Cooking and co-ingested polyphenols reduce *in vitro* methylmercury bioaccessibility from fish and may alter exposure in humans

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Running title

MeHg bioaccessibility is reduced by food preparation

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Key Words

Methylmercury, bioaccessibility, cooking, polyphenols, tea, risk assessment

Abstract

Fish consumption is a major pathway for mercury exposure in humans. Current guidelines and risk assessments assume that 100% of methylmercury (MeHg) in fish is absorbed by the human body after ingestion. However, a growing body of literature suggests that this absorption rate may be overestimated. We used an *in vitro* digestion method to measure MeHg bioaccessibility in commercially-purchased fish, and investigated the effects of dietary practices on MeHg bioaccessibility. Cooking had the greatest effect, decreasing bioaccessibility on average to 12.5 ± 5.6%. Polyphenol-rich beverages also significantly reduced bioaccessibility to 22.7 ± 3.8% and 28.6 ± 13.9%, for green and black tea respectively. We confirmed the suspected role of polyphenols in tea as being a driver of MeHg's reduced bioaccessibility, and found that epicatechin, epigallocatechin gallate, rutin and cafeic acid could individually decrease MeHg bioaccessibility by up to 55%. When both cooking and polyphenol-rich beverage treatments were combined, only 1% of MeHg remained bioaccessible. These results call for *in vivo* validation, and suggest that dietary practices should be considered when setting consumer guidelines for MeHg. More realistic risk assessments could promote consumption of fish as a source of fatty acids, which can play a protective role against cardiovascular disease.

1. Introduction

A large proportion of the world's population depends on fish. Indeed, fish are estimated to provide 17% of animal proteins consumed by humans (and 6.7% of all proteins consumed worldwide) (Food and Agriculture Organization, 2016), and are an important source of vitamins, minerals and fatty acids, which can protect from cardiovascular disease (Mahaffey et al., 2011). However, fish consumption is one of the major pathways of human exposure to mercury (Hg) (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States, 2010), which in its organic form of methylmercury (MeHg) is a potent neurotoxin (Clarkson and Magos, 2008). To protect at-risk populations, Hg blood guidelines have been established, derived from large-scale studies defining lowest adverse effect doses (Chapman and Chan, 2000; Legrand et al., 2010).

However, there is also a growing body of evidence suggesting that our understanding of Hg absorption in the body is incomplete. Current recommendations on fish consumption consider that the ingested dose of Hg from fish is equal to MeHg's – this assumes that 100% of Hg in fish is in the form of MeHg, and that MeHg's absorption rate is of 100% (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States, 2010; Ha et al., 2016). This stems from older studies performed on human volunteers (Aberg et al., 1969) and on rats (Miettinen et al., 1971) with methylmercuric nitrate ($MeHgNO_3$). However, this may not be representative of MeHg speciation in fish, which is more likely bound to thiol groups included in proteins (Clarkson and Magos, 2008; Harris, 2003). Indeed, assuming that nearly all of Hg in fish is bioavailable may overestimate intake by 50% (Ha et al., 2016): while the absorption rate of solubilized MeHg may be high, not all MeHg is necessarily freed from the fish matrix into digestive fluids (i.e. made bioaccessible) and made available for absorption by the body following metabolism in the intestine by the gut microbiome or in the liver (bioavailable) (Afonso et al., 2015a). Thus, to postulate near total MeHg bioavailability overlooks processes that may occur before absorption and into systemic circulation. This is supported by studies reporting that Hg bioaccessibility is not positively correlated to concentration in the consumed food (Laird and Chan, 2013; Laird et al., 2009a). While biomarkers like blood or hair Hg show robust relationships to Hg intake (Abdelouahab et al., 2008; Cole et al., 2004; Kosatsky et al., 2000; Legrand et al., 2005; Mahaffey and Mergler, 1998), in most of these studies, Hg intake is estimated from food frequency questionnaires and the literature on the consumed fish species, rather than direct Hg measurements (Abdelouahab et al., 2008; Sunderland, 2007), meaning that exact Hg intake is frequently unknown. Furthermore, there is evidence that populations exhibit toxicological

responses to Hg in different ways (Canuel et al., 2006a; Chapman and Chan, 2000). As Hg remains a contaminant of major concern (Mergler et al., 2007), it is critical we better understand its fate in the body. A cost-effective and non-invasive way of doing so is through *in vitro* bioaccessibility studies, to first investigate the fate of Hg in the gastrointestinal tract.

Many factors could be responsible for altering MeHg bioaccessibility from ingested food. Food matrix composition may affect the fate of MeHg in the body, with one study reporting that Hg from the flesh of a salmonid may be 6-fold more bioaccessible than that from marine mammalian organs (Laird et al., 2009a). Different levels of Hg bioaccessibility have also been reported for various fish species (H.-S. Wang et al., 2013). Fish handling by industries and by consumers could also alter bioaccessibility. While freezing can induce physicochemical changes to meat (Farouk et al., 2004; Sanza et al., 1999), it is widely used in fish processing to prevent spoilage (George, 1993), which could change MeHg bioaccessibility before fish become available on the market for purchase. Consumer-based food preparation can significantly transform meat, with cooking and drying reducing moisture, crude protein content and total lipids (Toyes-Vargas et al., 2016). Indeed, cooking has been found to reduce Hg and MeHg bioaccessibility (Afonso et al., 2015b; He and W.-X. Wang, 2011; Jadán Piedra et al., 2016; Ouédraogo and Amyot, 2011; Torres-Escribano et al., 2011; 2010). *In vitro* studies have also suggested that foods rich in plant polyphenols (such as tea) may reduce MeHg bioaccessibility (He and W.-X. Wang, 2011; Ouédraogo and Amyot, 2011; Shim et al., 2009). Dietary practices may thus alter the way MeHg is solubilized from food (bioaccessibility), and ultimately change its bioavailability. A better understanding of these processes could lead to easily implementable guidelines and recommendations to reduce Hg loading in fish-consuming populations.

The goal of this study was to explore how dietary practices can alter MeHg bioaccessibility, using an *in vitro* digestion model. We explored how various cooking techniques and the co-ingestion of polyphenol-rich foods could alter MeHg solubilization from food. We also investigated the role of specific polyphenols in driving this effect which had been hypothesized in the literature, but never confirmed. We also assessed the potential effect of combined dietary practices on MeHg bioaccessibility. Finally, we report how these dietary practices can affect MeHg intake and loading in the body, and propose ways to use this information to inform future research and guidelines.

2. Methods

2.1. Food items, co-ingested foods and polyphenols

Experiments were performed on swordfish, grouper, tuna and salmon filets obtained from fish markets in Montreal. These species were selected to reflect fish readily available to Canadian consumers year-round. Blueberries, coffee (Nescafé, Maxwell) and green and black teas of various brands (Twinnings, Stash, Green Sail, Salada) were purchased in Montreal supermarkets as were corn oil (Mazola) used for cooking treatments, and cornstarch (Ideal), used as a non-polyphenol control. Pure polyphenols (gallic acid (>97.5%), catechin >98%), epigallocatechin gallate (>80%), theaflavin (>80%), rutin (>94%)) were obtained from Sigma-Aldrich.

2.2. Food preparation methods

Three cooking methods were tested: grilling, frying and boiling. Grilling was performed on a Teflon-coated pan, at 100 and 150 °C for 1 min. Frying treatments were conducted in 1 mL of corn oil, in glass vials heated on a burner for 1 min. Samples were boiled in 2 mL of ultrapure MilliQ water (> 18.2 MΩ cm^{-1}) (EMD Millipore) in glass vials for 5 or 10 min. Temperature was monitored throughout cooking. For freezing, fish samples were subsampled immediately following their purchase and placed in glass vials, and kept at -20, -80 °C or flash frozen in liquid nitrogen (then kept at -80 °C). Glassware was rinsed with distilled water, soaked in a 45% HNO3, 5% HCl (Fisher Scientific, ACS-pure) bath overnight and rinsed 3 times with MilliQ water before use.

For co-ingestion experiments, fish samples were digested simultaneously with either beverages or pure polyphenols. Beverages (tea, coffee, instant coffee) were prepared as per the manufacturer's instructions, and lyophilized overnight into a powder (Freezone6, Labconco). Powdered beverages were solubilized in 2 mL of MilliQ water, in two different doses: 40 mg or 120 mg, and were added to fish at the start of *in vitro* digestion experiments. In these experiments, controls were amended with 2 mL of MilliQ water to adjust the volume. Pure polyphenols were solubilized in 2 mL of dimethyl sulfoxide (DMSO), in amounts of 5 or 10 mg, and used in *in vitro* digestions. Controls with no polyphenols were also amended with 2 mL of DMSO, to account for volume increase.

2.3. Physiologically-based extraction test

Many *in vitro* digestion protocols exist to assess bioaccessibility of nutrients and dietary compounds (Dong et al., 2016; Minekus et al., 2014; Van de Wiele et al., 2007). We selected the Physiologically-based extraction test (PBET), adapted from Ruby et al. (1996) and Ouédraogo

and Amyot (2011), to perform digestive simulations, as it has been used frequently for metals and Hg (Calatayud et al., 2012; Ouédraogo and Amyot, 2011; Siedlikowski et al., 2016). All digestive simulations were performed on 1.0 ± 0.1 g of fresh fish sample, in triplicate. Experimental solutions were prepared in acid-washed Teflon bottles prior to each PBET digestion. The gastric phase was prepared by combining 1.25 g porcine pepsin (>400 units/mg), 0.50 g sodium citrate (>99%), 0.50 g malic acid (>99%), 420 μL lactic acid (>85%) and 500 μL of acetic acid (99.7%) (purchased from Sigma-Aldrich and Fisher Scientific**)** in ultrapure MilliQ water in a final volume of 1 L, and pH was adjusted to 2 with HCl (OmniTrace Ultra, EMD). The intestinal phase contained 0.60 g bile salts and 0.15 g pancreatin (4 x USP grade, lipase >24 units/mg, protease >400 units/mg) (Sigma-Aldrich), in a final volume of 250 mL 1 M $NaHCO_3$.

Briefly, samples were placed in Falcon tubes with 40 mL of gastric solution, and were incubated at 37 °C with agitation (100 rpm) for 1 hour. pH was then adjusted to 7 using 5 M NaOH. Nine mL of intestinal solution were added to all samples, which were incubated at 37 °C with agitation (100 rpm) for 2 hours. Following incubation, samples were centrifuged for 15 minutes at 3,000 g. The supernatant, considered to contain the bioaccessible (solubilized) fraction of MeHg, was isolated and used for MeHg analyses.

