higher in males (0.68 ± 0.26 mg kg^{-1} dw) than females (0.60 ± 0.32 mg kg^{-1} dw), the difference was not significant.

Table 1. Number of specimens and total mercury (THg) levels, mg kg^{-1}, (mean ± standard deviation and range), reported as wet weight (ww) and dry weight (dw), found in *Merluccius merluccius* specimens caught in the Northern and Central Adriatic Sea.

Specimens	*n*	THg, mg kg^{-1} ww mean ± SD* (min-max)	THg, mg kg^{-1} dw mean ± SD* (min-max)
all	74	0.13 ± 0.06 (0.04-0.33)	0.64 ± 0.29 (0.20-1.53)
males	36	0.14 ± 0.05 (0.07-0.29)	0.68 ± 0.26 (0.34-1.39)
females	38	0.13 ± 0.07 (0.04-0.19)	0.60 ± 0.32 (0.20-0.88)

*SD, standard deviation

3.2 *Seasonality*

The effects of seasonality on Hg accumulation were investigated by dividing the year into the 4 seasons according to Artegiani *et al*. (1997a, 1997b) as follows: winter (January-March), spring (April-June), summer (July-September) and autumn (October-December). Total mercury level (THg, mg kg^{-1} dw) and body size of *Merluccius merluccius* are reported in Table 2 and Figure 1 according to seasonality and sex. In general, specimens caught in spring and summer showed similar Hg levels (respectively 0.78 ± 0.25 mg kg^{-1} dw and 0.78 ± 0.39 mg kg^{-1} dw). These concentrations were significantly higher (p=0.002) than those of specimens sampled in autumn and winter (respectively 0.56 ± 0.26 and 0.52 ± 0.17 mg kg^{-1} dw, Table 2 and Figure 1). Notably, levels were significantly higher (p=0.023) in females caught in summer (0.88 ± 0.40 mg kg^{-}1 dw) and spring (0.77 ± 0.39 mg kg^{-1} dw) than in those caught in autumn (0.45 ± 0.14 mg kg^{-1} dw) and winter (0.44 ± 0.10 mg kg^{-1} dw). In males, THg concentrations were largely similar throughout the year, except in spring, when they showed a peak (0.79 ± 0.16 mg kg^{-1} dw, (Table 2 and Figure 1). Comparison of Hg levels in females and males caught in the same season highlighted a significantly lower content in males (p=0.023) in summer. Although males caught in the cold season showed a higher Hg content than females, the difference was not significant (Figure 1b).

Table 2. Total mercury level (THg, mg kg^{-1} dw) and body size of *Merluccius merluccius* specimens caught in spring, summer, autumn and winter.

Sampling season	Sex	*n*	Weight, g Mean ± SD*	Length, cm Mean ± SD*	THg, mg kg^{-1} dw Mean ± SD* (min-max)
Spring 2018	all	15	79 ± 39	22 ± 4	0.78 ± 0.25 (0.35-1.39)
	males	10	83 ± 40	22 ± 4	0.79 ± 0.16 (0.57-1.11)
	females	5	73 ± 40	21 ± 4	0.77 ± 0.39 (0.35-1.39)
Summer 2018	all	17	166 ± 68	27 ± 4	0.78 ± 0.39 (0.31-1.53)
	males	7	135 ± 14	25 ± 1	0.62 ± 0.36 (0.34-1.38)
	females	10	203 ± 66	29 ± 4	0.88 ± 0.40 (0.33-1.53)

Autumn 2018	all	20	97 ± 66	21 ± 5	0.56 ± 0.26 (0.20-1.39)
	males	10	79 ± 61	21 ± 5	0.66 ± 0.31 (0.35-1.39)
	females	10	114 ± 69	24 ± 6	0.45 ± 0.14 (0.20-0.67)
Winter 2019	all	22	91 ± 72	22± 5	0.52 ± 0.17 (0.29-0.98)
	males	9	42 ± 9	18 ± 1	0.62 ± 0.20 (0.41-0.98)
	females	13	126 ± 76	24 ± 5	0.44 ± 0.10 (0.29-0.60)

[*] SD, standard deviation

3.3 *Biological factors*

The THg concentrations were also examined in relation to body size (TL and TW), sex, reproductive stage and total lipid content. TL and TW ranged from 16 to 35 cm (mean, 23 ± 5 cm) and from 20 to 311 g (mean, 108 ± 71 g), respectively (Table S1, Supplementary Material). Since the two parameters showed similar trends, and length is a good descriptor of body size and age because it is less subject to major fluctuations than weight (Yi and Zhang 2011), we decided to discuss our data in relation to TL alone. A significant and positive linear correlation ($r=0.4109$; $p=0.0115$, ANOVA) was seen in females between TL (cm) and THg levels (mg kg^{-1} dw), whereas in males the trend was not equally clear ($p > 0.05$, ANOVA) (Figure S2, Supplementary Material).

