CRediT authorship contribution statement

Patrícia Anacleto: Conceptualization, Formal analysis, Investigation, Visualization, Supervision, Writing – original draft, Writing - review & editing. **Vera Barbosa:** Formal analysis, Investigation, Writing - review & editing. **Ricardo N. Alves:** Formal analysis, Investigation, Writing - review & editing. **Ana Luísa Maulvault:** Investigation, Writing - review & editing. **Maria Rosário Bronze:** Investigation, Resources, Writing - review & editing. **António Marques:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.

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Abstract

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Keywords: Mercury, Bioaccessibility, Seafood, Green tea, Grilling, Risk assessment

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Abstract

Human exposure to mercury (Hg) and methylmercury (MeHg) through the ingestion of seafood raises human health-related concerns. In contrast, green tea has health benefits and its consumption potentially reduces bioaccessibility of dietary Hg. The present study aimed to assess the effect of green tea in total mercury (THg) and MeHg bioaccessibility in raw and cooked marine fish species commonly having high Hg levels. Preliminary results demonstrated that significantly higher reductions of bioaccessible THg were attained after the co-ingestion of green tea infusion (1 cup or more) in the oral and intestinal phases. Overall, the present findings clearly show that the co-ingestion of green tea along with seafood grilling strongly reduces THg and MeHg bioaccessibility in all fish species and consequently diminishes the probability of exceeding MeHg provisional tolerable weekly intakes through the consumption of these species with high Hg levels. Such results point out the need to better understand the beneficial/preventive role of green tea infusions and other food processing techniques in bioaccessibility reduction of other chemical contaminants present in food products. Such information is certainly useful to help consumers to wisely select their food, and to enable food safety authorities to integrate such information in risk assessment.

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1. Introduction

Seafood is recognized as an important source of high value protein and essential nutrients, including long chain polyunsaturated n-3 fatty acids, vitamin D, selenium and iodine (Shim et al., 2009). Regular and balanced consumption of seafood is generally recommended by worldwide food safety and health authorities due to its benefits in reducing coronary heart disease, ischemic stroke, the risk of congestive heart failure, and sudden cardiac death, but also by promoting fetal growth, cognitive development and bone health (Hsieh and Arjmandi, 2011; Rimm et al., 2018). However, some seafood can also accumulate chemical contaminants, including toxic elements, such as Hg, Cd, Pb, As, etc., present in the environment, posing health risks to seafood consumers (Maulvault et al., 2015). Indeed, fish has been recognized as the main source of methylmercury (MeHg), i.e. the organic and most toxic form of Hg, which is considered as neurotoxic, carcinogenic and teratogenic for humans (Ouédraogo and Amyot, 2011; Girard et al., 2018). Due to its high stability and slow elimination from tissues, MeHg can be bioaccumulated and biomagnified in aquatic food webs reaching high concentrations in predatory and carnivorous fish species, such as sharks (Rumbold et al., 2014). The current concern of increased Hg and MeHg exposure through seafood consumption due to the current consumption patterns in developed countries triggered the search for mitigation strategies able to reduce the amount of Hg that can reach the bloodstream during the digestion process (Marques et al., 2011; Jadán-Piedra et al., 2018).

Currently, risk assessment guidelines consider absorption rates of 100%, assuming that the ingested dose from a specific food item is equal to the dose found in the systemic circulation (Ouédraogo and Amyot, 2011; Girard *et al.*, 2018). Nevertheless, several studies demonstrated that the overall concentration of Hg and MeHg in seafood does not always reflect the amount that will become available for absorption at the human intestinal epithelium during the digestion process, defined as bioaccessibility (Laird et al., 2009; Alves et al., 2018). Hg and MeHg bioaccessibility can be affected not only by the food matrix composition (Alves et al., 2018; Girard et al., 2018), but also by several physical and chemical processes that occur during the

gastrointestinal digestion (Jadán-Piedra et al., 2016). Furthermore, bioaccessibility of Hg and MeHg present in fish products may be influenced by the co-ingestion of other food items (Jadán-Piedra et al., 2018), as well as by the culinary procedure applied, such as boiling, grilling or frying. Indeed, we previously demonstrated that cooking (steaming) generally reduces toxic elements bioaccessibility in fish (Alves et al., 2018). According to Torres-Escribano et al. (2011), the decrease in Hg bioaccessibility can be attributed to severe changes in the structural conformation of fish muscle proteins, due to the exposure to high temperatures and, consequently, the loss of water that occur during cooking. Also, elevated temperatures may affect the access of enzymes during the digestion process as well as the protein digestibility, which will hamper the release of Hg during cooking (Ouédraogo and Amyot, 2011; Girard et al., 2018).

Mercury toxicity may be reduced by several compounds such as selenium, tannic acid, cellulose, lignin or pectin, lactic acid bacteria and yeast (Cabañero et al., 2007; Jadán-Piedra et al., 2016, 2017a,b). Also, polyphenol-rich foods items, like coffee and tea, can influence Hg and MeHg bioaccessibility (Ouédraogo and Amyot, 2011; Girard et al., 2018). Plant-derived foods contain significant amounts of phytochemicals that are associated with health benefits, including the prevention of cardiovascular diseases, diabetes and cancer (Shim et al., 2009; Zhang et al., 2015). In green tea leaves, the most relevant phytochemicals available are catechins, which may act as chelating agents of redox-active metals (Ouédraogo and Amyot, 2011). The benefits of green tea catechins have been widely associated with antioxidant, antibacterial, antiviral and diuretic properties, contributing to the prevention of neurodegenerative diseases, diabetes, obesity (EFSA, 2018) and toxins elimination by the liver and kidney (Canuel et al., 2006). Several studies suggest that the consumption of green tea extracts protects the cardiovascular system (Basu and Lucas, 2007), lowers blood glucose and cholesterol levels (Hara, 1994), and has anti-inflammatory effects (Shapiro et al., 2009). Moreover, the ingestion of tea may contribute to the daily intake of essential minerals, such as Se, which can also contribute to reduce MeHg bioaccessibility (Schwalfenberg et al., 2013;

Girard et al., 2018). Yet, to our knowledge, there is a gap of studies regarding the implications of total mercury (THg) and MeHg exposure to human health, when consumers ingest cooked fish in combination with green tea.

In this context, the aim of the present study was to investigate the potential effect of green tea infusion isolated or combined with a traditional seafood culinary procedure (grilling) on THg and MeHg bioaccessibility in seven fish species (yellowfin tuna, common smooth-hound, swordfish, Atlantic wreckfish, black scabbardfish, blue shark and European conger) commonly showing high levels of Hg, using a well-recognized *in vitro* digestion model. Additionally, the effects of green tea and grilling on human health risks of exposure to inorganic Hg and MeHg intake were assessed. This research also intended to evaluate, for the first time, the concentration of catechins and gallic acid in the green tea infusion.

2. Materials and methods

2.1. Samples collection and preparation

Nineteen fish samples including yellowfin tuna (*Thunnus* sp., n = 3), common smooth-hound (*Mustelus mustelus*, n = 3), swordfish (*Xiphias gladius*, n = 3), Atlantic wreckfish (*Polyprion* sp., n = 3), black scabbardfish (*Aphanopus carbo*, n = 3), blue shark (*Prionace glauca*, n = 2) and European conger (*Conger conger*, n = 2) were collected in Portuguese local markets. The selection of fish species was based on the generally high Hg concentrations that can be found in these species. Table 1 shows the origin and moisture content (%) of the seven fish species used in this study. Fish samples were acquired frozen, with the exception of European conger samples that was fresh, and the muscle of each specimen fillets was used to assess bioaccessibility.

Each fillet was sliced in two portions that were weighted, and one was randomly assigned for raw assessment and the other one for cooking assessment (using common household practices). Grilling was selected as cooking procedure instead of steaming and frying, because it is the most common and traditional culinary practice used by Portuguese consumers to cook these

species. Culinary salt (NaCl, 1.5% of the fish portion weight) was added to each sample 15 min prior to the grilling procedure. Grilling (2000 W 421-FL Barbecue grill, Flama Sketch, Portugal) was performed at 175 °C during 10 min. Then, the yield after grilling (%) was calculated as follows: grilling yield (%) = weight of the grilled fillet (g) / weight of the fillet before grilling (g). The average yield obtained for the grilling procedure was around 78.1 ± 6.1% (data not shown). Raw and grilled samples were homogenized with a grinder (Retasch Grindomix GM200, Germany) using polypropylene cups and stainless-steel knives at 5,000 rpm until complete visual disruption of the tissue. Fish skin was removed from fillets prior to the homogenization step, except in black scabbardfish samples (as it is impossible to remove the skin in this species). Samples were kept at -20 °C until the *in vitro* digestion, and a portion of sample was freeze-dried (at −50 °C, low pressure – 10^{-1} atm; Power Dry 150 LL3000, Heto, Czech Republic) for THg and MeHg quantification prior to the *in vitro* digestion.