2.4. Bioaccessibility

Bioaccessibility was calculated after PBET simulations with the following equation:

[Equation 1]

$$\%\ bioaccessibility = \frac{[MeHg]\ in\ PBET\ \ (ng/L)\ \ x\ PBET\ volume\ (L)}{[MeHg]\ in\ fish\ (ng/g)\ x\ fish\ mass\ \ (g)}\ x\ 100$$

with *[MeHg] in PBET* being MeHg measured in the extract of the simulated digestion, *PBET volume* being PBET digestive fluids volume, *[MeHg] in fish* is [MeHg] in initial fish sample, and *fish mass* is the mass of fresh fish used as input into the PBET simulation.

Multiple bioaccessibility experiments were also performed using the same commercially-purchased fish filet over different days (stored at 4 °C between experiments). Bioaccessibility values for controls were compared across runs, and we found no statistical differences within a single fish filet (with the number of experimental days performed on each fish individual varying from 2 to 5) (Kruskal-Wallis, $P > 0.05$) (Figure S2). This showed us that we could use multiple samples from one individual fish filet over several days with minimal impact on bioaccessibility.To compare different sets of experiments, we normalized results within each experiment to raw

untreated fish muscle (using Equation 2), giving a percent of bioaccessible MeHg compared to controls (now normalized to 100%).

[Equation 2]

$$\%\ bioaccessibility\ compared\ to\ control = \frac{average\ treated\ \%}{average\ control\ \%}\ x\ 100$$

where average treated % refers to the mean bioaccessibility obtained across triplicates of a given treatment, and average control % refers to mean bioaccessibility calculated in untreated triplicates of raw fish muscle from the same run. Non-normalized control values are presented in Table 1.

2.5. MeHg analyses

MeHg in fish was measured in freeze-dried samples (Freezone6, Labconco), while MeHg following simulated digestion was analyzed in PBET fluids. Prior to analysis, both dried fish samples and PBET fluids were extracted overnight in 5 mL of 4 M HNO_3 (Fisher Scientific, ACS-pur) at 60 °C. MeHg in fish and PBET solutions was measured by gas chromatography and cold-vapor fluorescence spectrometry (CVAFS) (Tekran 2700, Tekran Instruments Corporation), according to U.S. EPA method 1630 (detection limit of 0.01 ng L^{-1}, defined as three times the standard deviation calculated on 10 ultrapure MilliQ blanks).

2.6. Fish matrix characterization

Lipids were quantified by gravimetry, using a method adapted from Folch et al., 1957. Nitrogen was used as a proxy for protein content and was quantified with a CHN Element Analyzer 1108 (Thermo Fisher). Moisture content in fish muscle was quantified by subtracting sample dry weight from wet weight after drying. Full methods on fish matrix characterization are presented in Supplementary Information.

2.7. Polyphenol analyses

Polyphenols were quantified by ultra-performance liquid chromatography with tandem mass spectrometer (UPLC-MSMS) at the Institute of Nutrition and Functional Foods (Quebec, Canada) using a Waters Acquity Ultra-Performance LC system (Waters). The full method is presented in Supplemental Information. The seven polyphenols that were quantified in co-ingested foods and subsequently tested in their purified form are presented in Table S2. Complete profiles of the 56 polyphenols analyzed in co-ingested foods are presented in Table S3.

2.8. Risk assessment

We estimated a probable daily intake (PDI) (ug kg^{-1}) for an average adult for each of the fish tested in this study, using the following equation:

[Equation 3]

$$PDI = \frac{[MeHg\ in\ fish\ ](ug/g)\ x\ average\ daily\ fish\ intake\ (g)}{average\ adult\ body\ weight\ (kg)}$$

where *[MeHg in fish]* is the MeHg concentration measured in the fish sample tested; *average daily fish intake* is based on fish consumption values from the Bureau of Chemical Safety of Canada (22 g for an adult) (Bureau of Chemical Safety Health Canada, 2004*)*; and the *average adult body weight* is based on values from Nutrition Canada (60 kg for an adult) (Health Canada, 2004). PDIs represent average exposure to MeHg, if an adult consumed each fish daily over a long period of time. We then calculated a bioaccessibility-corrected PDI (PDI_{BA}):

[Equation 4]

$$PDI_{BA} = PDI\ x\ \%\ bioaccessibility$$

where the *% bioaccessibility* is the soluble fraction calculated from our experiments. This PDI_{BA} accounts for the bioaccessibility of MeHg in the *in vitro* model and the effect of food preparation and co-ingestion treatments.

2.9. Statistical analyses

Statistical analyses were performed with R software (R Development Core Team) using nonparametric methods, as normality and homoscedasticity were not respected (tested with shapiro.test() and bartlett.test() functions). Differences in bioaccessibility across treatments were compared with Kruskal-Wallis analysis of variance (kruskal() in the agricolae{} package) (De Mendiburu, 2012), and Bonferonni corrections were used to correct for multiple hypothesis testing. Linear regressions were used to model bioaccessibility and lipid content, and ordinations was calculated on log-transformed data. Plots were prepared using the ggplot2{} (Wickham, 2009) and ggbiplot{} (Vu, 2011) packages. Letters on plots denote significantly different treatments ($P < 0.05$), bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

3. Results

3.1. Fish sample characterization and inter-fish and inter-simulation variation

Mean MeHg concentrations in fish samples tested were below Health Canada guidelines (Food Directorate Bureau of Chemical Safety, Health Canada) (500 ng g^{-1} for retail fish, 1000 ng g^{-1} for swordfish and tuna) (average concentration in swordfish = 439 ± 237 ng g^{-1}; in grouper: 612 ± 315 ng g^{-1}; in tuna = 694 ± 778 ng g^{-1} and in salmon = 20.1 ng g^{-1}), except for one grouper (835 ng g^{-1}) and one tuna (1244 ng g^{-1}) (Table S1). Protein, lipid and moisture content did not vary across fish species (Kruskal-Wallis, $P > 0.05$) (Table S1), and we found no correlation between MeHg bioaccessibility and lipid content in fish ($P > 0.05$) (Figure S1).

Multiple fresh filets from different individual fish were tested for each species. For grouper and tuna, we observed significant differences in MeHg bioaccessibility among individual fish (Kruskal-Wallis, $P < 0.05$) (Figure S2). Differential lipid content in the muscles tested did not account for this variation, as we observed no relationship between lipids and raw bioaccessibility percentages when considering the four fish species tested in this study (Figure S2). The use of only one salmon in this study may mask potential variation between fish, and inter-individual absolute bioaccessibility may vary in ways similar to grouper and tuna. However, this study does not aim to report absolute bioaccessibility values, and trends from treatments experiments were robust across individuals for all species (see Sections 3.2 & 3.3).

3.2. Effects of cooking and freezing on MeHg bioaccessibility

Cooking had a significant impact on MeHg bioaccessibility compared to raw controls in all fish species tested. In swordfish (Kruskal-Wallis, $P < 0.05$) (Figure 1A), on average, MeHg bioaccessibility was reduced to 12.6 ± 5.6% of control values across all cooking treatments. Grilling and frying at maximal temperatures (150 and 160 °C) reduced bioaccessibility to 18.0 ± 5.6 and 7.1 ± 1.2% respectively, while boiling decreased bioaccessibility to 8.4 ± 3.0% of control values (Figure 1A). Lower temperatures or cooking times did not yield significantly greater bioaccessibility losses ($P > 0.05$). The effect of cooking was consistent for grouper (18.6 ± 10.3%), tuna (12.2 ± 5.5%) and salmon (10.9 ± 6.7%) ($P < 0.05$) (Figure 1B-D). In all fish species, frying tended to be the most effective at reducing MeHg bioaccessibility, but this trend was not significant ($P > 0.05$) (Figure 1).

When swordfish samples were frozen, decreasing freezing temperatures, from -20 to -80 °C and flash freezing did not lead to significantly different MeHg bioaccessibility levels ($P > 0.05$) (Figure S3).

3.3. Effects of co-ingested foods and polyphenols on MeHg bioaccessibility

We tested the effects of co-ingested foods on MeHg bioaccessibility in swordfish and in tuna compared to unamended raw controls (Figure 2). As previous reports have suggested that polyphenols were responsible for altered MeHg bioaccessibility from fish (He and W.-X. Wang, 2011; Ouédraogo and Amyot, 2011; Shim et al., 2009), we selected several polyphenol-rich foods and beverages, and one food not enriched in polyphenols (cornstarch). Our results show that green tea led to the lowest MeHg bioaccessibility values in swordfish (22.7 ± 3.8% when 120 mg was used) (Figure 2A-B) and in tuna (34.8 ± 5.6% with 120 mg of tea) ($P < 0.05$) (Figure 2C). Black tea also significantly decreased MeHg bioaccessibility in swordfish (28.6 ± 13.9% with 120 mg of tea) ($P < 0.05$). The effect of tea was greater when 120 mg of dried tea (equivalent to approximately 375 mL of prepared tea) was used compared to 40 mg (approximately 125 mL). As expected, cornstarch, which contained negligible amounts of polyphenols (Table S2) had no effect on MeHg bioaccessibility (Figure 2B). Coffee and instant coffee had a slight, yet non-significant effect, while blueberries had no detectable impact ($P > 0.05$) (Figure 2C). Therefore, it appears only some polyphenol-rich beverages can alter MeHg bioaccessibility.

To identify which compounds may be responsible for this effect, we quantified 56 polyphenols in the foods that were used in bioaccessibility experiments. Of these, we found that gallic acid and flavonoids (including flavanol quercetins such as rutin, and flavon-3-ol catechins and theaflavins) were abundant in green and black tea, and in small or undetectable amounts in the other tested co-foods (Table S2). Multivariate analysis showed that treatments with the greatest decreases in bioaccessibility in this experiment were positively associated with certain polyphenol groups such as catechins, quercetins, thearubigins and kaempferols (Figure S4).