Analysis of THg concentrations in relation to the maturity stage showed that levels were lowest in immature females and highest in mature (actively spawning) females ($p < 0.05$). In males, the difference between immature and mature specimens was not significant (Figure 2). Given its high lipophilicity, hence its tendency to accumulate in lipids, we investigated whether the muscle lipid content correlated with THg levels. The mean lipid content was 0.3 ± 0.1 % ww (1.5 ± 0.6%, lipids expressed as dw), ranging from 0.14 to 0.54% ww and 0.31 ± 0.11 in females and 0.32 ± 0.09 % ww in males. Females showed a nearly constant lipid content throughout the year, which was slightly higher than in males (spring, 0.38 ± 0.15%; summer, 0.36 ± 0.15%, autumn, 0.34 ± 0.18%), except in winter (0.14 ± 0.03%), when the difference was significant ($p=0.0350$, Kruskal-Wallis test).

Conversely, in males the lipid content was significantly different (p=0.0384, Kruskal-Wallis test) among seasons, being highest in spring (0.42 ± 0.07%), followed by winter (0.35 ± 0.12%), autumn (0.29 ± 0.05%) and summer (0.21 ± 0.07%) (Figure 3). Comparison of the lipid content of females and males caught in the same season highlighted significantly lower values in females (p=0.035, Kruskal-Wallis test) in winter. A significant positive relationship between THg (mg kg^{-1} dw) and total lipid content (%, dw) was found in both sexes (females, r=0.6772; p= 0.0221; males, r=0.7673; p=0.0014). The analysis of THg in relation to TL and total lipid content (Figure 4) demonstrated that in males the highest THg levels were found in individuals with the highest lipid content and the largest body size (Figure 4a). In females, Hg accumulated mainly in large specimens regardless of their lipid content and in small specimens having a high lipid content (Figure 4b).

3.4 Risk assessment and benefits for consumers

The assessment of the risk associated with dietary Hg was based on Regulation EU 1967/2006 "concerning management measures for the sustainable exploitation of fisheries resources in the Mediterranean Sea", which sets the minimum catch and marketing size of European hake at 20 cm. Notably, the mean THg value found in our sample, 0.13 ± 0.06 mg kg^{-1} ww (range, 0.04-0.33), was well under the 0.5 mg kg^{-1} ww set by Commission Regulation (EU) No 420/2011 (Figure 5).

As regards the risk in relation to the TWI of 4 µg kg^{-1} bw, the weekly average amount of hake consumed in the EU (18.03 g, *i.e.* 0.94 kg/inhabitant/year) (EUMOFA, 2019) contains 2.39 µg Hg. Since a person with an average body weight of 70 kg can assimilate up to 280 µg Hg a week without an appreciable risk of adverse effects, our samples contained a safe amount of Hg.

4. Discussion

The mercury level in tissues is strongly affected by the interaction of numerous parameters, both abiotic (water, sediment, geographic location) and biotic (specimen size, sex, age, reproduction stage), as well by ecological factors (*e.g.* growth rate, feeding habits, position in the trophic chain), which may affect biomagnification processes (Adams and Onorato, 2005; Bank *et al*., 2007; Tremain and Adams, 2012).

In this study, Hg levels in muscle (range, 0.20-1.53 mg kg^{-1} dw) were comparable to the values reported in specimens of the same size caught in the Adriatic Sea by Grgec *et al*. (2020) and Storelli *et al*. (2005) and in other areas of the Mediterranean (Aksu *et al*., 2011; Hornung *et al*., 1980; Kontas, 2006) and well below those reported by Jureša and Blanuša (2003) and Perugini *et al*. (2014) in the Adriatic Sea.

In our sample, Hg levels were not significantly different between the sexes, but in females they showed significant differences between the warm and the cold season, peaking in summer. Seasonal fluctuations are well documented both in freshwater and in marine environments (Burger and Gochfeld, 2011; Frapiccini et al., 2020, 2021; Park and Curtis, 1997; Renieri *et al*., 2019; Smylie *et al*., 2016).

Fluctuations in Hg muscle content could be due to changes in its dietary uptake in the course of the year, in relation to its different availability in the environment, for instance through changes in water temperature, feeding sources or local pollution (Harmelien-Vivien *et al*., 2012; Park and Curtis, 1997). They may also reflect variations in metabolic activity between the sexes, possibly in relation to the different energetic cost of reproduction, growth and physiological processes. All such factors affect muscle tissue composition (in terms of lipid content) through the seasons and reproduction stages (Chouvelon *et al*., 2018; Cossa *et al*., 2012; Cresson *et al*., 2015; Domínguez-Petit *et al*., 2010a, 2010b; Harmelien-Vivien *et al*., 2012).