Green tea (Gorreana, Azores, Portugal) was obtained in local markets. A sachet/pocket of tea (2 g) was infused during 10 minutes at 90–100 °C in 250 mL of Milli-Q water. Green tea was always prepared before the *in vitro* digestion protocol and cooled at room temperature until reaching 30-37 °C before being used in the *in vitro* digestion process.

2.2. *In vitro* human digestion model

2.2.1. Reagents

The reagents used to prepare the digestion fluids were the following: Inorganic: NaCl (Merck, 99.5% m/v), $NaHCO_3$ (Merck, 99.5% m/v), $CaCl_2 \cdot 2H_2O$ (Sigma, C3881), KCl (Merck, 99.5% m/v), KSCN (Sigma, P2713), NaH_2PO_4 (Merck, 99.5% m/v), Na_2SO_4 (Merck 90% m/v), NH_4Cl (Riedel-de Haen, 99.5% m/v), KH_2PO_4 (Merck, 99.5%), $MgCl_2$ (Riedel-de Haen, 99.5% m/v), HCl (Merck, 37% m/v); Organic: urea (Sigma, U5128), glucose (Sigma, G5400), glucuronic acid (Sigma, G5269), D-(+)-Glucosamine hydrochloride (Sigma, G1514), uric acid (Sigma, U2625), albumin from bovine serum (Sigma, A7906), α-amylase, from *Aspergillus oryzae*

(Sigma, 86250), mucin from porcine stomach (Sigma, M2378), pepsin from porcine stomach mucosa (Sigma, P7125), lipase from porcine pancreas type II (Sigma, L3126), pancreatin from porcine pancreas (Sigma, P1625), trypsin from porcine pancreas (Sigma, T0303), α-chymotrypsin from bovine pancreas (Sigma, C4129), bile porcine extract (Sigma, B8631), gallic acid (Sigma, G7384), gallocatechin (Extrasynthese, 0973S), epigallocatechin (Extrasynthese, 0979S), catechin (Sigma, C1251), epicatechin (Extrasynthese, 0977S), epigallocatechin gallate (Extrasynthese, 0981S), gallocatechin gallate (Extrasynthese, 0974S), epicatechin gallate (Extrasynthese, 0978S) and catechin gallate (Extrasynthese, 0972S).

2.2.2. Procedure

Each raw and grilled fish sample was digested in duplicate using the same *in vitro* digestion protocol described by Alves et al. (2018). To evaluate the effect of green tea co-ingestion, the digestion of raw and grilled fish samples was performed with or without the addition of green tea infusion.

Briefly, 1.5 g of each fish homogenized sample were digested in Nalgene™ high-speed PPCO centrifuge tubes at 37 °C using a Rotary Tube Mixer with Disc (25 rpm; LSCI, Portugal) in an incubator (Genlab, UK). Three digestion steps were performed: i) oral phase, where 4 mL of saliva fluid was added to the fish sample and incubated for 5 min at pH 7.0 ± 0.2); ii) gastric phase, where 8 mL of gastric fluid was added to the fish sample and incubated for 2 h at pH 2.0 ± 0.2); and iii) intestinal phase, where 8 mL of duodenal fluid and 4 mL of bile fluid were added to the fish sample and incubated for 2 h at pH 7.0 ± 0.2. For the green tea co-ingestion assay (i.e. fish samples with green tea), green tea infusion (1.87 mL, i.e. equivalent to 1 cup of tea of 250 mL; please see detailed information in section 2.2.3) was added to fish samples before the oral phase. Also, Milli-Q water (1.87 mL) were added to fish samples without green tea in order to adjust the volume before the oral phase.

Enzyme degradation/inhibition was prevented by preparing each digestion fluid before starting the digestion protocol, and the pH was adjusted immediately before each digestion step with NaOH (1 M) or HCl (1 M). In the end, the digestion process was stopped by placing the reaction tubes on ice, followed by centrifugation at 2,750 *g* and 10 °C during 10 min to separate the bioaccessible fraction (i.e. supernatant; BIO) from sample residues/pellet (non-bioaccessible fraction - NBIO). Negative controls containing the digestion fluids without fish sample were also performed. BIO and NBIO fractions were kept at −20 °C until analysis.

Total protein levels in samples before digestion (BD) and in both BIO and NBIO fractions were analyzed to confirm the digestion efficiency of our protocol, as previously described in Alves et al. (2017). Protein bioaccessibility ranged between 80 and 95%.

2.2.3. Optimization of green tea utilization during *in vitro* digestion

A preliminary assay was performed with one fish species to optimize the method and to evaluate the effects of green tea in THg bioaccessibility. In this way, the digestion of raw blue shark was performed in the presence or absence of green tea. This fish species was selected because presented one of the highest levels of Hg. Sub-samples were collected at the end of each digestion step to determine in which step green tea reduced the bioaccessible THg concentration (Fig. 1). In addition, to evaluate the effect of different green tea concentrations, raw and grilled blue shark samples were also co-digested with different amounts of green tea infusion, corresponding to ½, 1, 2 or 3 cups of 250 mL (using respectively in the digestion reaction, 0.94, 1.87, 3.75 and 5.62 mL) and THg bioaccessibility was analysed at the end of the intestinal phase (Fig. 2). Afterwards, the best green tea concentration conditions were employed to all fish species samples (raw and cooked).

2.3. THg and MeHg analyses

206 The methodology used to determine THg and MeHg in samples before digestion (BD) and in
207 both BIO and NBIO fractions is described by Alves et al. (2018). Briefly, for THg
208 determination, 10 mg dry weight (dw) BD or NBIO samples, or 100 µL BIO sample, were used.
209 After drying and combustion, samples undergo amalgamation at 700 °C. The elemental mercury
210 (Hg0) was pre-concentrated, released and detected at a wavelength of 254 nm. Methylmercury
211 was extracted from fish samples as described by Maulvault and colleagues (2015). Briefly,
212 hydrolysation with hydrobromic acid (10 mL, 47% w/w; Merck) of BD (150 mg of freeze-dried
213 sample) or BIO (5 g) fractions were performed, followed by MeHg extraction in two steps of
214 purification with toluene (99.8% w/w, Merck). A cysteine solution (1% L-cysteine chloride in
215 12.5% anhydrous sodium sulphate and 0.8% sodium acetate) was added to extract MeHg
216 (Maulvault et al., 2015).

217 THg and MeHg (through cysteine extracts) were quantified by atomic absorption spectrometry
218 (AAS), following the method 7473 of the US EPA (2007), using an automatic Hg analyser
219 (AMA 254, Leco, St. Joseph, MI, USA). Mercury concentrations were calculated from linear
220 calibration with an Hg (II) nitrate standard solution (1,000 mg L^{-1}, Merck, Darmstadt, Germany)
221 diluted in nitric acid (0.5 mol/L, Merck) at concentrations ranging between 0.10 and 40 ng of
222 Hg.

223 The methodology accuracy for THg and MeHg quantification was checked through the analysis
224 of the certified reference material for trace metals DORM-4 (fish protein, National Research
225 Council of Canada, Canada), and the results obtained in the present study were within the
226 certified range values (THg: certified value = 0.410 ± 0.055 mg kg^{-1}; value obtained in the
227 present work = 0.390 ± 0.025 mg kg^{-1}; MeHg: certified value = 0.354 ± 0.031 mg kg^{-1}; value
228 obtained in the present work = 0.353 ± 0.062 mg kg^{-1}). Also, the limits of detection (LOD) for
229 THg and MeHg in the BD and BIO of raw or grilled fish were 1.93-3.24. Three replicate
230 measurements were performed for each sample and results were reported as µg g^{-1}, ww,
231 according to sample moisture content (results shown in Table 1).

Total Hg recovery (%) was defined as the following ratio:

(*Hg BIO* + *Hg NBIO*) × 100/*Hg BD*,

where Hg BIO + Hg NBIO correspond to the sum of Hg contents detected in BIO and NBIO fractions, whereas Hg BD is the amount of Hg detected in the sample BD. Total Hg recovery after the digestion process was 103 ± 9% for raw samples, 105 ± 7% for grilled samples, 95 ± 10% for raw samples without the infusion of green tea in fish and 103 ± 10% for grilled samples combined with green tea infusion.