To verify the hypothetical role of these polyphenols on MeHg bioaccessibility, we repeated PBET simulations using individual purified polyphenol compounds. First, we observed that the effect of polyphenols increased with the amount added to digestion experiments, from 5 to 10 mg (Figure 3). However, while catechin is cited in the literature as a hypothetical driver of reduced MeHg bioaccessibility (He and W.-X. Wang, 2011), we found its purified form had no significant effect ($P > 0.05$) (Figure 3A). Other forms of catechin, including cis-configuration epicatechin and epigallocatechin gallate (EGCG) did significantly limit MeHg bioaccessibility to 61.7 ± 4.5 and 47.0 ± 15.5% of unamended control, respectively ($P < 0.05$). Another flavon-3-ol (rutin) and a

hydroxycinnamic acid (cafeic acid) also significantly reduced bioaccessibility to 55.6 ± 1.9%, and 44.8 ± 12.5%, respectively ($P < 0.05$) (Figure 3).

3.4. Effects of multiple dietary practices on MeHg bioaccessibility

Applied separately, cooking and polyphenols (as beverages or purified compounds) both impacted MeHg bioaccessibility compared to raw, unamended controls (Figures 1-3). We tested these treatments together within the same experiment, to assess their combined effects. Cooking swordfish (by grilling, frying or boiling) and digesting it with 120 mg of green or black tea led to less than 1% of MeHg remaining bioaccessible ($P < 0.05$, Figure 4A and B). Combining cooking and 10 mg of purified gallic acid or catechin also significantly decreased MeHg bioaccessibility, to 2-17% of bioaccessible MeHg compared to raw unamended controls (Figure 4C and D) ($P < 0.05$). The combined effects of cooking and co-ingested polyphenols (as beverages or purified extracts) were also observed in grouper ($P < 0.05$, Figure S6) and tuna ($P < 0.05$, Figure S7). In all cases, boiling combined with green or black tea were the most effective combination to reduce MeHg bioaccessibility, decreasing bioaccessibility by 99% compared to raw, unamended controls ($P < 0.05$, Figure 4, S6 and S7).

3.5. Bioaccessibility and risk assessments

Finally, we compared PDIs for an average adult calculated with the MeHg concentration measured in tested fish, to corrected PDI_{BAs} considering bioaccessibility and the effect of dietary practices. This is a theoretical exercise in the absence of *in vivo*-validated results, and is presented here only to compare the impact of different treatments, rather than to estimate realistic PDIs. Table S4 shows that the estimated daily intake for an adult is greatly reduced when bioaccessibility is considered. All values calculated from MeHg concentrations were below the provisional tolerable daily intake (the maximum amount of MeHg that can be ingested daily over a lifetime without increasing risk of health effects) established by the World Health Organization (0.23 ug kg^{-1} d^{-1}) (Joint FAO/WHO Expert Committee on Food Additives, 2007).

4. Discussion

4.1. Cooking reduces MeHg bioaccessibility from fish; freezing has no effect

Several studies have shown that cooking increases Hg concentrations in fish (Burger et al., 2003; Morgan et al., 1997; Perelló et al., 2008). It has been suggested that this may be due

to weight loss due to moisture and fat loss during cooking (Morgan et al., 1997). When considering bioaccessibility however, studies consistently show a reduction of solubilized Hg (Ouédraogo and Amyot, 2011; Torres-Escribano et al., 2011) and MeHg (He and W.-X. Wang, 2011) from cooked fish muscle. Our results on MeHg bioaccessibility were consistent with these earlier findings (Figure 1). Here we expanded on these studies by comparing multiple temperatures and cooking times for each cooking treatment, and we found no significant differences between 100 and 150 °C for grilling, 100 and 160 °C and frying, nor for 5 and 10 minutes for boiling (Figure 1). All temperatures tested were greater than 70 °C, the safe internal cooking temperature recommended by Health Canada (Health Canada First Nations Branch, Government of Canada, 2016). However, while fish cooked by consumers may reach this safe internal threshold, higher temperatures are typically used when preparing meals. Indeed, temperatures greater than 100 °C are typically used in studies measuring the effects of cooking on metals (Devesa et al., 2001; Ersoy et al., 2006) and heterocyclic amines (Oz et al., 2007). It is likely that the impact of heat on MeHg solubilization increases until a given temperature (below 100 °C), and further increases have no effect on MeHg bioaccessibility within the range of temperatures commonly used in cooking. Protein aggregation induced by high temperatures, leading to lowered pepsin digestibility, may explain these results. Cooking has also been found to induce the formation of disulfide bonds in proteins, which may further limit digestibility (Duodu et al., 2002; He et al., 2010; Kulp et al., 2003). This is supported by observations that heat induces structural changes to meat: at temperatures greater than 100 °C, oxidation can cause protein aggregation, slowing enzymatic digestion by pepsin (Bax et al., 2012).

It is important to note that these experiments were performed on small (1 g) sub-samples of fish muscle, which were thoroughly cooked during treatments. It is likely that in a thicker and larger fish filet more representative of an adult portion (150 g as per Health Canada) (Bureau of Chemical Safety Health Canada, 2007), cooking has more heterogeneous effects: temperature may not be even throughout the portion, and structural protein changes that might control MeHg bioaccessibility may vary across filet thickness. Nonetheless, these experiments on small test meals do provide insight into the effect of temperature on MeHg solubilization from proteins. Future experiments on cooking and MeHg should include portion-sized fish for a more realistic portrait of bioaccessibility.

Freezing can induce protein denaturation and physicochemical changes to meat (Farouk et al., 2004; Sanza et al., 1999). In a study of 27 samples of frozen swordfish, Torres-Escribano et al. suggested that variations in Hg bioaccessibility from 38-83% (64 $\pm$ 14%) may be attributable to protein denaturation caused by different freezing and thawing rates, or varying storage

temperatures (Torres-Escribano et al., 2010). In this study, we observed no relationship between bioaccessibility and colder temperatures. However, since all the fish tested in this study were purchased commercially, they had all likely been frozen before, as freezing is widely used in fish processing to prevent spoilage (George, 1993). While this industrial freezing performed after catch to conserve fish may alter bioaccessibility, our results suggest that a second freezing has no impact. Therefore, consumers who freeze commercially-purchased fish are unlikely to further impact MeHg bioaccessibility (Figure S3).

4.2. Plant polyphenols found in tea can limit MeHg solubilization

As plant metabolites present in all plant organs, polyphenols are an important component of human diets (Bravo, 2009), and dietary intake of flavonoids alone (which include the different catechins) has been estimated at 187 mg d^{-1} in the US (Chun et al., 2007). While they are of great interest in nutrition due to their ability to bind and precipitate certain molecules, and for their antioxidant effects in humans (Bravo, 2009), it is likely their role in metal chelation (Graham, 1992; Hider et al., 2001; Ragan et al., 1979; T. Wang et al., 2009) that drives their impact on MeHg bioaccessibility. We found that certain polyphenol-rich foods, such as green and black tea, could significantly reduce MeHg bioaccessibility, and that the effect increased with the amount of tea added (Figure 2). However, not all foods limited bioaccessibility: blueberries (rich in anthocyanidins) (Table S3), had no significant impact on MeHg bioaccessibility, suggesting that not all polyphenols have metal chelating properties.

The amounts of dried tea used in our experiments roughly reflect the ratio of one cup of tea consumed with one portion (150 g) of fish. The polyphenol molecules found in tea are suspected to reduce MeHg and Hg bioaccessibility (He and W.-X. Wang, 2011; Ouédraogo and Amyot, 2011; Shim et al., 2009) but the causal role of polyphenols was not directly investigated. Here, we tested a wide range of purified polyphenols to confirm their role in decreasing MeHg bioaccessibility, and to identify which compounds may be responsible for the effects of tea. Our results suggest that EGCG, caffeic acid and rutin likely have the greatest chelating properties (Figure 3), possibly forming insoluble complexes with MeHg and decreasing bioaccessibility. Indeed, metals chelated to polyphenols are considered to have low bioavailability: in humans, consumption of large amounts of tea or polyphenols is associated with poor iron absorption and anemia (Baynes and Bothwell, 1990). Our findings from fish and polyphenols support the results of Jadan Piedra et al., who tested the effects of catechin and tannic acids on bioaccessibility in standard MeHg aqueous solutions (Jadán Piedra et al., 2016). Other polyphenols that were measured in co-foods (Table S3) but that were not tested in their purified form could also

contribute to the effect of tea. Indeed, kaempferols, thearubigins or quercetins other than rutin may have a similar effect as EGCG for example (Figure S4). Their role should be investigated, by testing more compounds in bioaccessibility assays. Furthermore, tea may include other compounds that could limit MeHg bioaccessibility. Tea leaves growing in certain areas of China has been found to be enriched in selenium (Molan et al., 2009), and selenium can interfere with Hg toxicity and bioaccessibility (Cabañero et al., 2006; 2004). While our results from purified polyphenols suggest that these compounds are the main drivers of the effects of tea on MeHg bioaccessibility, other molecules in the beverage may also have a role to play.

Tea polyphenols may also have other impacts on the fate of MeHg in the body: *in vivo* assays have suggested that tea could accelerate enterohepatic cycling of MeHg in the body (Canuel et al., 2006b) or that tea extracts may limit oxidative stress induced by MeHg in rats and alter its pharmacokinetics (Black et al., 2011). Green tea has also been found to increase Hg load in blood after consumption of MeHg-contaminated fish (Janle et al., 2015). These mechanisms should be further investigated through controlled *in vivo* experiments.

4.3. Bioaccessibility studies can be used to inform current guidelines

Studies performed on Hg and MeHg bioaccessibility in fish report values that range from 9-100%, with large variations often seen within a single species (Table 1). However, over half of the values reported in Table 1 are below 50%, suggesting that only a fraction of mercury is solubilized (bioaccessible) during simulated digestion, and thus potentially available for absorption and metabolism in the intestine and liver (bioavailable) (Afonso et al., 2015a). Current guidelines and recommendations assume that 100% of MeHg in fish is readily absorbed by the gastrointestinal tract (Committee on Toxicological Effects of Methylmercury, National Research Council of the United States, 2010; Ha et al., 2016). This value is based on early work on the excretion of an orally administered radio-labelled MeHg nitrate solution from the human body (Aberg et al., 1969). However, mercury in fish is more typically bound to sulfur-rich groups such as thiols (Clarkson and Magos, 2008; Harris, 2003), and may behave differently. This suggests that the form of Hg and its complexation to food or other elements may reduce its absorption, and that the 100% MeHg absorption rate likely overestimates what is bioavailable from fish. When considering raw tuna, our PDI estimate which accounts for incomplete bioaccessibility (PDI_{BA}) is 71.7% lesser than PDI (Table S4).