The higher Hg content observed in mature spawning females could therefore be due to greater lipid stores, accumulated to promote oocyte development. After spawning, Hg levels decreased, partly as a result of a detoxification process related to the transfer of a quota of the contaminants accumulated in lipids through oocyte release (Mille *et al*., 2020, 2021), and partly due to a slower metabolic rate. The possible elimination of Hg through spawning has also been described in several other species (del Carmen Alvarez *et al*., 2006; Hammerschmidt *et al*., 1999; Hammerschmidt and

Sandheinrich, 2005; Johnston *et al*., 2001; Sackett *et al*., 2013). By contrast, the season did not affect THg concentration in males, whose lipid content and energy reserves did not change greatly during the year. This is likely due to continuous spermatogenesis in adult specimens, where the "resting phase" is almost absent (Candelma *et al*., 2018).

Our data also highlighted a close correlation between Hg levels and body size, though only in females. In males the smaller difference is probably due to the narrower size range of our sample (20–26 g and 16–17 cm in males *vs* 30–34 g and 17–18 cm in females). Females generally grow much faster than males and achieve a larger body size (Cossa *et al*., 2012; Cresson *et al*., 2015). Consequently, males of the same size as females are older and their higher THg concentrations may be due to longer Hg exposure (Barghigiani *et al*., 2000; Mellon-Duval *et al*., 2010). Higher Hg levels have also been described in other marine fish (Barghigiani *et al*., 2000; Cossa *et al*., 2012; Cresson *et al*., 2015; Grgec *et al*., 2020; Harmelin-Vivien *et al*., 2012; Jureša and Blanuša, 2003; Karimi *et al*., 2013; Kontas *et al*., 2006; Storelli *et al*., 2005). Altogether, in fish species THg concentrations tend to increase with age, at a rate that depends on their growth rate (Cossa *et al*., 2012). Some studies have found a non-significant or even negative correlation between Hg level and size, especially in species with a limited size range (Al-Yousuf *et al*., 2000; Burger and Gochfeld 2011; Canli and Atli 2003; Freeman *et al*., 1974; Hornung *et al*., 1980; Perugini *et al*., 2014).

The lipid content determined in *M. merluccius* muscle was comparable to the nutritional composition reported by National Institute for research on Food and Nutrition (INRAN) (Italy) (0.3 g lipids per 100 g of fillet), but is slightly lower than the values reported for the Adriatic Sea (0.89-1.62 % ww; Soriguer *et al*., 1997) and the Aegean Sea (1.2 % ww; Tornatiris *et al*., 1994).

Similar to the THg levels, fat storage and content also depends on reproductive stage and sex. The significant differences found in our specimens reflect lipid mobilisation from muscle to gonads in spring and through the reproductive period. In females, the lipid content was highest before spawning, it declined during egg release, when the available energy had to be shared between body fitness and reproduction, and were lowest after spawning. In winter the lipid stores, which have supported the body through a period of food scarcity, are depleted (Mendez and Gonzalez, 1997).

A possible explanation for the differences in body size and lipid storage found between male and female hake may be the presence of sexually mature males throughout the year. Moreover, it has been clearly documented that feeding behaviour in different stages of the life cycle (juvenile and adult) and the trophic position of the species in the food chain are critical for Hg accumulation. Indeed, according to our data THg rose steeply in individuals entering adulthood, owing to the combined effects of slower growth and change in feeding habits, since at 15−20 cm TL they switch from crustaceans and benthic fish to pelagic species, which contain 5-10 times more Hg (Bozzano *et al*., 1997; Ferraton *et al*., 2007; Guichet, 1995; Papaconstantinou *et al*., 1987).

Spawning females were the largest specimens. Their size usually depends on diet composition and on the availability of large, abundant and varied prey. This generates greater interaction with the ecosystem and increases the probability of exposure to contaminated prey (Mille *et al*., 2021). Our data agree with studies describing hake feeding habits in the Mediterranean Sea (Cossa *et al*., 2012; Ferraton *et al*., 2007; Stagioni *et al*., 2011). Juveniles mainly feed on suprabenthic crustaceans and small benthic fish. Specimens 15−20 cm in TL are more piscivorous and eat increasingly larger prey as they grow (Ferraton *et al*., 2007). The adult diet is mostly based on fish with a high lipid content (*e.g.* blue whiting, horse mackerel, sardine; Bozzano *et al*., 1997). Older individuals may even feed on young conspecifics (Mille *et al*., 2021).