Bioaccessible THg (or MeHg) (%) with and without green tea was defined as the following ratio:

THg (or *MeHg*)*BIO* × 100/T*Hg* (or *MeHg*)*BD*,

where THg (or MeHg) BIO corresponds to THg (or MeHg) levels detected in BIO, whereas THg (or MeHg) BD is the amount of THg (or MeHg) levels detected in BD sample.

2.4. Determination of catechins and gallic acid

Catechins and gallic acid (GA) from green tea (Gorreana, Azores, Portugal) were infused in Milli-Q water (2 g in 250 mL and concentrated 5 times, i.e. 2 g in 50 mL) and quantified by high-performance liquid chromatography (HPLC) with Diode Array Detection (DAD). The identification of GA and catechins was carried out by comparison with the retention times of each standard. Compounds were quantified using a calibration curve prepared with the corresponding standard compound at a concentration range from 0.781 to 50 mg L^{-1}, except for gallocatechin (3.1 to 50 mg L^{-1}) and epigallocatechin (1.6 to 50 mg L^{-1}) (Table 2). The detection limit of the method was determined considering the lowest concentration that gave a signal that corresponded to 3 times the noise evaluated with a blank solution.

2.4.1. HPLC conditions

The LC system used was a Thermo Scientific (Ultimate 3000 model, USA) equipped with an autosampler, pump and DAD. Chromatographic separation of compounds was carried out on a Lichrocart RP-18 column (250 x 4 mm, particle size 5 µm, Merck) using a Manu-cart® RP-18 pre-column, that were in a thermostated oven at 35 ºC. DAD was programmed for scanning between 190 and 580 nm at a speed of 5 Hz with a bandwidth of 2 nm. The detection was monitored using three channels (280, 320 and 360 nm) at a speed of 5 Hz with a bandwidth of 2 nm. The injection volume applied was 20 µL. The auto sampler's temperature was 12 ºC. The mobile phase consisted of water-formic acid (99.5%:0.5%) (eluent A) and acetonitrile-water-formic acid (90%:9.5%:0.5%) (eluent B) at a flow rate of 1 mL min^{-1}. The system was run with the following gradient program: 0-60 min, 96-75% A; 60-90 min, 75-60% A; 90-90.1 min, 60-94% A and from 90.1-95 min, 96% A in order to reestablish the original conditions.

2.5. Mercury exposure assessment and human health risk characterization

The potential human health risks associated with the intake of MeHg through the consumption of raw and grilled fish species with or without green tea infusion before and after the *in vitro* digestion process were calculated according to the guideline values recommended by the European Food Safety Authority (EFSA, 2012), i.e. the Tolerable Weekly Intake (TWI) of 1.3 µg kg^{-1} body weight (bw). Since currently there is no TWI established for THg, because was replaced by a TWI for inorganic mercury of 4 µg/kg bw, the consumer's health risk to inorganic mercury was evaluated considering that 20% of THg in fish is in the form of inorganic mercury (EFSA, 2012). The percentage of TWIs obtained with the consumption of the studied fish species was calculated for children (3-<10 years old; considering an average bw of 23 kg), adolescents (10-<18 years old; considering an average bw of 43 kg), pregnant women (18-35 years old, considering an average bw of 55 kg) and adults (≥18 years old; considering an average bw of 70 kg) taking into account a consumption of one weekly portion of muscle fish

(equivalent to 150 g of fish per week for the last three age groups and 75 g of fish per week for children). Moreover, a deterministic risk assessment was performed for inorganic Hg and MeHg bioaccessibility.

2.6. Statistical analyses

For each analysis (THg, MeHg and MeHg/THg) and fish species, significant differences in elemental concentration and bioaccessibility between culinary treatments (raw and grilling) and presence or absence of green tea infusion were analysed by *t*-test student for dependent samples. Data were transformed, whenever necessary, to comply with the assumptions of normality of distribution and homogeneity of variances, using Kolmogorov-Smirnov's and Levene's tests, respectively. Additionally, one-way analysis of variance (ANOVA), followed by post-hoc tests (Tukey HSD), were performed in order to detect significant differences between fish species for each treatment (raw or grilled and presence or absence of green tea). Non-parametric analysis of variance (Kruskall–Wallis) and multiple comparisons tests were applied whenever transformed data could not meet these requirements. Statistical significance was established at a probability level of $p < 0.05$, using STATISTICA™ software (Version 10.0, StatSoft Inc., Tulsa, Oklahoma, USA). All data were expressed as mean ± standard deviation (mean ± SD).

3. Results

3.1. Green tea infusion optimization conditions

The effect of THg bioaccessibility throughout the *in vitro* digestion process (oral, gastric and intestinal phases) and the effect of different green tea concentrations on THg bioaccessibility (at the end of the intestinal phase) were assessed in raw and grilled blue shark samples, in order to select the optimal conditions to employ to all fish species samples. Results indicate that bioaccessibilities of THg under *in vitro* oral conditions in raw samples had a mean of 12% (Fig. 1A), which were significantly less than that under *in vitro* gastric (80%) and intestinal (74%)

digestion phases (Fig. 1A). Similarly, in grilled samples THg bioaccessibilities under *in vitro* oral conditions had a mean of 1.1%, being significantly less than that under *in vitro* gastric (64%) and intestinal (65%) conditions (Fig. 1B). Significantly lower levels of THg bioaccessibilities were observed in grilled samples in comparison with the raw counterpart, with the only exception in samples with the presence of green tea in the intestinal phase. It was detected a significant effect of green tea on reducing THg bioaccessibility both in raw and grilled samples during the three *in vitro* digestion phases, particularly in the oral (ranging between 7% and 12% with and without green tea, respectively) and intestinal phases (ranging between 35% and 74% with and without green tea, respectively) in raw samples (Fig. 1A) as well in the gastric phase in grilled samples (ranging between 47% and 64% with and without green tea, respectively; (Fig. 1B). Concerning the trial performed with raw and grilled blue shark samples co-digested with different concentrations of green tea infusion (½, 1, 2 or 3 cups of 250 mL; Fig. 2), it was observed significantly higher reductions of bioaccessible THg concentration above 1/2 cup of green tea, whose reduction was even higher in grilled samples. Since the bioaccessible THg concentration above 1 cup of green tea (equivalent to 250 mL) lead to significantly higher reductions in blue shark either in raw (varying between 15% and 78% with 1 cup of green tea and without green tea, respectively) and grilled samples (varying between 7% and 61% with 1 cup of green tea and without green tea, respectively), this concentration was used in all fish samples (Fig. 2).

3.2. Green tea composition

The concentration of catechins and gallic acid in the green tea infusion was calculated and is presented in Table 2. Besides gallic acid, the eight catechins identified in the green tea were in the following order of concentration: (-)-gallocatechin gallate (GCG), (-)-gallocatechin (GC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), (-)-epicatechin (EC), (-)-catechin gallate (CG) and (-)-epigallocatechin (EGC). Therefore, the most

abundant catechins detected in green tea extracts were GCG (43.8 mg L^{-1}) and GC (37.3 mg L^{-1}), which accounted 43.7% of the total catechin content (Table 2). The chromatographic profiles corresponding to a standard mixture and the sample are presented in Supplementary Fig. S1.

3.3. Green tea effect on THg and MeHg bioaccessibility

In general, results showed that the co-ingestion of green tea infusion significantly diminished THg and MeHg bioaccessibility in almost all fish species (the only exception was the raw European conger in both elements and grilled European conger only in THg; Figs. 3 and 4; Supplementary Tables S1 and S2). A significant reduction was observed when green tea infusion was added in THg bioaccessibility of raw black scabbardfish (ranging between 21% and 50% for samples with green tea and without green tea, respectively), followed by yellowfin tuna (ranging between 29% and 53% for samples with green tea and without green tea, respectively) and swordfish (ranging between 30% and 49% for samples with green tea and without green tea, respectively; Fig. 3; Supplementary Table S1). Furthermore, the addition of green tea reduced THg bioaccessibility to values ranging between 21% (black scabbardfish) and 37% (common smooth-hound) in raw fish. Concerning MeHg bioaccessibility, the addition of green tea significantly decreased the levels in raw samples, with the exception of common smooth-hound and European conger, where no significant differences were detected (Fig. 4; Supplementary Table S2). Similarly to THg, a significantly high reduction in MeHg bioaccessibility after the green tea addition was mainly observed in black scabbardfish (ranging between 22% and 50% for samples with green tea and without green tea, respectively), followed by yellowfin tuna (ranging between 38% and 67% for samples with green tea and without green tea, respectively). Moreover, the addition of green tea reduced MeHg bioaccessibility to values ranging between 21% (swordfish) and 39% (common smooth-hound) in raw fish.