These results are amplified when considering not only bioaccessibility, but also dietary practices. Indeed, when the effects of cooking are included in PDI calculations, bioaccessibility-

corrected PDI_{BAs} are reduced by 70.6 – 98.1% across all fish species (Table S4). As most fish consumed in North America is cooked (with the exception of sushi as well as traditional practices in indigenous communities), not including this factor in guidelines leads to overestimating MeHg exposure in most populations. If PDIs are corrected for green and black tea consumption, the bioaccessibility-corrected PDI_{BA} is reduced by 74.6 – 94.4% (Table S4). Finally, when correcting PDI for bioaccessibility data from a meal that is both cooked and consumed with a cup of tea, PDI_{BA} drops by 99%, to less than 1 ng kg^{-1} (Table S4). All PDIs presented here, calculated from commercially-purchased fish, were below the WHO's maximal provisional tolerable daily intake (PTDI) (0.23 ug kg^{-1}) (Joint FAO/WHO Expert Committee on Food Additives, 2007).

It is important to note that these PDI_{BAs} are calculated from the results of a simplified *in vitro* system. While these estimates do not replace *in vivo* or epidemiological studies, they provide valuable insight into the ways Hg is solubilized from food in the gut, and may explain population-based variations in Hg toxicological responses (Canuel et al., 2006a; Chapman and Chan, 2000). These bioaccessibility-based results can be used to guide more informative, but more costly *in vivo* studies, and *in vitro* assays may be of particular relevance to populations who frequently consume fish that are more heavily contaminated than commercially-monitored species. This includes recreational fishermen, coastal populations who often depend heavily on fish (Cisneros-Montemayor et al., 2016), and indigenous groups like the Inuit who's reliance on marine mammals and fish exposes them to higher dietary MeHg intake (Laird et al., 2013). In communities where food insecurity is a major public health issue, such as in the Canadian Arctic, advisories can have negative outcomes (Laird et al., 2013). If validated by *in vivo* studies, cooking and polyphenol-rich foods could thus be easily implementable recommendations to reduce exposure to Hg. This could promote safe consumption of fish as a source of fatty-acids, which could protect from cardiovascular disease (Mahaffey et al., 2011; Rideout and Kosatsky, 2017).

While *in vitro* bioaccessibility assays suggest that guidelines may overestimate MeHg exposure from fish, it is important to consider that these guidelines are designed to be overly conservative, in order to to protect especially vulnerable groups. These individuals, including children and pregnant women, are particularly sensitive to MeHg-induced health risks (Committee on the Toxicological Effects of MeHg). Criticism of guidelines should thus keep in mind the specific needs of vulnerable sub-groups.

4.4. Other factors may alter MeHg bioaccessibility

Other factors may also impact MeHg bioaccessibility, that were not taken into account here. The majority of bioaccessibility studies performed on MeHg in fish use commercially-

purchased filets, hence the type of muscle and the area of the fish sampled are unknown. Fish muscles are diverse in terms of physiological function and cellular composition (Sänger and Stoiber, 2001). Furthermore, other elements found in fish could influence bioaccessibility, such as selenium and its ratio to Hg (Cabañero et al., 2006; 2004). Future research into within-individual variations of MeHg bioaccessibility should include gradients along muscle tissues, and perform full characterizations of muscles, to further our understanding of how Hg binds fish muscle tissue in fish.

MeHg bioaccessibility has also been found to be limited by co-ingested plant products such as dietary fibers (Shim et al., 2009) and plant cell walls compounds (lignin, methylcellulose, pectin) (Jadán Piedra et al., 2016). Studies conducted on rats fed with intrinsically MeHg-contaminated food matrices also show that plant compounds can limit absorption (Yannai and Sachs, 1993). Current guidelines do not take into consideration complex diets, accounting only for MeHg measured in fish muscle. Our results combined with others from the literature suggest that ignoring plant-based co-foods, which contain polyphenols and dietary fiber may lead to overestimations of Hg intake from fish. Risk assessments also overlook cooking and the combined effect of different processes involved in preparing a meal, contributing to this lack of realism.

Host genetics, including the gut microbiome could also potentially alter the fate of Hg in the body, as has been observed with other metals. For example, toxic species of arsenic and bismuth can be produced by gut bacteria prior to absorption by the epithelial lining (Diaz-Bone and Van de Wiele, 2010; Van de Wiele et al., 2010), and Laird et al. observed increased arsenic bioaccessibility in the presence of a simulated gut microbiome community, compared to sterile conditions (Laird et al., 2009b). While *in vivo* methylation would increase Hg toxicity, this pathway has not yet be observed in primates (Gilmour et al., 2013; Martín-Doimeadios et al., 2017). Evidence for MeHg demethylation by the microbiome has been reported from mice models, producing poorly-absorbed inorganic Hg (Rowland, 1988). Meanwhile the *mer* operon, responsible for mercury resistance and cell membrane transport, is frequent: a study of 800 antibiotic-resistance plasmids from Gram- bacteria have been found to carry the operon (Schottel et al., 1974). This may allow it to alter Hg cycling in the gut. Lactic acid bacteria have been found to reduce Hg bioaccessibility in mushrooms and aqueous solutions, but not in seafood (Jadán-Piedra et al., 2017a; 2017b). Interactions between dietary Hg and the microbiome are thus unclear, and should be explored further.

4.5. In vitro *findings must be validated to improve current risk assessments*

In vitro gastrointestinal models are useful to understand the fate of food components and contaminants in the human body, as they are inexpensive and easy to use. They allow for screening high numbers of samples in a controlled setting, can be validated with reference materials, and avoid ethical considerations of using model animals, which can be more or less relevant to humans (Fernández-García et al., 2009).

Bioaccessibility assays have been suggested as a way to improve risk assessments (Cardoso et al., 2014) and guidelines (Ángeles García et al., 2016). However, *in vitro* models are rarely validated because of the lack of *in vivo* experiments using consumer products in contaminant studies (Brandon et al., 2006; Hur et al., 2011). *In vitro* studies could be improved by integrating cell cultures such as Caco-2, to account for membrane transport (Hur et al., 2011; Moreda-Piñeiro et al., 2011), but this does not take into account whole-body processes that could alter the fate of Hg, such as stimulation of the enterohepatic cycle (Canuel et al., 2006b) or interactions with the gut microbiome, which are also determinants of bioavailability. Future work should involve *in vivo* experiments, to validate the effects of dietary practices and co-foods on Hg bioaccessibility. *In vitro* investigations remain useful, as they offer insight into the structural and complexation changes that Hg undergoes during food preparation and digestion, and provide evidence that it would be worthwhile to embark on costly and ethically-loaded *in vivo* assays. We recommend that further work on the fate of MeHg in the body should be performed in animal models such as swine, which are considered appropriate for human health risk assessments (Moreda-Piñeiro et al., 2011).

Culturally-specific guidelines may also be necessary: for example, current pharmacokinetic models are poor predictors of Hg burden in Canadian indigenous populations (Canuel et al., 2006a), who have a different genetic background, but who also have specific dietary practices regarding food preparation and co-foods. This supports observations made by a European study on MeHg risk assessment, which showed that guidelines should be population and country-specific (Jacobs et al., 2016). While smaller, low-trophic level fish could be recommended over more contaminated species to promote fish intake, this solution may not be implementable in developing countries or coastal populations who rely on high-trophic level organisms. Risk assessments based from validated bioaccessibility data could provide specific recommendations for various populations, who are exposed to dietary MeHg in different ways. This may lead to easily applicable, non-invasive guidelines that allow a population to adapt its food preparation to limit exposure to Hg in culturally important foods. This could be of critical importance for coastal indigenous populations, which have a per capita fish consumption 15 times greater than non-indigenous peoples (Cisneros-Montemayor et al., 2016). Altering MeHg

bioaccessibility through dietary practices in these populations could have important consequences on Hg absorption and health. Since fish are an excellent source of protein, vitamins, fatty acids and minerals which feed a significant portion of the world's population and are associated with cardiac health, it is critical to better understand the risk that Hg in fish represents to human health.

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Table legends

Table 1. Total Hg and MeHg bioaccessibility (%) measured in fresh, raw fish muscle in this study and from the literature. Values presented are averages and standard deviations (SD)