Altogether, in all hake specimens caught in the Adriatic Sea the THg levels were under the level set by the EU regardless of the season. Hake from this area is thus safe for human consumption.

5. Conclusion

Fish is a major source of dietary contaminants, particularly heavy metals such as Hg, which can accumulate in marine organisms through the food chain. Therefore, seafood security is an important concern worldwide. This study provides fresh information on the amount of Hg found in muscle tissue in European hake (*M. merluccius*), an important commercial species, which is a consequence of environmental Hg contamination in a highly productive fishing ground in the Adriatic Sea.

The mercury muscle content was related to seasonality, the main biological parameters (body size, sex, sexual maturity) and muscle lipid content. Although they deeply influence Hg accumulation in edible tissue, these factors have not been thoroughly investigated in the northern-central Adriatic. Seasonal Hg fluctuations seem to be chiefly related to the reproduction cycle and the different cost of reproduction, growth and physiological processes for the sexes, and are reflected in tissue composition (in terms of lipids content). The investigation of Hg dynamics in the gonads may thus provide valuable information to understand its potential effects on reproduction and on the population dynamics of this species.

Muscle mercury concentrations were consistently under EU legal levels. Yet, chronic albeit relatively low-level exposure to Hg through regular consumption may entail health risks both for humans and animals. It is therefore essential to monitor contaminant levels in seawater and in the main commercial species, as recommended by descriptor 9 of the Marine Strategy Framework Directive, which focuses on contaminants in fish and other seafood for human consumption.

A greater understanding of the spatial and temporal variation of tissue Hg levels and fish sampling in a wider area can help develop standardised sampling procedures for long-term programs, thus allowing to evaluate potential health hazards for humans and to monitor the aquatic ecosystem health and the effects of Hg exposure on fish in the Mediterranean Sea.

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Figure captions

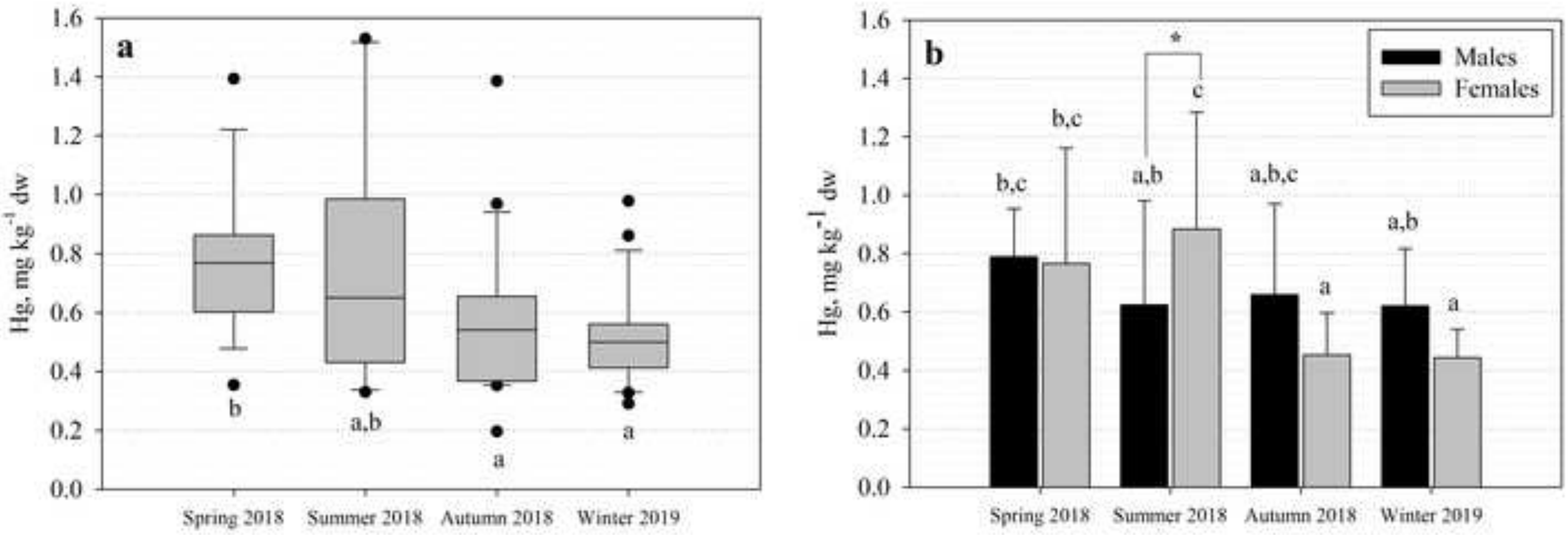
Figure 1. Figure 1. Mean THg levels (mg kg-1 dw) in Merluccius merluccius muscle considering all samples (a) and males and females (b) in relation to catch season.
Low-case letters ($p < 0.05$) indicate statistically significant differences between groups. * $p < 0.05$ indicates statistically significant difference between males and females caught in the same season.