3.4. Cooking and green tea effects on THg and MeHg bioaccessibility

The effect of culinary treatment (grilling) on THg and MeHg levels in fish species before *in vitro* digestion are presented in Table 1. In almost all fish species, THg and MeHg levels significantly increased after grilling ($p < 0.05$), particularly in common smooth-hound and blue shark (increase of 44% and 40% for THg and MeHg, respectively). However, an opposite trend was observed when considering THg and MeHg bioaccessible fraction of *in vitro* digested fish (Figs. 3 and 4; Supplementary Tables S1 and S2). Moreover, the combination of green tea infusion with grilling further significantly diminished THg and MeHg bioaccessibility values in almost fish species. Indeed, in grilled samples in the presence of green tea, THg bioaccessibility varied between 9% (Atlantic wreckfish) and 19% (yellowfin tuna) in comparison with raw samples without the addition of green tea that varied between 38% (European conger) and 53% (yellowfin tuna; Fig. 3; Supplementary Table S1). Moreover, significantly higher reductions in THg bioaccessibility in the presence of green tea infusion and grilling were observed for yellowfin tuna, with levels ranging between 19% (with green tea) and 31% (without green tea) and for Atlantic wreckfish, with levels ranging between 9% (with green tea) and 20% (without green tea; Fig. 3; Supplementary Table S1). Concerning MeHg bioaccessibility, lower levels were also observed in grilled samples combined with green tea infusion [(range: 9% (European conger) and 28% (yellowfin tuna)] compared to raw samples with green tea [range: 21% (swordfish) and 39% (common smooth-hound)] or without green tea [range: 35% (common smooth-hound) and 67% (yellowfin tuna); Fig. 4; Supplementary Table S2]. Similarly to THg, the fish species that showed significantly higher reductions in MeHg bioaccessibility with both conditions (i.e. in the presence of green tea infusion and grilling) were grilled blue shark, with values varying between 15% (with green tea) and 29% (without green tea) and grilled yellowfin tuna, with values varying between 28% (with green tea) and 40% (without green tea; Fig. 4; Supplementary Table S2). Noteworthy, for grilled European conger the decrease was minor, only ranging between 9% (with green tea) and 12% (without green tea).

3.5. Human health risk assessment of MeHg and inorganic Hg exposure

Tables 3 and 4 show the exposure of consumers to the tolerable weekly intakes (TWIs) set for MeHg and inorganic Hg, taking into account the consumption of one portion of fish per week (equivalent to 150 g for adolescents, pregnant women and adults; and equivalent to 75 g for children). When considering MeHg and inorganic Hg concentrations before *in vitro* digestion (i.e. disregarding compound bioaccessibility), the percentage of TWIs was very high particularly in vulnerable population groups (i.e. children, adolescents and pregnant women) and for MeHg. Among the different fish species, grilled blue shark (with or without the green tea infusion) yielded the highest intake of mercury, largely surpassing 100% of the TWI in MeHg (329% in children, 352% in adolescents, 275% in pregnant women and 216% in adults; Table 3), whereas the TWI in inorganic Hg never surpassed 100% and the values were very low (below 28%; Table 4). Other species that showed particularly higher TWIs associated to MeHg exposure were raw or grilled swordfish and European conger (all samples with or without green tea) in most demographic groups (particularly in children and adolescents). Additionally, the percentage of TWI in MeHg was slightly above 100% for common smooth-hound, yellowfin tuna and black scabbardfish, particularly in grilled samples in more vulnerable population groups (Table 3). It is also noteworthy that Hg intake tended to increase after grilling with or without the inclusion of green tea, particularly in yellowfin tuna (47% and 51% increase in MeHg and inorganic Hg, respectively).

However, when considering THg and MeHg concentrations in the bioaccessible fraction of *in vitro* digested fish (i.e. accounting for compound bioaccessibility), raw samples yielded higher Hg intakes than grilled ones, except in yellowfin tuna. More specifically, raw swordfish presented the highest percentage of TWI set for inorganic Hg in all populations groups (children and adolescents: 10%; pregnant women: 8%; adults: 6%; Table 4), while raw blue shark displayed the maximum percentage of TWI set for MeHg (children: 107%; adolescents: 114%; pregnant women: 89%; adults: 70%; Table 3). Overall, the percentage of TWI in the bioaccessible Hg fraction considerably decreased in relation to the one found before *in vitro* digestion, with a reduction above 50% reached in all fish species (Tables 3 and 4). Moreover,

414 this reduction was higher (>80%) in the majority of grilled fish samples with or without the
415 inclusion of green tea. In what concerns MeHg, the great majority of fish species reduced TWIs
416 below 100% in the bioaccessible fraction in comparison with samples before *in vitro* digestion
417 (TWI>100%), since the TWIs for inorganic Hg remained even lower in the bioaccessible
418 fraction. However, TWIs in the MeHg remained slightly above 100% for more vulnerable
419 population groups, as the case of adolescents for raw swordfish (104%) and raw/grilled blue
420 shark (114% and 103%, respectively) as well as in children for raw blue shark (107%) (Table
421 3). When grilling was combined with green tea, a further decrease in the percentages of TWIs
422 for both MeHg and inorganic Hg bioaccessibility were registered, except in yellowfin tuna,
423 where an increase occurred after grilling. Additionally, for all population groups, a highest
424 reduction of TWI was reached in raw/grilled swordfish and blue shark for MeHg with the
425 addition of green tea (e.g. for children TWI range between 58% and 98% in raw swordfish and
426 between 31% and 58% in grilled swordfish, with and without the addition of green tea,
427 respectively).
428

429 **4. Discussion**

430 *4.1. Green tea composition*

431 The most abundant catechins detected in green tea extracts was GCG and GC, accounting with
432 44% of the total catechin content (Table 2). Several studies report the effects of GCG and GC in
433 the reduction of dietary cholesterol absorption, as well as in scavenging effects of these
434 galloylated catechins (Ikeda, 2008; Lee et al., 2014), which may have contributed to the
435 beneficial results on Hg retention and consequently Hg bioaccessibility reduction. Most
436 published studies indicate that EGCG and EGC are the most abundant catechins found in green
437 tea leaves, accounting 70% of the total amount of catechins (e.g. Graham, 1992; Lee et al.,
438 2014; Yashin et al., 2015; EFSA, 2018). According to Friedman et al. (2009), there is wide
439 variability in catechin content of commercial teas, which can be due to genetic variability
440 among plants from which leaves were harvested and/or due to soil composition, climate,

441 harvesting practices, postharvest storage, sampling and manufacturing practices (Rusak et al.,
442 2008; Sultana et al., 2008). Additionally, tea varieties are harvested in different ways and in
443 distinct seasons (Friedman et al., 2009).
444
445 *4.2. THg and MeHg bioaccessibility before cooking and green tea infusion (Baseline)*
446 Results demonstrated that almost all raw fish samples presented a THg and MeHg
447 bioaccessibility below 50% (except for tuna; Table 1). The obtained levels are lower than
448 bioaccessibility usually found for MeHg in fish (higher than 80%) stated by EFSA (2012).
449 Nevertheless, previous authors (Cabañero et al., 2004; Leufroy et al., 2012; Alves et al., 2018)
450 have also obtained lower Hg bioaccessibility (around 20% for raw tuna). Overall, the
451 bioaccessibility of Hg or MeHg in fish is highly variable, ranging from 13 up to 87% (reviewed
452 by Chiocchetti *et al.*, 2017). The high Hg variability between species reported in literature may
453 be due to different *in vitro* digestion methodologies used, distinct nutritional composition of the
454 food matrix, different Hg/MeHg accumulation rates in seafood, seafood feeding habitats and
455 other biotic parameters (Cabañero et al., 2007; Torres-Escribano et al., 2011; Calatayud et al.,
456 2012; Cano-Sancho et al., 2015). Other potential factors affecting Hg bioaccessibility can be the
457 thawing conditions, freezing rates and storage temperature, which are related to protein
458 denaturation (Wang et al., 2013).
459
460 *4.3. Cooking and green tea effects on THg and MeHg bioaccessibility*
461 After the *in vitro* digestion, a different baseline pattern was observed in grilled fish species, i.e.
462 when bioaccessibility was taken into account the THg and MeHg concentrations in almost all
463 fish species were reduced after grilling in opposition of the higher concentrations initially
464 observed in grilled samples prior *in vitro* digestion (Figs. 1 and 2; Table 1). These findings were
465 consistent with other studies performed in several fish species (swordfish, grouper, tuna,
466 salmon, shark, mackerel, bonito, rabbitfish, grouper, mullet, sillago, yellow croaker, golden
467 thread and horsehead; He and Wang, 2011; Ouédraogo and Amyot, 2011; Torres-Escribano et