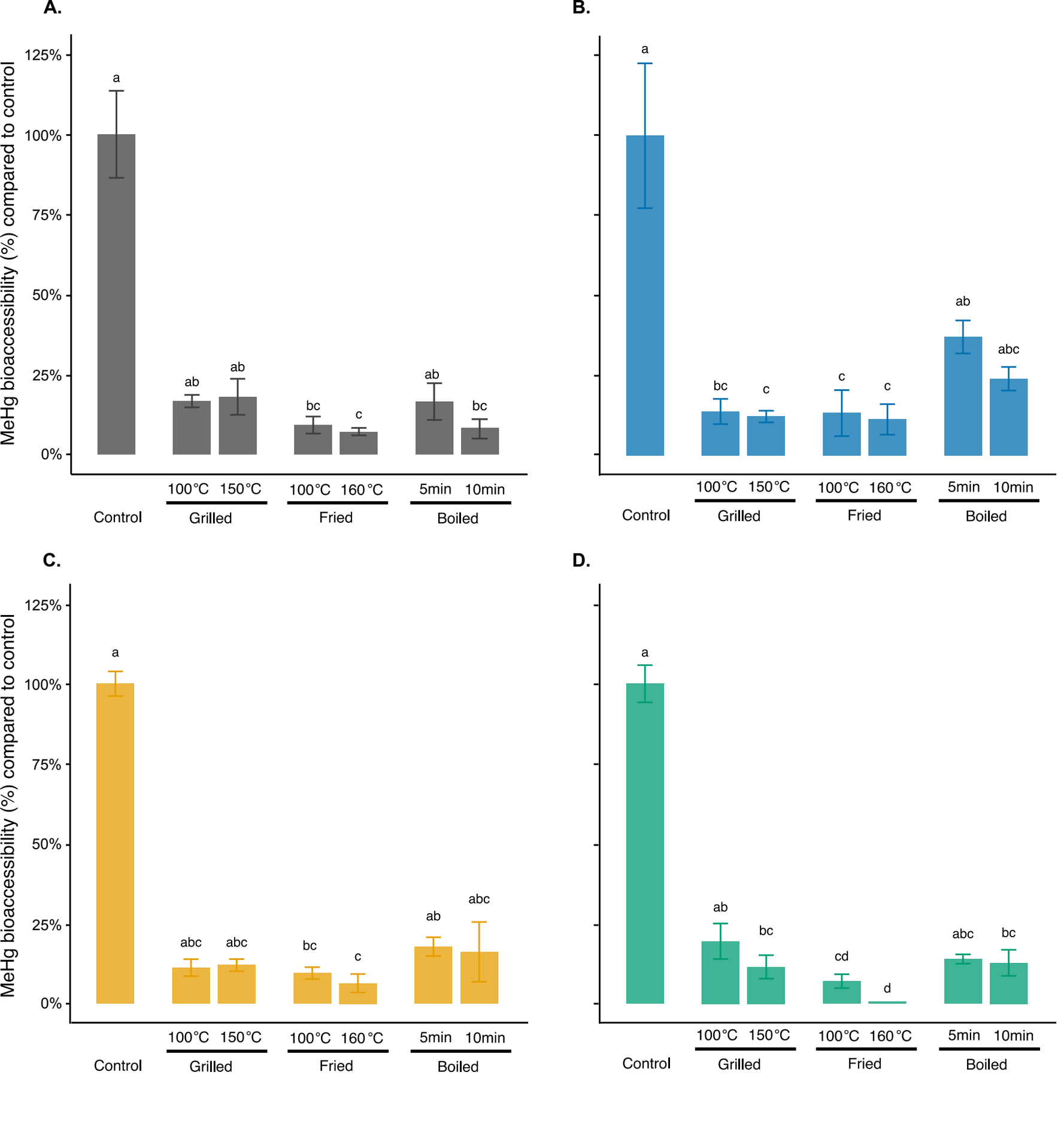
Figure legends

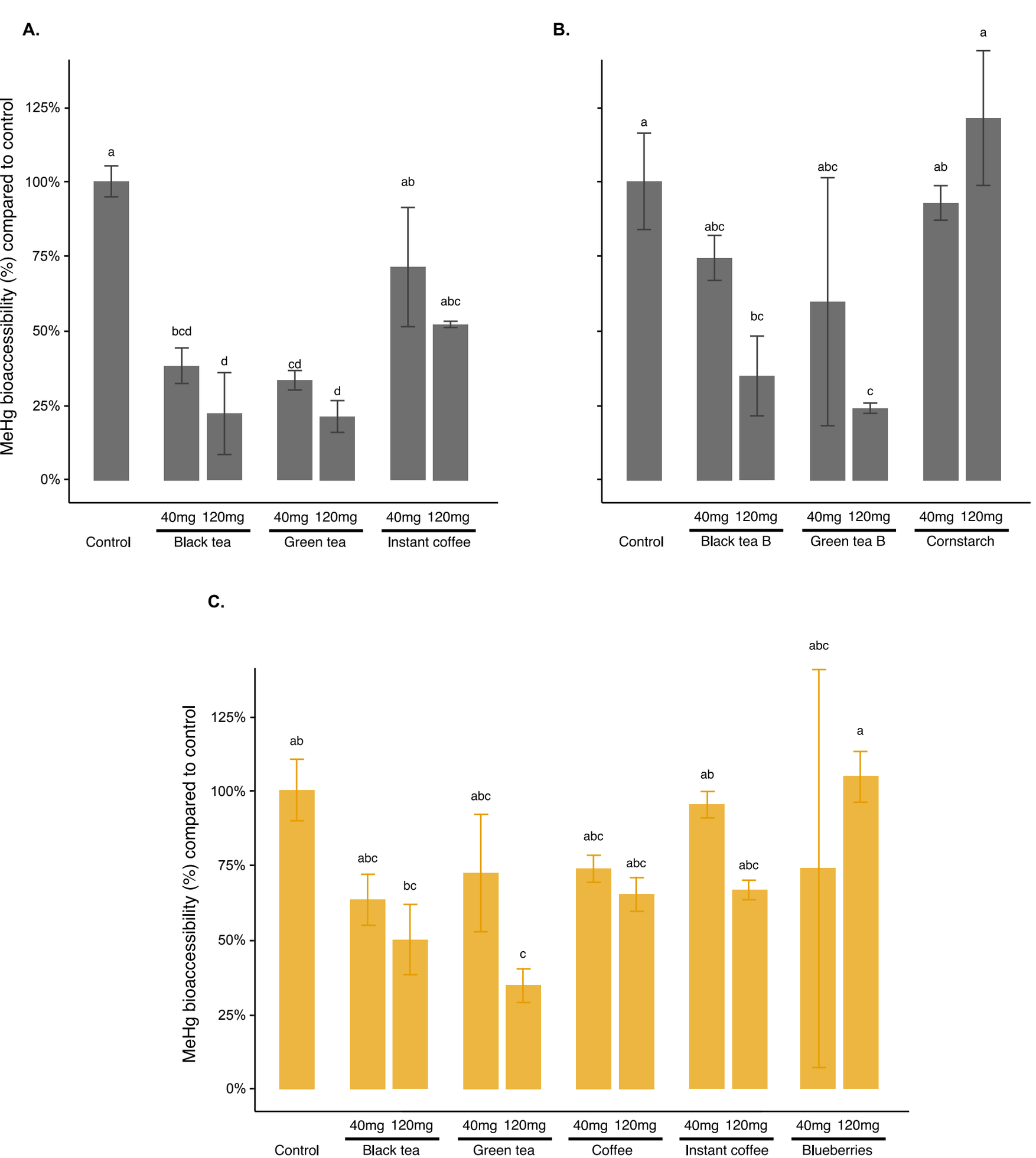
Figure 1. Effect of cooking on MeHg bioaccessibility in **A.** swordfish, **B.** grouper, **C.** tuna and **D.** salmon. Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

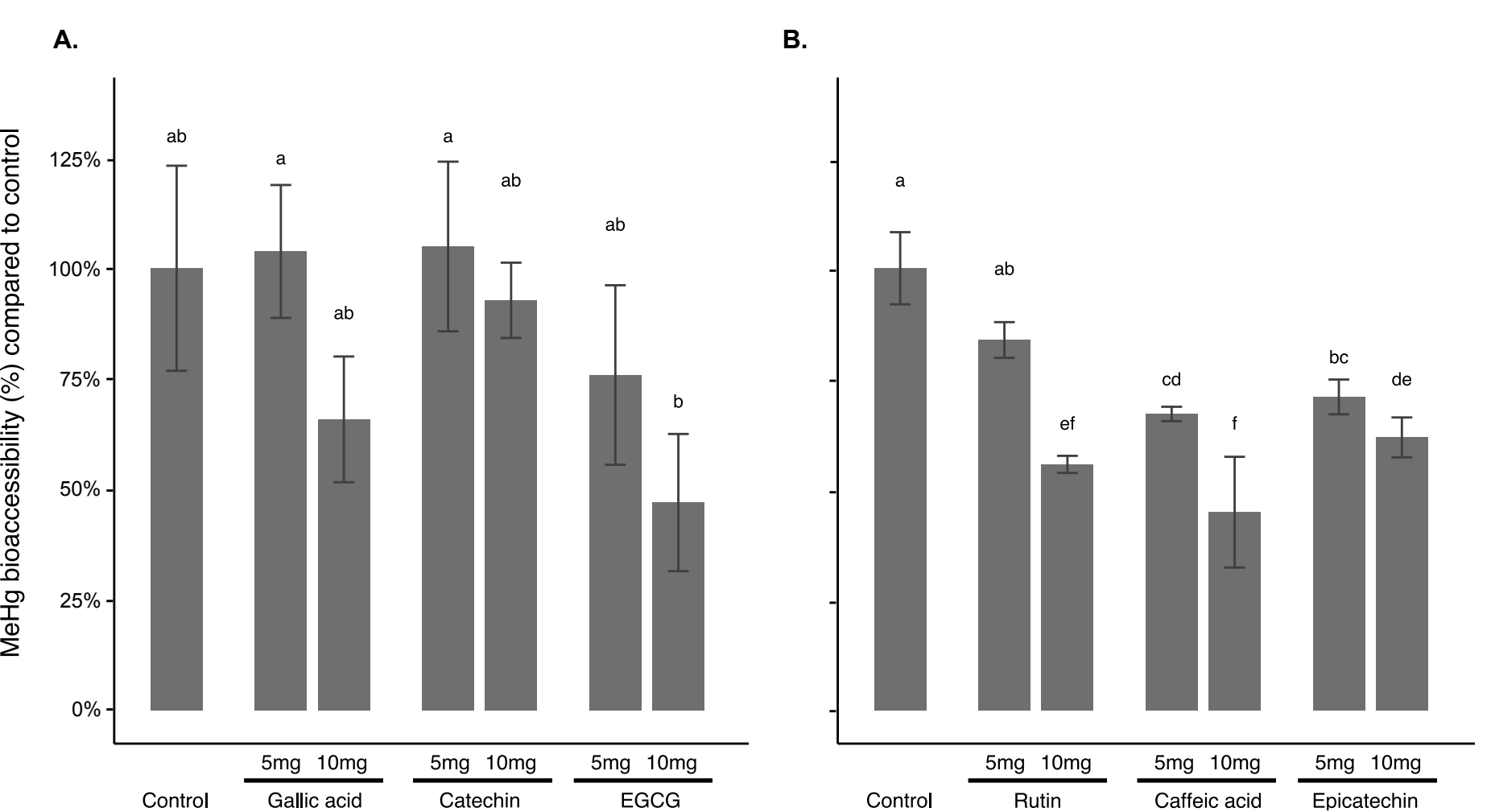
Figure 2. Effect of polyphenol-rich beverages and foods on MeHg bioaccessibility in **A.** & **B.** swordfish and **C.** tuna. Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