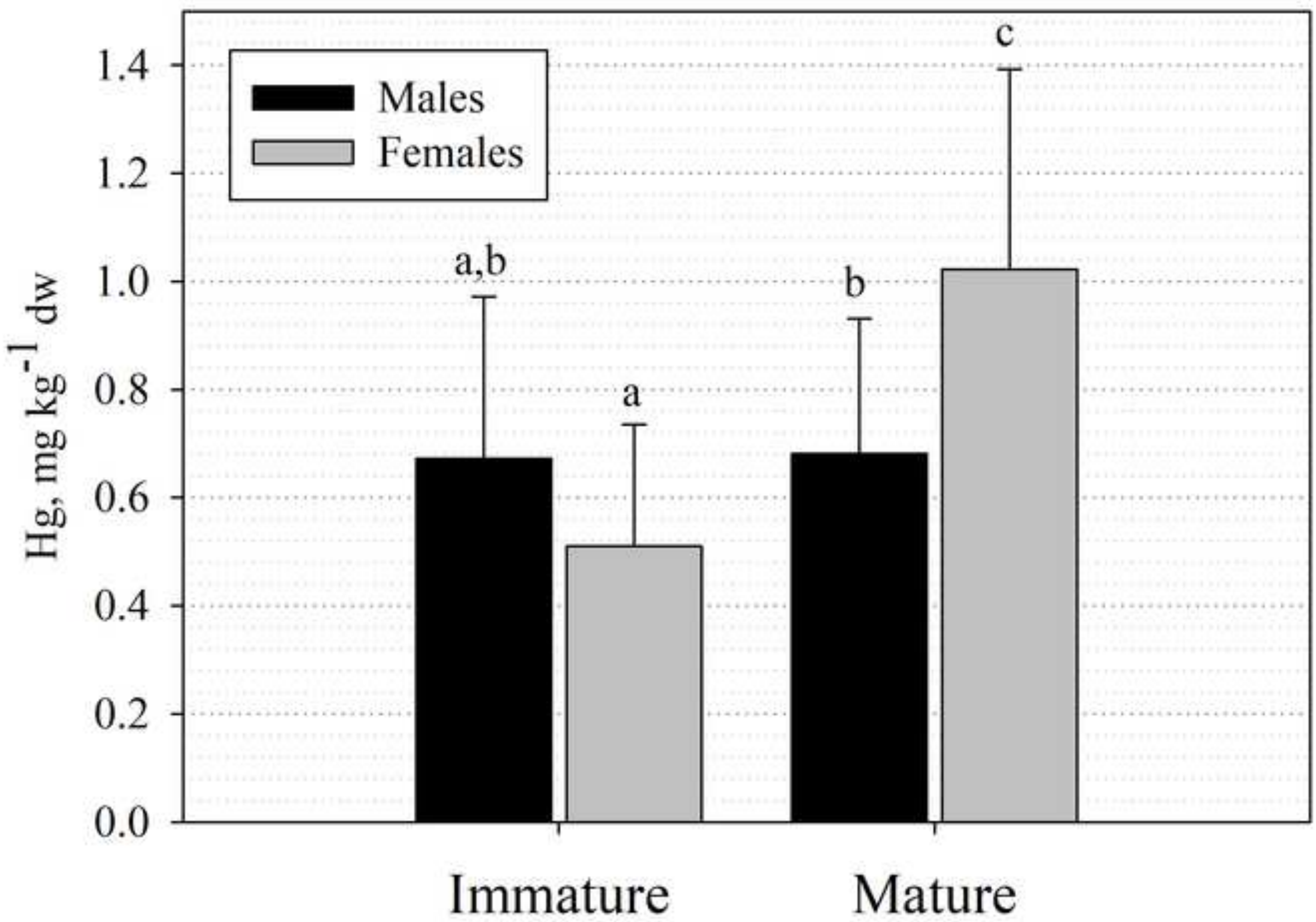
Figure 2. Mean Hg levels (mg kg^{-1} dw) in *Merluccius merluccius* muscle in relation to maturity (Candelma *et al.*, 2021). Letters indicate significant differences ($p < 0.05$) between the groups.

Figure 3. Mean lipid content (% lipid ww) in *Merluccius merluccius* muscle in relation to sampling season and sex.
Low-case letters ($p < 0.05$) indicate statistical significant differences between groups. * $p < 0.05$ indicates statistical significant difference between males and females caught in the same season.