al., 2011; Afonso et al., 2015; Girard et al., 2018). It is well known that Hg has high affinity to proteins (Harris et al., 2003; George et al., 2008), but has relatively weak lipophilic nature (Mason et al., 1996). In this sense, it was argued that cooking may modify protein structure due to heat exposure, rendering Hg-protein complexes less accessible to digestive enzymes, and subsequently reduce their solubilisation during digestion (Ouédraogo and Amyot, 2011). This can probably explain the lower levels of THg and MeHg bioaccessibility observed in all grilled fish species in comparison with raw samples. Additionally, protein denaturation promoted by the exposure to high temperatures, might have led to lower pepsin digestibility, altering the proteins' ability to bind to Hg, either through the creation of additional bindings sites or through Hg sequestration from active protein sites (Ouédraogo and Amyot, 2011; Girard et al., 2018). Some authors reported that cooking also induces the formation of disulfide bonds in proteins, which may further limit protein digestibility (Duodu et al., 2002; Kulp et al., 2003; He et al., 2010). Bax et al. (2012) indicated that, at temperatures above 100 °C, oxidation can cause protein aggregation, thus slowing enzymatic digestion by pepsin.

In this study, we also found that green tea infusion significantly reduced THg and MeHg bioaccessibilities in fish (Figs. 3 and 4), which is in accordance with previous reports (Canuel et al., 2006; Shim et al., 2009; He and Wang, 2011; Ouédraogo and Amyot, 2011; Jadán-Piedra et al., 2016; Girard et al., 2018). These authors suggested that polyphenols, one of the most biologically active components in green tea, most of which are flavonols that are commonly known as catechins, may act as chelating and scavenging agents of redox-active metals (e.g. ability to bind and precipitate certain molecules). Thus, they potentially limiting Hg and MeHg absorption in the intestine and, therefore, being responsible for changes in Hg and MeHg bioaccessibility from fish (Graham, 1992; Record et al., 1996; Wang et al., 2009). Shim and colleagues (2009) obtained a reduction rate of 82–92% of Hg bioaccessibility in uncooked fish (King mackerel) with the addition of green tea, while in our study a decrease of 17-66% and 14-57% was obtained in raw and grilled fish samples, respectively (Fig. 3). Taking into account that these authors used the catechins in powder, this can greatly influence the Hg

bioaccessibility. Some authors (Shim et al., 2009; Kumar et al., 2010) hypothesized that this reduction could be related to the chelating role of some green tea components rich in phytates that can establish complexes with proteins, consequently, altering protein structure which may then result in decreased protein solubility. Furthermore, Girard et al. (2018) suggested that EGCG, the most relevant catechin in green tea, has higher chelating properties, possibly forming insoluble complexes with MeHg and therefore decreasing its bioaccessibility. These authors observed that EC and EGCG could individually decrease MeHg bioaccessibility by up to 55% in swordfish. In our study, the major constituent of green tea was GCG, which generally results in the conversion from EGCG to GCG, an epimerization change that occurs in a high temperature environment (> 120 °C; Ikeda et al., 2003). Girard et al. (2018) mentioned that other polyphenols like caffeic acid, rutin have the same chelating proprieties and similar effect as EGCG.

It is also noteworthy that the combined effect of grilling and green tea infusion co-ingestion lead to very low levels of THg and MeHg absorption when consuming fish, decreasing THg bioaccessibility by 57% in Atlantic wreckfish and 47% in black scabbardfish. Other authors found similar effects on MeHg bioaccessibility when simulating the co-ingestion of grilled swordfish or tuna and green tea infusion (Girard et al., 2018). Besides that, these authors also compared the effect of other polyphenol-rich beverages, as black tea, and observed a similar decrease as with green tea in MeHg bioaccessibility of swordfish and a higher decrease in tuna.

Nevertheless, our study evaluates, for the first time, the concentration of catechins in green tea infusion after optimization the tea concentration, and estimated how the utilization of green tea and grilling can influence human health risks of exposure to THg and MeHg intake, considering four distinct vulnerable demographic groups (children, adolescents, pregnant women and adults).

4.4. Effects of exposure to MeHg and inorganic Hg intake on human health risks

Results show that the consumption by adults, once a week, of 150 g of any of the studied fish species does not yield inorganic Hg exposure values above the TWI set for this element (4 μg kg^{-1} of individual bw), regardless of applying a cooking procedure or ingesting fish in combination with green tea infusion. It is also noteworthy that the percentage of TWI for inorganic Hg remained very low in all fish samples (below 28%). Nevertheless, our results revealed that the risk is even greater for TWI of MeHg (1.3 μg kg^{-1} of individual bw) in all demographic groups. In particular, some fish species largely exceedeed 100% of TWI like swordfish, blue shark and European conger, especially grilled samples (with or without the co-ingestion of green tea infusion) and in more vulnerable groups (i.e. children, adolescents and pregnant women). Hence, it is advisable a parsimonious consumption of these three species in particular. Yet, if the Hg content in the bioaccessible fraction is considered instead (a more realistic scenario), the exposure to this element drastically decreases regardless of species, cooking procedure and co-ingestion of green tea infusion. Thus, a weekly consumption of 150 g/75 g of the studied species does not represent a risk of exceeding the TWIs for both MeHg and inorganic Hg, even in the case of the three species (swordfish, blue shark and European conger), as well as grilled yellowfin tuna, common smooth-hound and grilled black scabbardfish that previously exceeded this recommend value before *in vitro* digestion. The only exceptions are MeHg in raw blue shark for children and adolescents, as well as in raw swordfish and grilled blue shark only in adolescents (all samples without green tea), where the risk remained relatively high (slightly above 100% of the TWI). Therefore, the studied fish species can be parsimoniously consumed by childs, adolescents, pregnant woman or adults with 23, 43, 55 and 70 kg, respectively (once per week) with limited health hazards, and consumers should preferably use combined co-ingestion of green tea infusion and grilling as cooking procedure as far as MeHg and inorganic Hg are concerned. Maulvault et al. (2011) also reported that a evident decrease in the risk of exceeding the TWI for MeHg in black scabbardfish (*A. carbo*), but the values exceed in the grilled samples for children, suggesting that this fish species might be consumed once per month in order to have few health hazards to the consumers.

548

549 **Conclusions**

550 The findings of the present study suggest that grilling and/or co-ingestion of green tea infusion
551 can effectively reduce THg and MeHg bioaccessibilities in the studied fish species, particularly
552 Atlantic wreckfish (57%) for THg and yellowfin tuna (54%) for MeHg. Therefore, the dietary
553 exposure to these toxic compounds (THg and MeHg) through the consumption of contaminated
554 seafood can significantly diminish in the presence of both variables (grilling and green tea).
555 Such information is crucial to consumers, particularly those with high consumption frequencies
556 of predatory fish species, as well as to health authorities and policy makers. Also, results of risk
557 assessment evidenced that fish species with MeHg contents that would yield average weekly
558 intakes above the TWI set for this element (particularly swordfish, blue shark and European
559 conger) may still be consumed without representing a risk to consumers (particularly adults and
560 pregnant women), if these fish products are ingested after grilling and in combination with
561 green tea infusion. Some exception still remains for more vulnerable groups (children and
562 adolescents), where there is a high concern of consumption of specific fish species, like
563 swordfish and blue shark. Nevertheless, a better understanding is needed in what concerns the
564 interactive effect of green tea in the bioacessibility and protective role against different chemical
565 contaminants. Therefore, further studies should be undertaken, particularly integrating human
566 intestinal cell models (bioavailability). Such information will also be useful to help consumers
567 to make wiser choices when it comes to the preparation of healthy meals, as well as it will
568 enable food safety and health authorities to integrate this information in risk assessment and
569 communication activities.

570

571 **CRediT authorship contribution statement**

572 **Patrícia Anacleto:** Conceptualization, Formal analysis, Investigation, Visualization,
573 Supervision, Writing – original draft, Writing - review & editing. **Vera Barbosa:** Formal

analysis, Investigation, Writing - review & editing. **Ricardo N. Alves:** Formal analysis, Investigation, Writing - review & editing. **Ana Luísa Maulvault:** Investigation, Writing - review & editing. **Maria Rosário Bronze:** Investigation, Resources, Writing - review & editing. **António Marques:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

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.