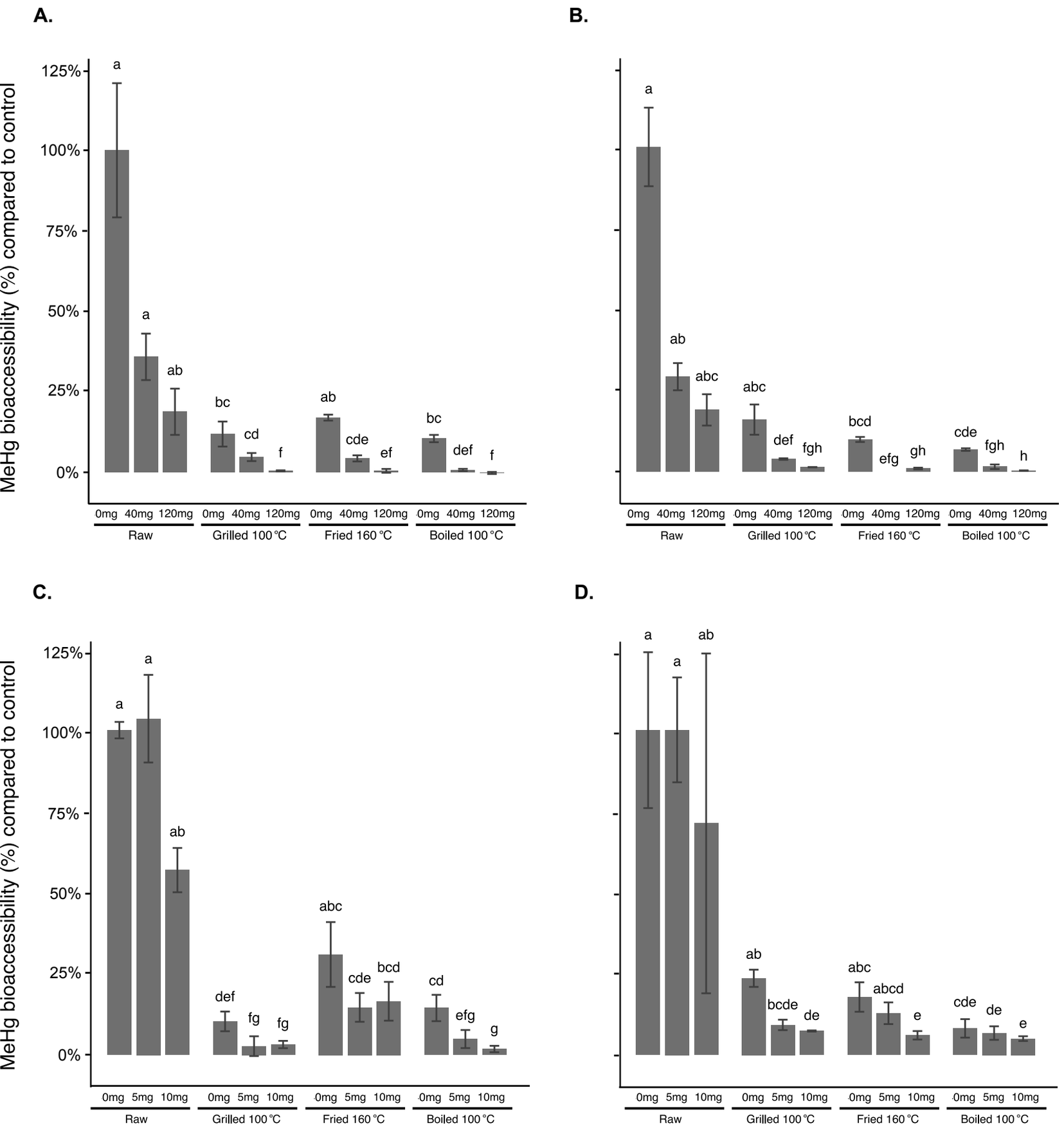
Figure 3. Effect of pure polyphenols on MeHg bioaccessibility in swordfish. **A** & **B.** Gallic acid, epigallocatechin gallate, rutin and caffeic acid lead to significant decreases in MeHg bioaccessibility (Kruskal-Wallis, $P < 0.05$). Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Figure 4. Mixed effect of cooking and polyphenols/polyphenol-rich beverages on MeHg bioaccessibility in swordfish for polyphenol-rich beverages and foods (**A.** black tea, **B.** green tea) and for pure polyphenols (**C.** gallic acid, **D.** catechin). Results were normalized to controls at 100%, to allow comparison across experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.


A.
B.
C.
D.
MeHg bioaccessibility (%) compared to control
125%
100%
75%
50%
25%
0%
a
ab
ab
bc
c
ab
bc
a
bc
c
c
c
ab
abc
a
abc
abc
bc
c
ab
abc
a
ab
bc
cd
d
abc
bc
100°C
150°C
100°C
160°C
5min
10min
Control
Grilled
Fried
Boiled


A.
MeHg bioaccessibility (%) compared to control
125%
100%
75%
50%
25%
0%
a
bcd
d
cd
d
ab
abc
40mg 120mg
Control
Black tea
Green tea
Instant coffee
B.
a
abc
bc
abc
c
ab
a
Control
Black tea B
Green tea B
Cornstarch
C.
MeHg bioaccessibility (%) compared to control
ab
abc
bc
abc
c
abc
abc
ab
abc
abc
a
Control
Black tea
Green tea
Coffee
Instant coffee
Blueberries


A.
B.
MeHg bioaccessibility (%) compared to control
125%
100%
75%
50%
25%
0%
ab
a
ab
a
ab
ab
b
a
ab
ef
cd
f
bc
de
5mg
10mg
Control
Gallic acid
Catechin
EGCG
Rutin
Caffeic acid
Epicatechin


A.
B.
C.
D.
MeHg bioaccessibility (%) compared to control
125%
100%
75%
50%
25%
0%
0mg 40mg 120mg
0mg 5mg 10mg
Raw
Grilled 100 °C
Fried 160 °C
Boiled 100 °C

Supplemental Material

Cooking and co-ingested polyphenols reduce *in vitro* methylmercury bioaccessibility from fish and may alter exposure in humans

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Table of content

Supplemental material contains supplementary methods, 4 supplementary tables (Tables S1-S4) and 7 supplementary figures (Figures S1-S7).

Supplementary Methods

MeHg analyses

Calibration curves were prepared using certified MeHg solutions. In brief, a MeHg stock solution (1000 ppm, certified by Alfa Aesar) was diluted in methanol (Fished Scientific, HPLC grade) to prepare a 1 mg L^{-1} MeHg solution. Intermediate working solutions (10.0 μg L^{-1}, 600 ng L^{-1} and 10 ng L^{-1}) were freshly prepared in MilliQ water and preserved with 0.3% acetic acid (Fisher Scientific, ACS-pur) and 0.2% HCl (EMD, Omni-trace ultra).

Fish matrix characterization

Lipids were quantified by gravimetry, using a method adapted from Folch et al. Briefly, 5 mL of a 2:1 chloroform:methanol solution (>99.8 and 99.9%, Fisher Scientific) were added to a Falcon tube containing 0.5 – 1 g of lyophilized and grinded fish sample. Tubes were vortexed for 15 seconds, then left under a fumehood overnight. Samples were vortexed again for 15 seconds, and centrifuged for 5 minutes at 1,500 g. Supernatant was poured into pre-weighed aluminum vessels, the pellet was washed with 2:1 chloroform:methanol solution and centrifuged once more, and washing solution was added to the vessel. Solutions were air-dried under a fumehood overnight, and were weighed. Percent lipid content was obtained with the following equation:

[Equation S1]

$$\%\ lipid\ content = \frac{mass\ of\ vessel\ with\ sample\ (g) - mass\ of empty\ vessel\ (g)}{mass\ of fish\ extracted\ (g)}$$

Nitrogen was used as a proxy for protein content, and was quantified using a CHN Element Analyzer 1108 (Thermo Fisher). Samples were weighed in tin capsules and then burnt in a combustion column at 1,040 °C and reduced in a reduction column at 650 °C, converting nitrogen into N_2. N_2 was separated from other gases generated during reduction by gas chromatography, then quantified by a thermo-electric detector. Calibrations were performed with acetanilide. Atropine and sulfanilamide (Elemental Microanalysis Limited) were used as standards.

Moisture content in fish muscle was quantified by subtracting sample dry weight from wet weight after drying.

Polyphenol analyses

Polyphenols were quantified by UPLC-MSMS using a Waters Acquity Ultra-Performance LC system with a quaternary pump system and an Acquity high-strength silica T3 column (120 mm x 2.1 mm, 1.8 mm particle size) (Waters). The stationary phase was 100% silica particles. Compounds were separated with a mobile phase of 0.2% acetic acid (eluent A) and acetonitrile (eluent B), with a flow rate of 0.4 mL min^{-1}. The gradient elution was as follows: initial 5% B, 5-20% B (0 – 4.5 min), isocratic 20% B (4.50 – 6.45 min), 20-45% B (6.45 – 13.50 min), 45-100% B (13.5 – 16.5 min), isocratic 100% B (16.5-19.5 min), 100-5% B (19.5 – 19.52 min), isocratic 5% B (19.52 – 22.5 min). MS analyses were performed on a TQD mass spectrometer (Waters) with a Z-spray electrospray interface in negative mode, with data acquisition carried out by multiple reactions monitoring (MRM). Ionization source parameters were as follow: capillary voltage at 2.5 kV, source temperature at 140 °C, cone gas flow rate of 80 L h^{-1}, desolvation gas flow rate of 900 L h^{-1} and desolvation temperature of 350 °C. Nitrogen (>99%) was used as a nebulizing gas, and argon (>99%) as a collision gas. Data acquisition was performed with MassLynx 4.1 software. Results were quantified as gallic acid equivalents, and detection limits were calculated at 0.9 ug g^{-1} using standard gallic acid.

Supplementary References

Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **1957**, *226* (1), 497–509.

Supplementary Tables

Table S1. Composition of fish matrix tested in bioaccessibility experiments. Values are reported for an individual fish, which was used in numerous subsequent analyses. Means are shown with standard deviation, with range in brackets and number of individual fish per species (*n*).

Fish	MeHg (ng g^{-1})	Protein (%)	Lipids (%)	Moisture (%)
Swordfish	439.20 ± 237.47 ng g^{-1} (152.48-679.8) *n*=4	86.35 ± 3.41% (13.25-14.33) *n*=3	11.85 ± 2.36% (8.79-14.89) *n*=4	70.06 ± 1.18% (69.13-72.23) *n*=4
Grouper	612.42 ± 315.32 ng g^{-1} (389.45-835.39) *n*=2	86.35 ± 0.36% (13.77-13.88) *n*=3	8.55 ± 1.20% (6.40-9.09) *n*=2	64.43 ± 20.35% (50.04-78.82) *n*=2
Tuna	694.00 ± 777.80 ng g^{-1} (144.01-1243.98) *n*=2	87.42 ± 0.34% (13.93-14.04) *n*=3	10.05 ± 0.13% (9.91-10.16) *n*=2	60.66 ± 11.88% (52.26-69.06) *n*=2
Salmon	20.16 ng g^{-1} (-) *n*=1	n/a	26.54% (-) *n*=1	70.00% (-) *n*=1

Table S2. Polyphenol content in different beverages and foods used in MeHg bioaccessibility, measured by UPLC-MS/MS. Polyphenols presented here are those we subsequently tested in their purified form. EGCG: epigallocatechin gallate; ND: undetected (detection limit = 0.9 ug g^{-1}).