Figure 4. Correlation between mean THg level (mg kg^{-1} dw), fish length (cm) and lipid content (% lipid dw) determined in *Merluccius merluccius* muscle: (**a**) males and (**b**) females.

Figure 5. Mean Hg level (mg kg^{-1} ww) in *Merluccius merluccius* muscle in relation to the level of 0.5 mg kg^{-1} ww set by Commission Regulation (EU) No 420/2011 (red dashed line).


a
b
Hg, mg kg^{-1} dw
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Spring 2018
Summer 2018
Autumn 2018
Winter 2019
b
a,b
a
a
Males
Females
*
b,c
b,c
a,b
c
a,b,c
a
a,b
a


Males
Females
Hg, mg kg-1 dw
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
a,b
a
b
c
Immature
Mature

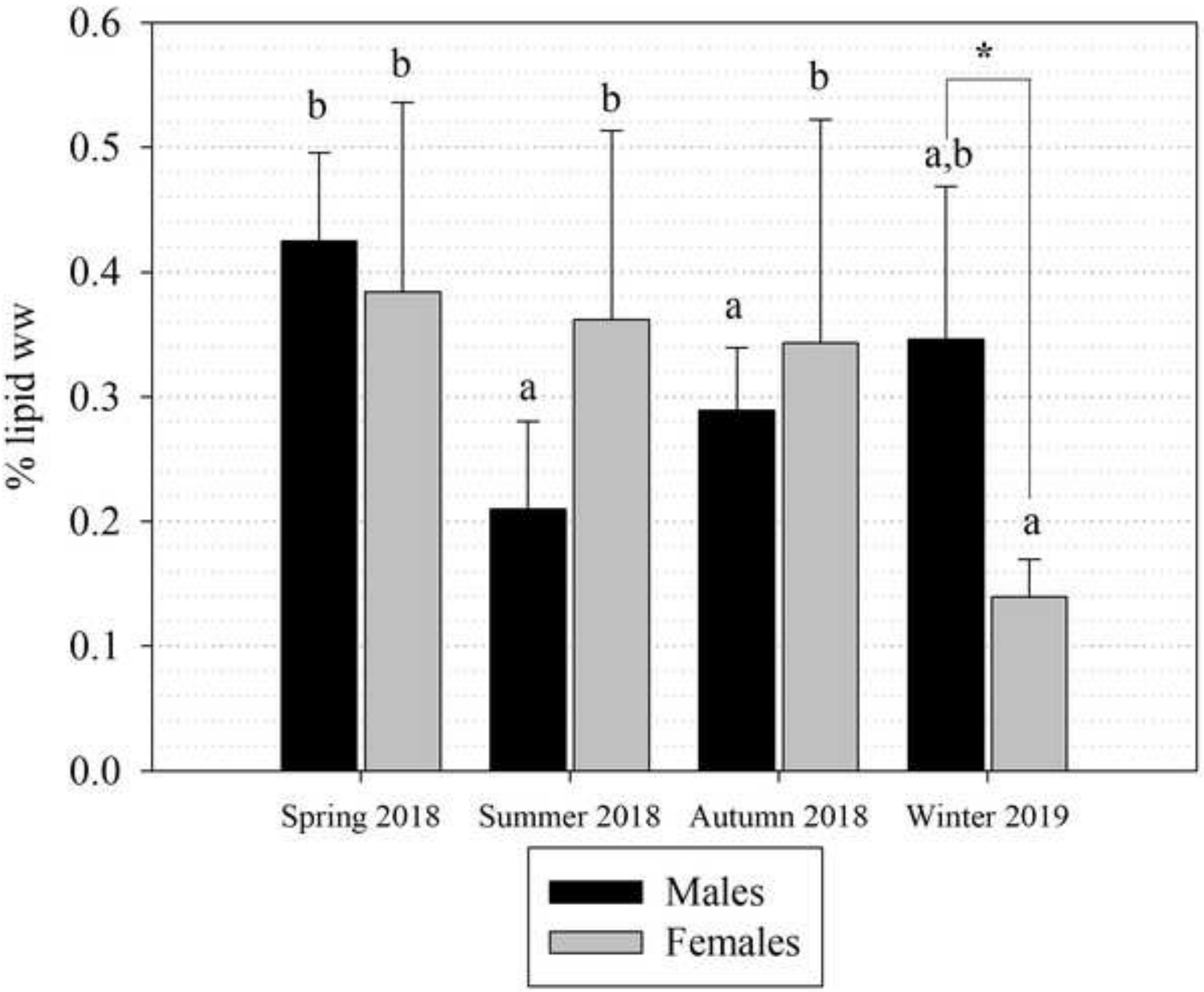
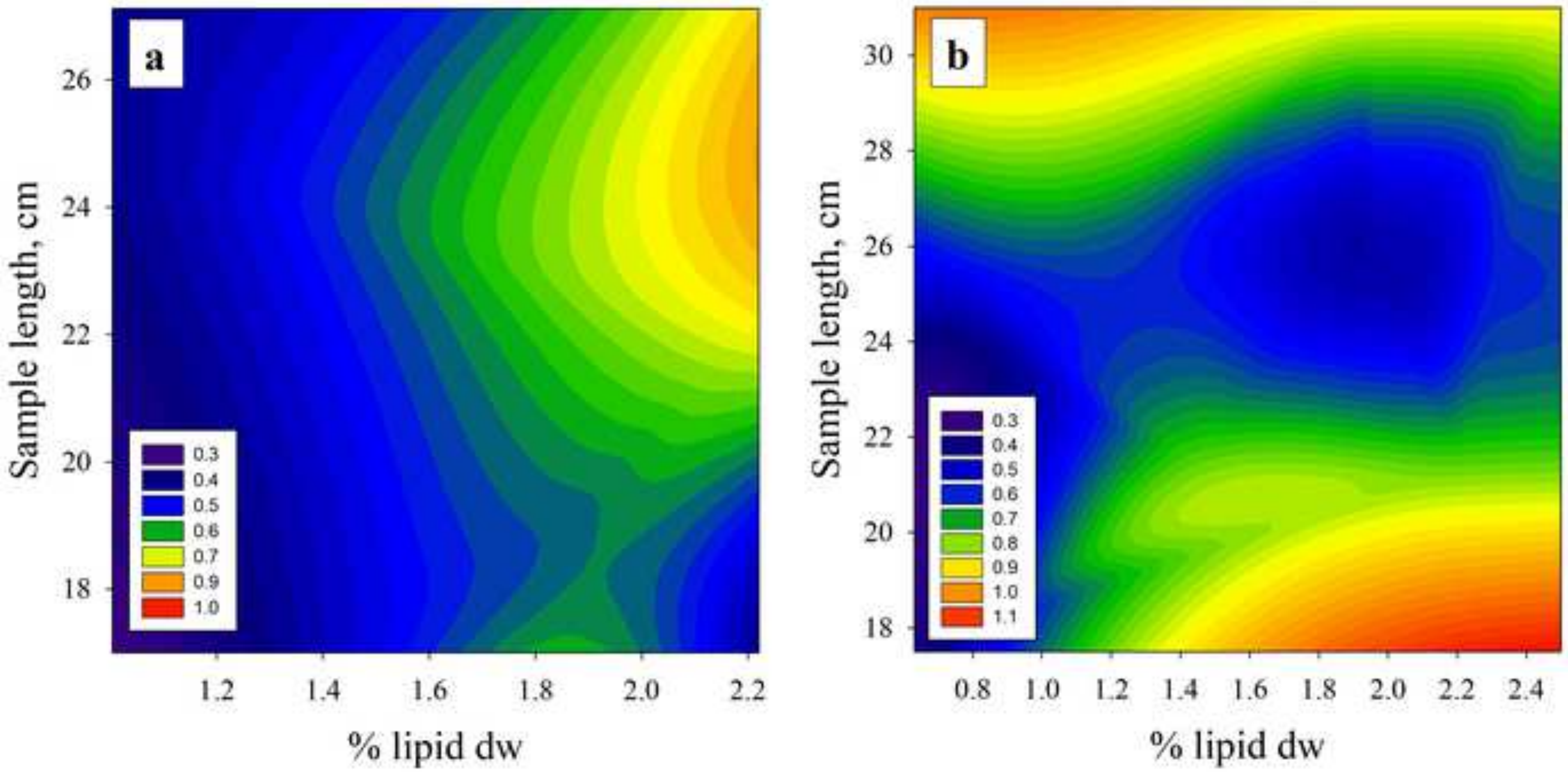
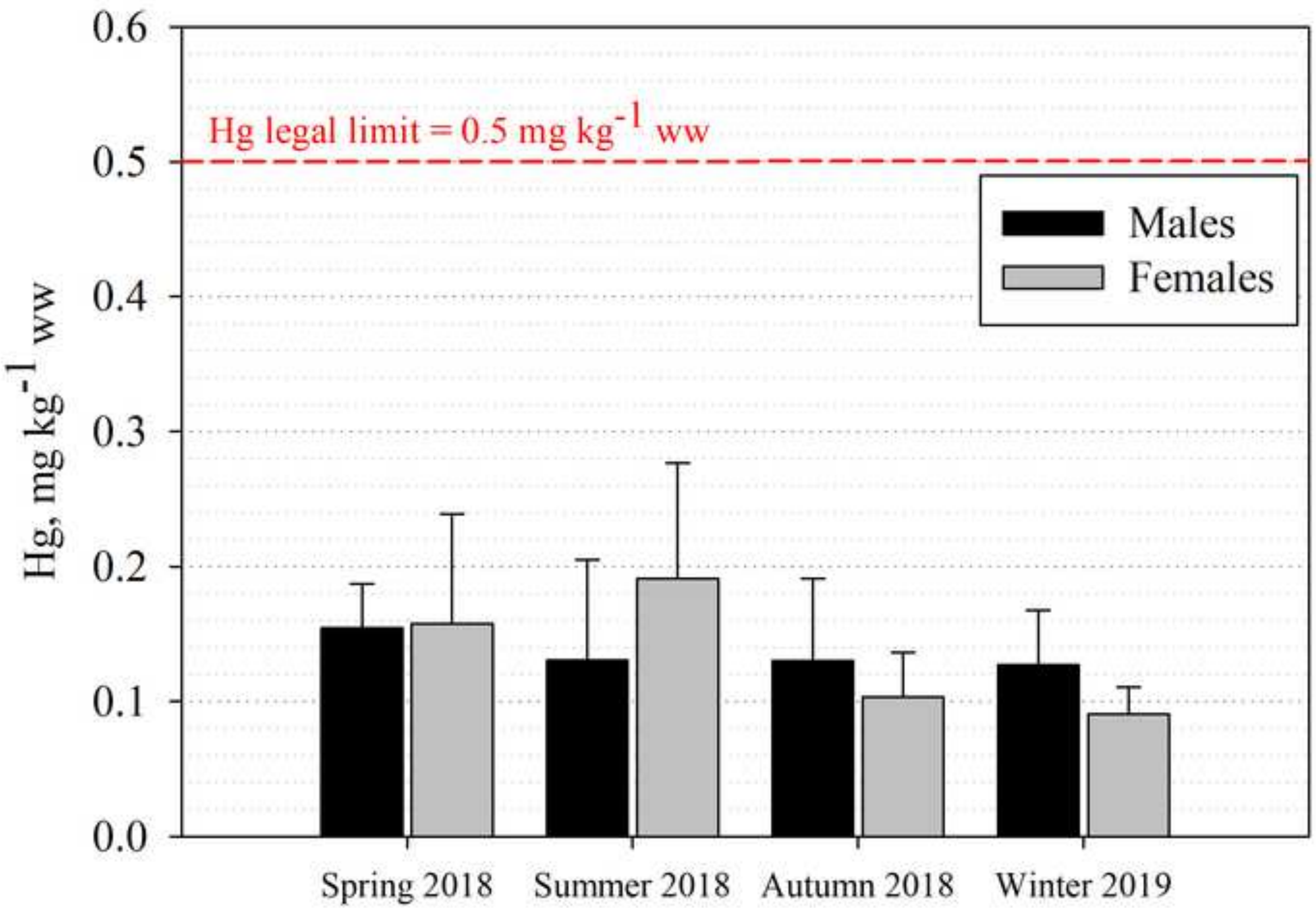