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771 **Figures legend**

772

773 **Fig. 1.** THg bioaccessibility (%) in raw (A) and grilled blue shark (B) in the presence or absence
774 of green tea infusion during the different *in vitro* digestion process phases (oral, gastric and
775 intestinal). Values represent mean ± standard deviation (n = 2). * represents significant
776 differences between samples without and with green tea in each *in vitro* digestion process phase
777 and different letters represents significant differences between the three *in vitro* digestion
778 process phases without (a-b) or with (A-C) green tea samples, respectively ($p < 0.05$).

779

780 **Fig. 2.** THg bioaccessibility (%) in raw and grilled blue shark in the presence or absence of
781 different concentrations of green tea infusion (n = 2). Different letters represents significant
782 differences between different concentrations of green tea for raw (a-b) or grilled (A-C) samples,
783 respectively ($p < 0.05$).

784

785 **Fig. 3.** Effect of green tea and grilling on THg bioaccessibility (%) in A) yellowfin tuna, B)
786 common smooth-hound, C) swordfish, D) Atlantic wreckfish, E) black scabbardfish, F) blue
787 shark and G) European conger. Values represent mean ± standard deviation (n = 3 except for
788 blue shark and European conger, n = 2). * represents significant differences between samples
789 without and with green tea and # represents significant differences between raw and grilled
790 samples for each fish species (ANOVA, $p < 0.05$). Please see detailed information in
791 Supplementary Table S1.

792

793 **Fig. 4.** Effect of green tea and grilling on MeHg bioaccessibility (%) in A) yellowfin tuna, B)
794 common smooth-hound, C) swordfish, D) Atlantic wreckfish, E) black scabbardfish, F) blue
795 shark and G) European conger. Values represent mean ± standard deviation (n = 3 except for
796 blue shark and European conger, n = 2). * represents significant differences between samples
797 without and with green tea and # represents significant differences between raw and grilled for

798 each fish species (ANOVA, $p < 0.05$). Please see detailed information in Supplementary Table

799 S2.

Table 1. Selected fish species from Portuguese local markets used to evaluate the effect of the green tea on the THg and MeHg bioaccessibility. THg and MeHg concentrations (µg g^{-1}, ww) and MeHg/THg (%) in raw and grilled fish species prior to *in vitro* digestion (Baseline)

Common name	Scientific name	Origin	Moisture (%)		THg (µg g^{-1}, ww)		MeHg (µg g^{-1}, ww)		MeHg/THg (%)	
			Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Yellowfin tuna [B]	*Thunnus* sp.	Pacific Ocean	72.8 ± 0.6^{a}	66.1 ± 1.9^{b}	1.953 ± 0.026^{a,*}	2.488 ± 0.008^{a}	1.360 ± 0.037^{a,*}	1.558 ± 0.064^{a}	69.7 ± 2.8^{c,*}	62.6 ± 2.8^{c}
Common smooth-hound [A]	*Mustelus mustelus*	Northeast Atlantic Ocean	79.6 ± 2.6^{a}	71.4 ± 2.7^{b}	0.532 ± 0.098^{ab,*}	0.759 ± 0.086^{ab}	0.392 ± 0.063^{ab,*}	0.549 ± 0.076^{ab}	75.5 ± 3.1^{c}	72.1 ± 2.3^{c}
Swordfish [B]	*Xiphias gladius*	Indic Ocean and Central Pacific Ocean	71.8 ± 4.6^{a}	63.7 ± 4.4^{b}	1.221 ± 0.013^{ab,*}	1.107 ± 0.520^{ab}	1.088 ± 0.084^{a}	0.898 ± 0.416^{ab}	82.9 ± 1.4^{abc}	81.4 ± 2.4^{ab}
Atlantic wreckfish [A]	*Polyprion* sp.	Northeast Atlantic Ocean and Pacific Ocean	79.3 ± 0.3^{a}	71.9 ± 1.7^{b}	0.163 ± 0.013^{b,*}	0.208 ± 0.025^{b}	0.134 ± 0.015^{b,*}	0.165 ± 0.019^{b}	79.7 ± 1.1^{abc}	79.4 ± 1.7^{b}
Black scabbardfish [B]	*Aphanopus carbo*	Northeast Atlantic Ocean	76.3 ± 3.0^{a}	72.9 ± 1.9^{b}	0.428 ± 0.023^{ab,*}	0.513 ± 0.054^{ab}	0.334 ± 0.021^{ab,*}	0.407 ± 0.037^{ab}	78.1 ± 5.7^{bc}	79.3 ± 2.6^{b}
Blue shark [B]	*Prionace glauca*	West Indic Ocean	79.3 ± 1.0^{a}	72.6 ± 1.0^{b}	1.099 ± 0.156^{a,*}	1.589 ± 0.329^{a}	0.973 ± 0.064^{a,*}	1.312 ± 0.208^{a}	89.3 ± 7.1^{ab}	83.3 ± 6.3^{ab}
European conger [A]	*Conger conger*	Northeast Atlantic Ocean	78.9 ± 1.9	72.7 ± 3.0	0.836 ± 0.765^{ab}	1.052 ± 0.957^{ab}	0.726 ± 0.644^{ab}	0.907 ± 0.824^{ab}	91.4 ± 8.7^{a}	86.5 ± 2.2^{a}

Values are expressed as mean ± standard deviation (n = 3 except for blue shark and European conger, n = 2). For each culinary treatment and analysis, different letters (a-c) indicate significant differences between fish species ($p < 0.05$). * indicate significant differences between culinary treatments for each fish species and analysis ($p < 0.05$). A and B indicate maximum levels (MLs) of 0.5 µg g^{-1} ww for fish in general and 1.0 µg g^{-1} ww for larger predatory fish species, respectively (EC, 2008).

Table 2. Concentrations (mg L^{-1}) of catechins and gallic acid in green tea infusions by HPLC and respective retention times and limit of detection.

	Retention time (min)	**Limit of Detection (mg L^{-1})**	**Concentration (mg L^{-1})**
Gallic acid (GA)	5.9	0.04	12.31
Gallocatechin (GC)	10.6	0.76	37.26
Epigallocatechin (EGC)	18.8	0.48	2.55
Catechin (C)	19.9	0.16	24.62
Epicatechin (EC)	28.09	0.18	15.37
Epigallocatechin gallate (EGCG)	29.2	0.12	24.92
Gallocatechin gallate (GCG)	33.8	0.18	43.82
Epicatechin gallate (ECG)	41	0.10	26.98
Catechin gallate (CG)	43.8	0.12	10.33

Table 3. Percentage of MeHg tolerable weekly intakes (TWIs; %) in different population groups accomplished with the consumption of 150 g of fish per week for adolescents, pregnant women and adults and with the consumption of 75 g of fish per week for children.

Fish species	Culinary treatment	Presence of tea	Children		Adolescents		Pregnant women		Adults	
			BD (%)	BIO (%)	BD (%)	BIO (%)	BD (%)	BIO (%)	BD (%)	BIO (%)
Yellowfin tuna	Raw	No tea	66	44	70	47	55	36	43	29
	Grilled		**123**	49	**131**	53	**103**	41	81	32
	Raw	Green tea	66	25	70	27	55	21	43	16
	Grilled		**123**	35	**131**	37	**103**	29	81	23
Common smooth-hound	Raw	No tea	98	34	**105**	37	82	29	65	23
	Grilled		**138**	27	**147**	29	**115**	23	90	18
	Raw	Green tea	98	38	**105**	41	82	32	65	25
	Grilled		**138**	16	**147**	17	**115**	13	90	10
Swordfish	Raw	No tea	**273**	98	**292**	**104**	**228**	82	**179**	64
	Grilled		**316**	58	**338**	62	**264**	48	**207**	38
	Raw	Green tea	**273**	58	**292**	63	**228**	49	**179**	38
	Grilled		**316**	31	**338**	34	**264**	26	**207**	21
Atlantic wreckfish	Raw	No tea	34	15	36	16	28	12	22	10
	Grilled		41	10	44	11	35	9	27	7
	Raw	Green tea	34	12	36	13	28	10	22	8
	Grilled		41	6	44	7	35	5	27	4
Black scabbardfish	Raw	No tea	84	42	90	45	70	35	55	28
	Grilled		**102**	20	**109**	22	85	17	67	13
	Raw	Green tea	84	18	90	19	70	15	55	12
	Grilled		**102**	12	**109**	13	85	10	67	8
Blue shark	Raw	No tea	**244**	**107**	**261**	**114**	**204**	89	**160**	70
	Grilled		**329**	96	**352**	**103**	**275**	80	**216**	63
	Raw	Green tea	**244**	76	**261**	81	**204**	64	**160**	50
	Grilled		**329**	49	**352**	52	**275**	41	**216**	32
European conger	Raw	No tea	**182**	65	**195**	70	**152**	55	**120**	43
	Grilled		**228**	26	**243**	28	**190**	22	**150**	17
	Raw	Green tea	**182**	53	**195**	57	**152**	45	**120**	35
	Grilled		**228**	21	**243**	22	**190**	17	**150**	14

Abbreviations: BD, before digestion; BIO, bioaccessible fraction.