Food item	Gallic acid (ug g^{-1})	Catechin (ug g^{-1})	Epicatechin (ug g^{-1})	EGCG (ug g^{-1})	Theaflavin (ug g^{-1})	Rutin (ug g^{-1})	Cafeic acid (ug g^{-1})
Black tea A	8218.01	1338.73	4428.45	17505.91	153.97	41138.98	58.21
Black tea B	7892.87	1226.50	3816.15	25131.52	52.71	47043.56	104.86
Green tea A	1431.36	1770.37	25154.20	96256.16	167.06	38485.35	30.29
Green tea B	3376.76	2269.17	16921.85	110499.05	133.59	15277.43	75.13
Coffee	ND	ND	ND	ND	ND	ND	331.34
Instant coffee	ND	ND	ND	ND	ND	ND	497.10
Blueberries	11.52	33.49	ND	84.35	ND	147.57	50.89
Cornstarch	ND	ND	ND	31.31	ND	ND	ND

Table S3. Raw polyphenol content in beverages and foods tested in bioaccessibility experiments, measured by UPLC-MS/MS.

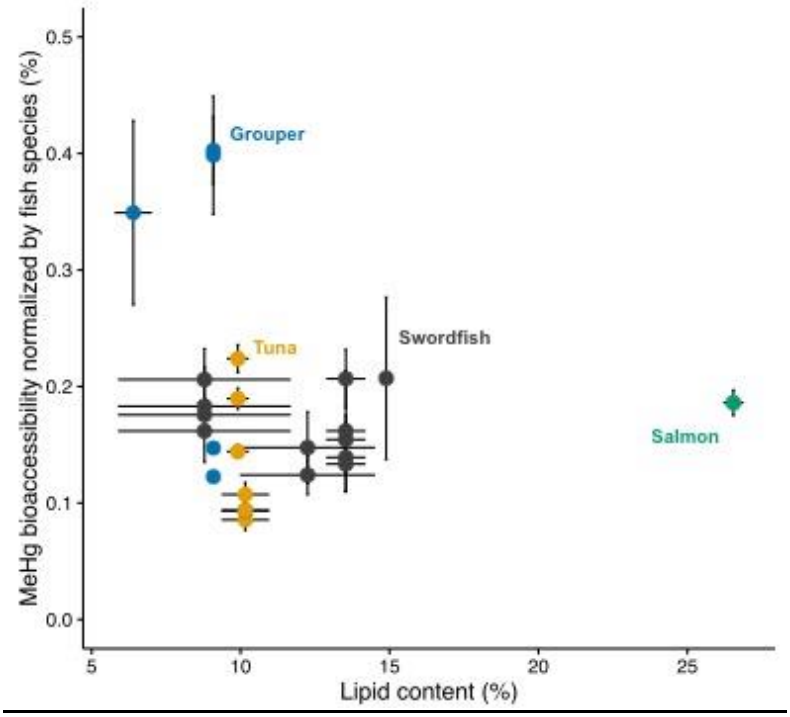
SuppTableS3.xlsx is available at https://github.com/cgir/PBET_MeHg

Table S4. Provisional daily intake (PDI) and bioaccessibility-corrected PDI (PDI_{BA}) for all fish and treatments tested in this experiment.

Treatment	Swordfish		Grouper		Tuna		Salmon	
	PDI ug kg^{-1}	PDI_{BA} ug kg^{-1}	PDI ug kg^{-1} [1]	PDI_{BA} ug kg^{-1}	PDI ug kg^{-1}	PDI_{BA} ug kg^{-1}	PDI ug kg^{-1}	PDI_{BA} ug kg^{-1}
Control (raw, unamended)	0.126	0.037	0.143	0.096	0.053	0.015	0.007	0.003
Grilling		0.007		0.012		0.002		<0.001
Frying		0.003		0.011		0.001		<0.001
Boiling		0.003		0.023		0.002		<0.001
Control (raw, unamended)	0.126	0.032	0.306	0.072	0.456	0.075	-	-
Green tea		0.007		0.017		0.026		-
Black tea		0.007		0.025		0.037		-
Control (raw, unamended	0.249	0.084	-	-	-	-	-	-
EGCG		0.040		-		-		-
Control (raw, unamended)	0.126	0.050	-	-	0.456	0.082	-	-
Grilling + green tea		0.001		-		<0.001		-

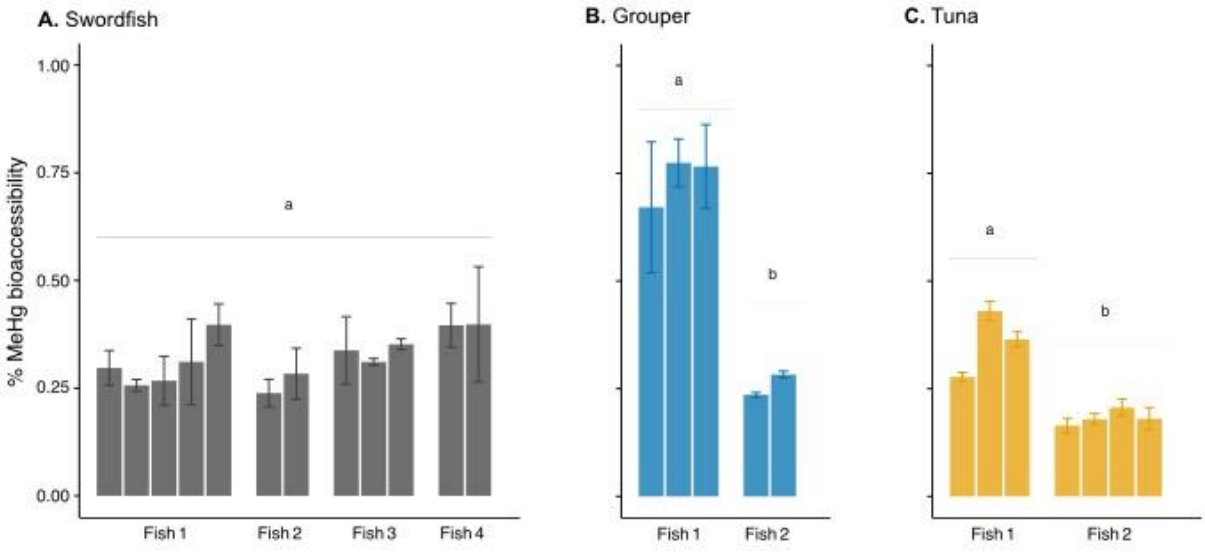
Supplementary Figures

Figure S1



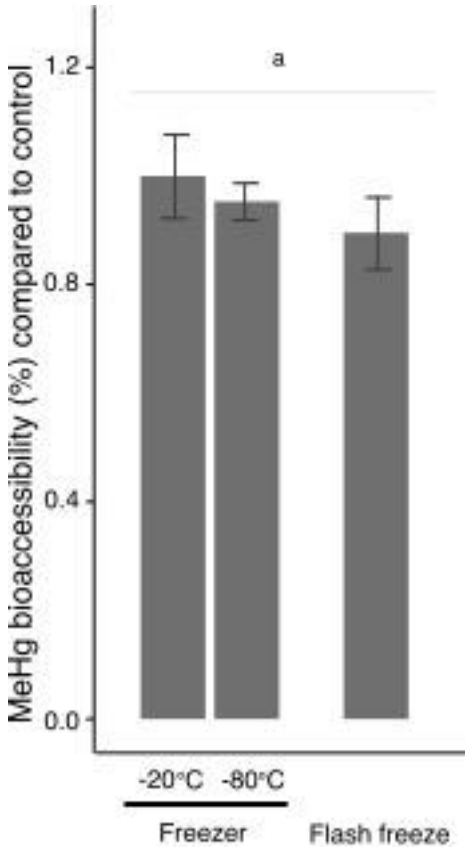
Supplementary Figure S1. MeHg bioaccessibility in relation to lipid content in different fish species (linear regression, $P > 0.05$). Points present averages from individual fish, and error bars show standard deviation for lipid content (x-axis) and MeHg bioaccessibility (y-axis).

Figure S2



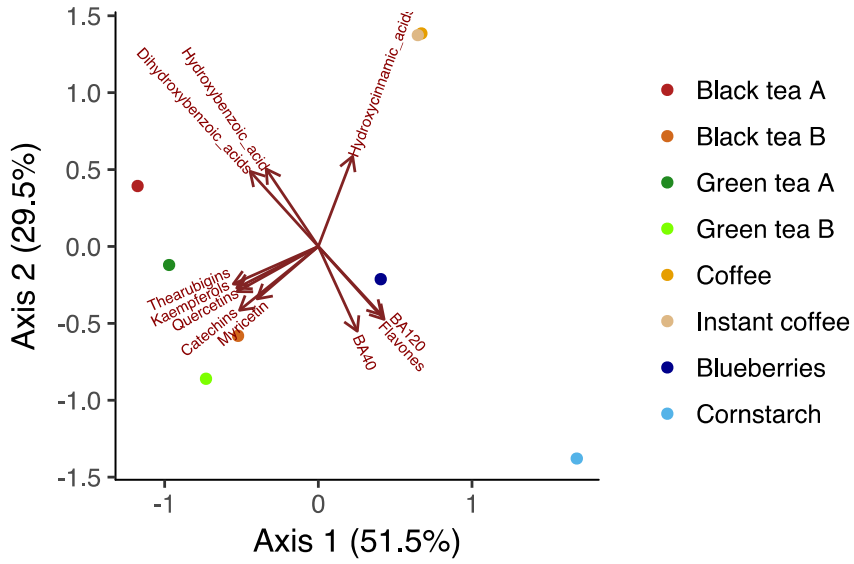
Supplementary Figure S2. MeHg bioaccessibility in raw fish from different individuals. Within a given individual, bioaccessibility did not vary significantly (Kruskal-Wallis, $P < 0.05$). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Figure S3



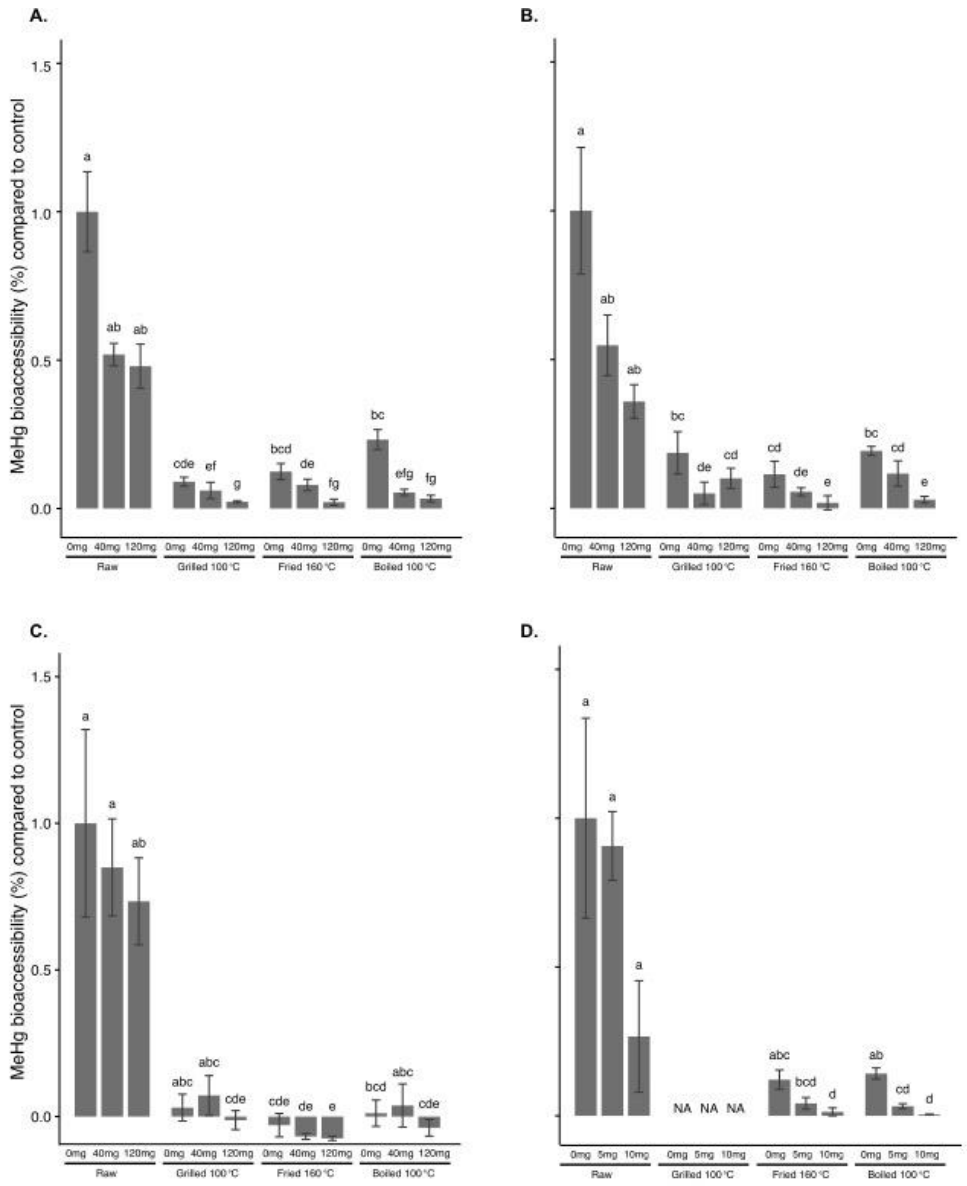
Supplementary Figure S3. Effect of freezing on MeHg bioaccessibility in swordfish. Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Figure S4



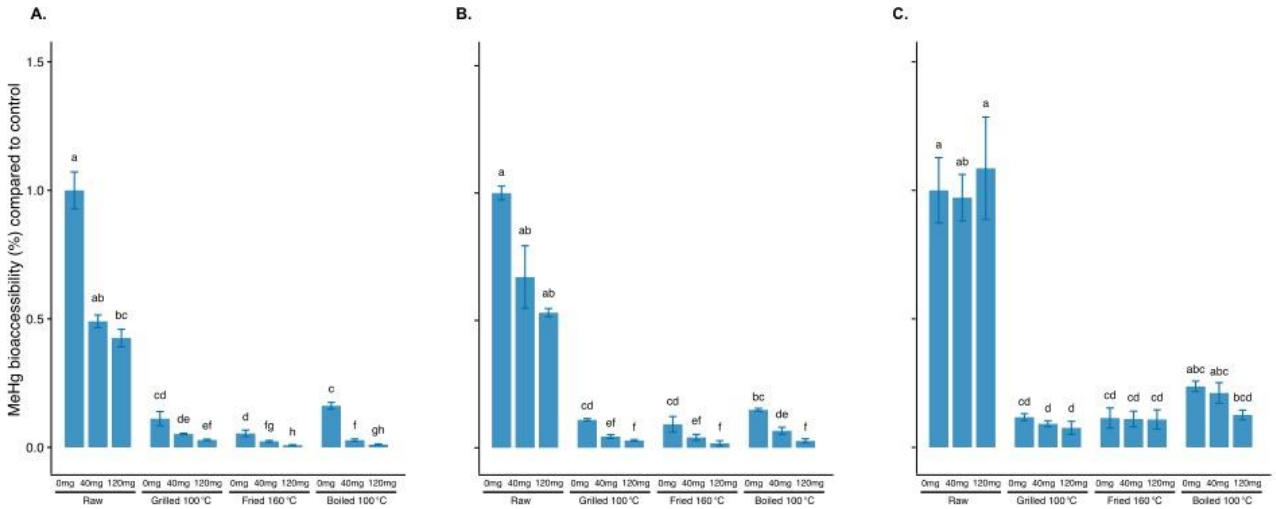
Supplementary Figure S4. Principal components analysis (PCA) correlation biplot showing bioaccessibility from experiments performed with polyphenol-rich treatments (colored points) and polyphenol content, broken down into 9 categories (red arrows). The PCA accounts for 81% of total variation from Axes 1 and 2.

Figure S5



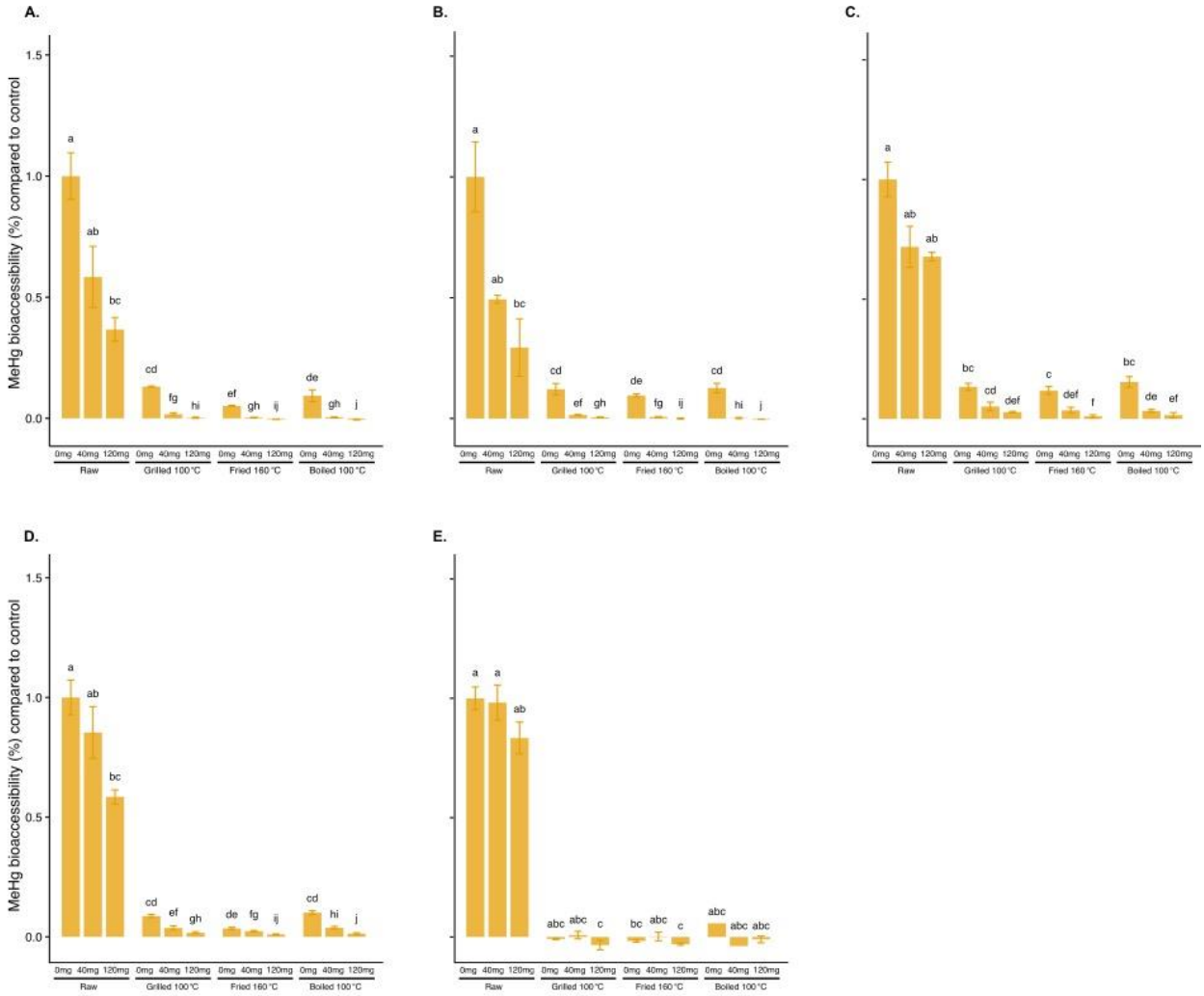
Supplementary Figure S5. Mixed effect of cooking and polyphenol/beverages on MeHg bioaccessibility in swordfish for polyphenol-rich beverages and foods (**A.** coffee, **B.** instant coffee and **C.** blueberries) and for pure polyphenols (**D.** epigallocatechin gallate). Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Figure S6



Supplementary Figure S6. Mixed effect of cooking and polyphenols/beverages on MeHg bioaccessibility in grouper. **A.** Coffee, **B.** instant coffee and **C.** blueberries. Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

Figure S7



Supplementary Figure S7. Mixed effect of cooking and polyphenols/beverages on MeHg bioaccessibility in tuna. **A.** Black tea, **B.** green tea, **C.** coffee, **D.** instant coffee and **E.** blueberries. Letters denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.