0.6
0.5
0.4
0.3
0.2
0.1
0.0
% lipid ww
b
b
b
a
b
a
a,b
a
*
Spring 2018
Summer 2018
Autumn 2018
Winter 2019
Males
Females

Figure 4


a
Sample length, cm
26
24
22
20
18
1.2
1.4
1.6
1.8
2.0
2.2
% lipid dw
0.3
0.4
0.5
0.6
0.7
0.9
1.0
b
Sample length, cm
30
28
26
24
22
20
18
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
% lipid dw
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1


Hg, mg kg-1 ww
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Hg legal limit = 0.5 mg kg-1 ww
Males
Females
Spring 2018
Summer 2018
Autumn 2018
Winter 2019

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement

Federico Girolametti: Writing - Original Draft, Formal analysis, Visualization. **Monica Panfili:** Investigation, Writing - Review & Editing, Resources. **Sabrina Colella:** Investigation, Writing - Review & Editing, Resources. **Emanuela Frapiccini:** Corresponding, Conceptualization, Investigation, Writing - Review & Editing, Resources. **Anna Annibaldi:** Writing - Review & Editing. **Silvia Illuminati:** Writing - Review & Editing. **Mauro Marini:** Writing - Review & Editing, Resources. **Cristina Truzzi:** Conceptualization, Data Curation, Writing - Review & Editing, Supervision.