Values are expressed as percentage of the tolerable weekly intake (TWI) set by EFSA (EFSA, 2012) for MeHg (1.3 μg kg^{-1} of individual b.w.), considering an average b.w. in Europe of 23, 43, 55 and 70 kg for children (3–<10 years old), adolescents (10–<18 years old), pregnant women's (18–35 years old) and adults (≥18 years old), respectively.

When TWI percentages are above 100%, the values are shown in bold.

Table 4. Percentage of inorganic Hg tolerable weekly intakes (TWIs; %) in different population groups accomplished with the consumption of 150 g of fish per week for adolescents, pregnant women and adults and with the consumption of 75 g of fish per week for children.

Fish species	Culinary treatment	Presence of tea	Children		Adolescents		Pregnant women		Adults	
			BD (%)	BIO (%)	BD (%)	BIO (%)	BD (%)	BIO (%)	BD (%)	BIO (%)
Yellowfin tuna	Raw	No tea	6	3	7	3	5	3	4	2
	Grilled		13	4	13	4	10	3	8	3
	Raw	Green tea	6	2	7	2	5	1	4	1
	Grilled		13	2	13	3	10	2	8	2
Common smooth-hound	Raw	No tea	9	4	9	5	7	4	6	3
	Grilled		12	2	13	2	10	2	8	1
	Raw	Green tea	9	3	9	3	7	3	6	2
	Grilled		12	1	13	2	10	1	8	1
Swordfish	Raw	No tea	20	10	21	10	17	8	13	6
	Grilled		25	4	27	5	21	4	17	3
	Raw	Green tea	20	6	21	6	17	5	13	4
	Grilled		25	2	27	3	21	2	17	2
Atlantic wreckfish	Raw	No tea	3	1	3	1	2	1	2	1
	Grilled		3	1	4	1	3	1	2	0
	Raw	Green tea	3	1	3	1	2	1	2	1
	Grilled		3	0	4	0	3	0	2	0
Black scabbardfish	Raw	No tea	7	3	7	4	6	3	5	2
	Grilled		8	2	9	2	7	1	6	1
	Raw	Green tea	7	1	7	2	6	1	5	1
	Grilled		8	1	9	1	7	1	6	1
Blue shark	Raw	No tea	18	8	19	9	15	7	12	5
	Grilled		26	7	28	8	22	6	17	5
	Raw	Green tea	18	6	19	6	15	5	12	4
	Grilled		26	4	28	5	22	4	17	3
European conger	Raw	No tea	14	5	15	5	11	4	9	3
	Grilled		17	2	18	2	14	1	11	1
	Raw	Green tea	14	4	15	4	11	3	9	3
	Grilled		17	2	18	2	14	1	11	1

Abbreviations: BD, before digestion; BIO, bioaccessible fraction.

Values are expressed as percentage of the tolerable weekly intake (TWI) set by EFSA (EFSA, 2012) for inorganic Hg (4 μg kg^{-1} of individual body weight; b.w.), considering an average b.w. in Europe of 23, 43, 55 and 70 kg for children (3–<10 years old), adolescents (10–<18 years old), pregnant women's (18–35 years old) and adults (≥18 years old), respectively.

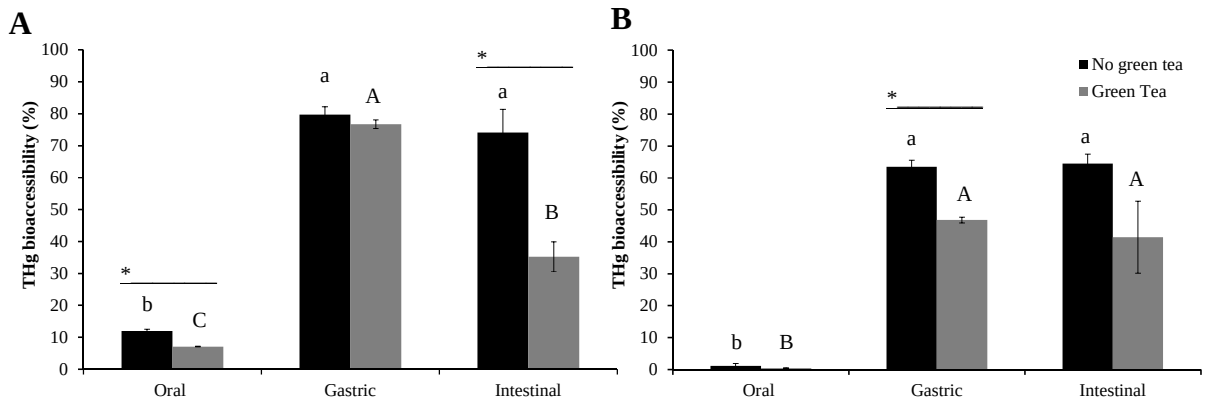

Fig. 1. THg bioaccessibility (%) in raw (A) and grilled blue shark (B) in the presence or absence of green tea infusion during the different *in vitro* digestion process phases (oral, gastric and intestinal). Values represent mean ± standard deviation (n = 2). * represents significant differences between samples without and with green tea in each *in vitro* digestion process phase and different letters represents significant differences between the three *in vitro* digestion process phases without (a-b) or with (A-C) green tea samples, respectively ($p < 0.05$).

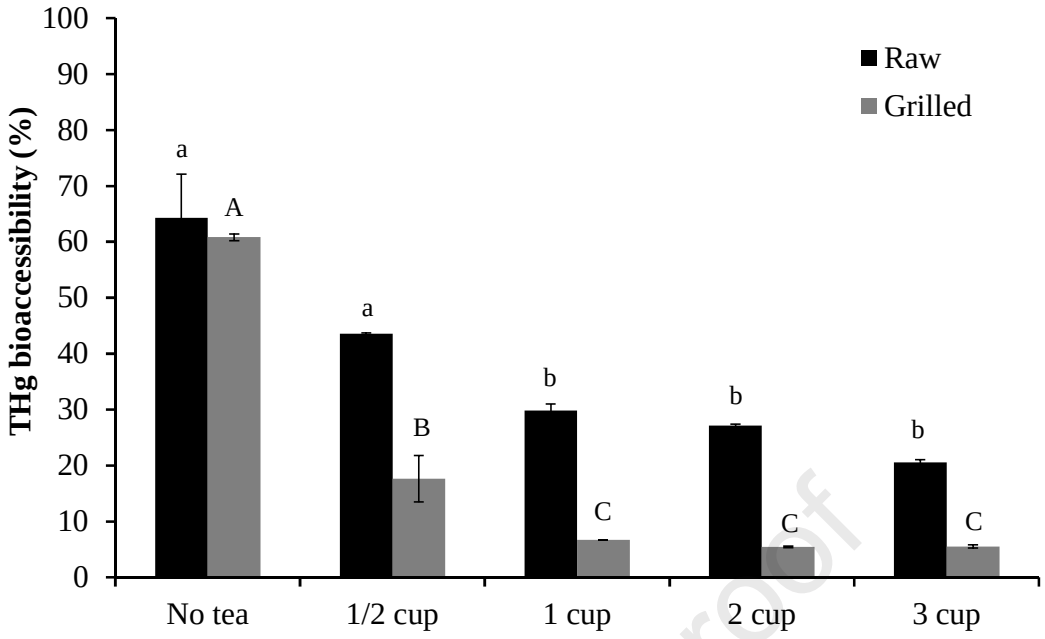

Fig. 2. THg bioaccessibility (%) in raw and grilled blue shark in the presence or absence of different concentrations of green tea infusion (n = 2). Different letters represents significant differences between different concentrations of green tea for raw (a-b) or grilled (A-C) samples, respectively ($p < 0.05$).

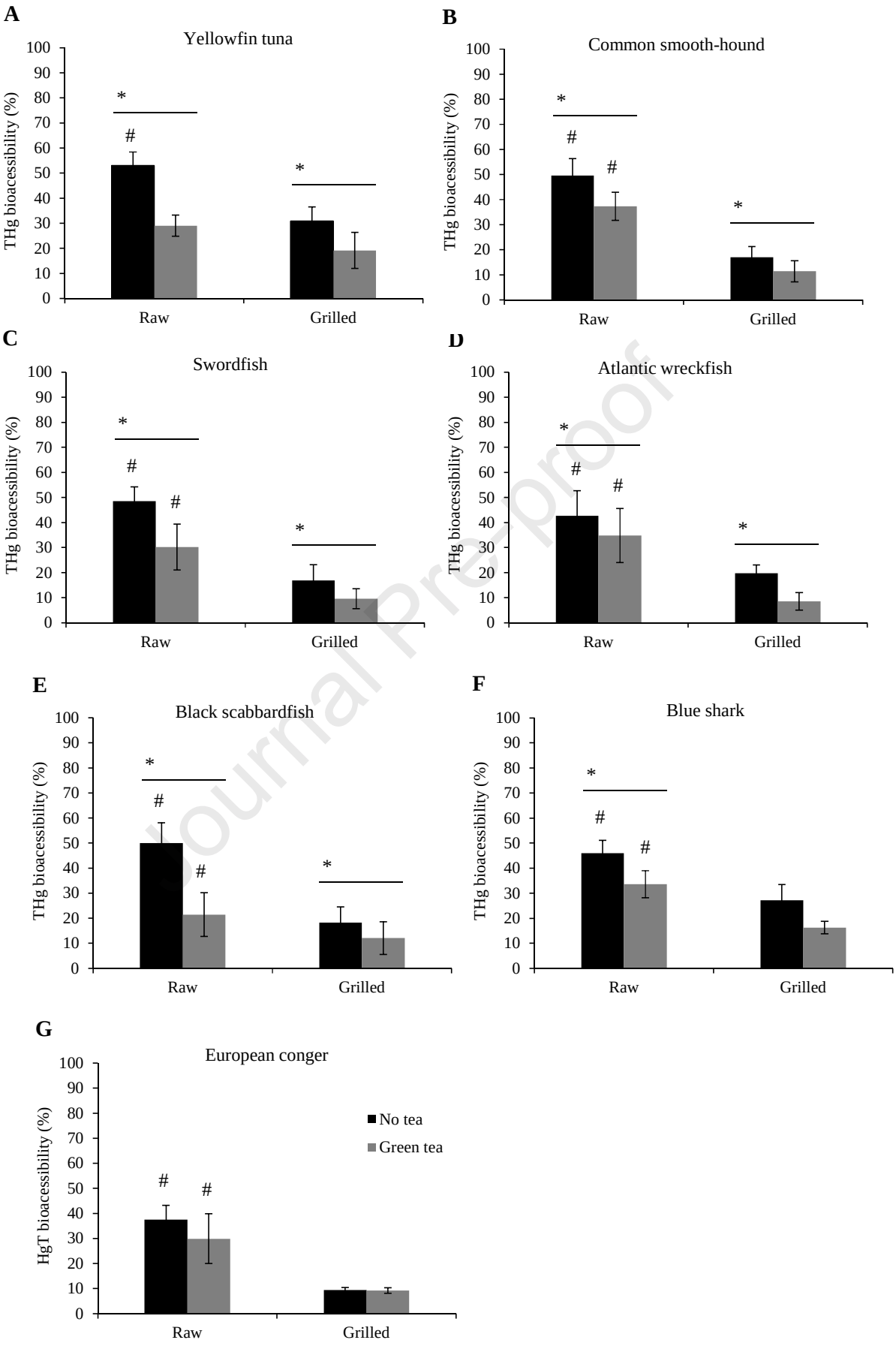

Fig. 3. Effect of green tea and grilling on THg bioaccessibility (%) in A) yellowfin tuna, B) common smooth-hound, C) swordfish, D) Atlantic wreckfish, E) black scabbardfish, F) blue shark and G) European conger. Values represent mean ± standard deviation (n = 3 except for blue shark and European conger, n = 2). * represents significant differences between samples without and with green tea and # represents significant differences between raw and grilled samples for each fish species (ANOVA, $p < 0.05$). Please see detailed information in Supplementary Table S1.

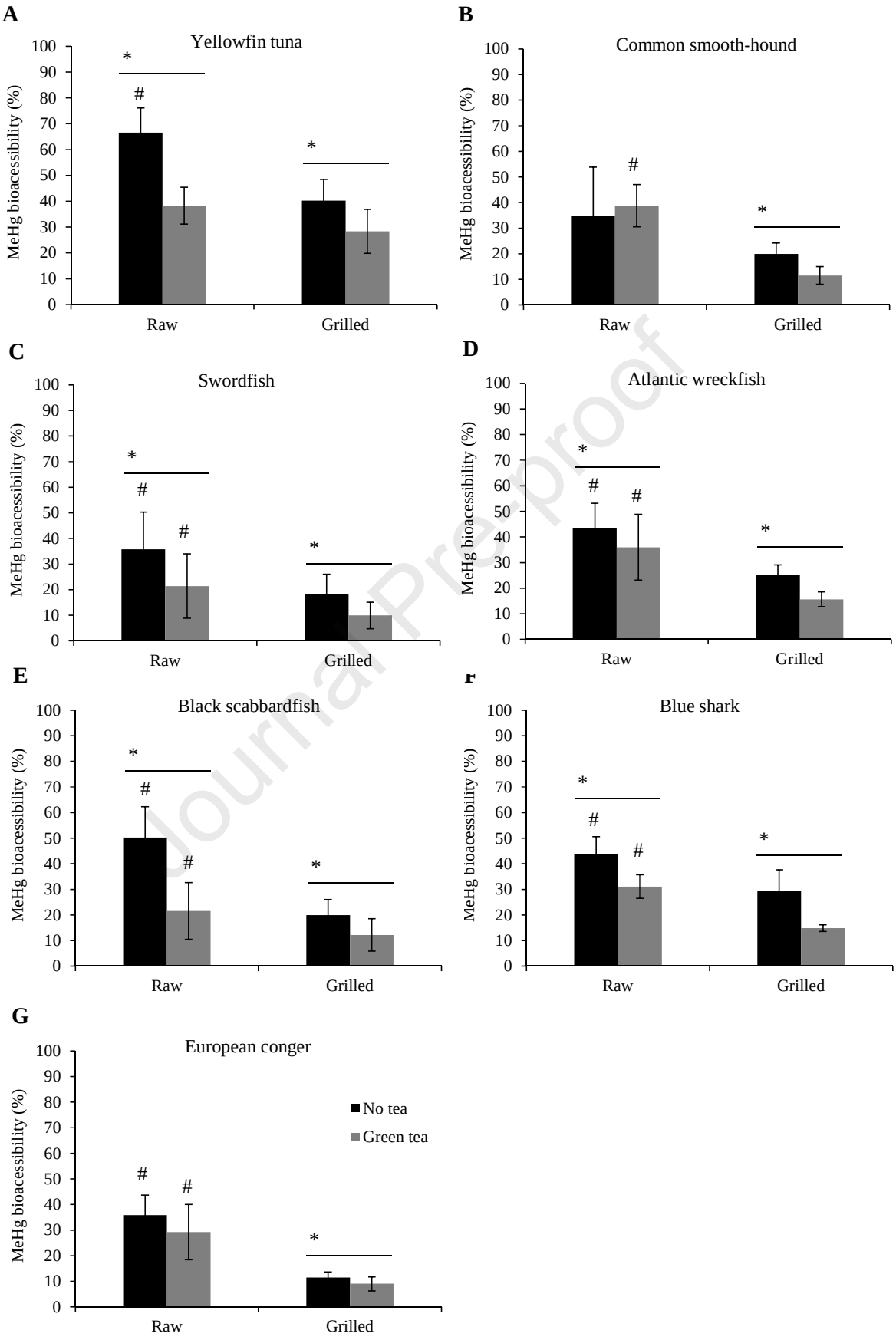

Fig. 4. Effect of green tea and grilling on MeHg bioaccessibility (%) in A) yellowfin tuna, B) common smooth-hound, C) swordfish, D) Atlantic wreckfish, E) black scabbardfish, F) blue shark and G) European conger. Values represent mean ± standard deviation (n = 3 except for blue shark and European conger, n = 2). * represents significant differences between samples without and with green tea and # represents significant differences between raw and grilled for each fish species (ANOVA, $p < 0.05$). Please see detailed information in Supplementary Table S2.

Highlights

- One cup or more of green tea diminished bioaccessible THg in oral/intestinal phases
- Grilling in combination with green tea strongly reduces THg and MeHg bioaccessibility
- Green tea infusion with grilling diminishes the probability of exceeding MeHg TWI
- Useful to help consumers to make wiser choices in the preparation of healthy meals
- Applicable to seafood safety authorities integrate this info in risk assessment

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: