

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

Catherine Girard^a, Tania Charette^{b,c}, Maxime Leclerc^{b,c}, B. Jesse Shapiro^c, Marc Amyot^{a,b,c}

a. Center for Northern Studies (CEN), Département de sciences biologiques, Université de Montréal, 90 Vincent-d'Indy, Montreal, H2V2S9, Canada.

b. ÉcoLac, Département de sciences biologiques, Université de Montréal, 90 Vincent-d'Indy, Montreal, H2V2S9, Canada.

c. Groupe de recherche interuniversitaire en limnologie et en environnement aquatique (GRIL), Département de sciences biologiques, Université de Montréal, 90 Vincent-d'Indy, Montreal, H2V2S9, Canada.

#Address correspondence to Marc Amyot, m.amyot@umontreal.ca, 514-343-7496. Université de Montréal, Département de sciences biologiques. Pavillon Marie-Victorin, C.P. 6128, succ Centre-ville, Montreal (Quebec) H3C3J7 Canada.

Running title

MeHg bioaccessibility is reduced by food preparation

Acknowledgments

The authors thank Dominic Bélanger, Shirley Atoche, Fei Tao Zhou, Valérie de Munck and Mélissande Gaucher for assistance in the laboratory, and Antoine Caron and Maikel Rosabal for advice. Research was funded through NSERC Discovery grant (217099-2012) and the Canada Research Chair program (950-230679) to MA. Student funding was provided by FRQNT and NSERC doctoral scholarships to CG.

Competing financial interests

All authors declare they have no actual or potential competing financial interest.

Key Words

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

33 **Abstract**

34

35 Fish consumption is a major pathway for mercury exposure in humans. Current guidelines and
36 risk assessments assume that 100% of methylmercury (MeHg) in fish is absorbed by the human
37 body after ingestion. However, a growing body of literature suggests that this absorption rate may
38 be overestimated. We used an *in vitro* digestion method to measure MeHg bioaccessibility in
39 commercially-purchased fish, and investigated the effects of dietary practices on MeHg
40 bioaccessibility. Cooking had the greatest effect, decreasing bioaccessibility on average to 12.5
41 $\pm 5.6\%$. Polyphenol-rich beverages also significantly reduced bioaccessibility to $22.7 \pm 3.8\%$ and
42 $28.6 \pm 13.9\%$, for green and black tea respectively. We confirmed the suspected role of
43 polyphenols in tea as being a driver of MeHg's reduced bioaccessibility, and found that
44 epicatechin, epigallocatechin gallate, rutin and cafeic acid could individually decrease MeHg
45 bioaccessibility by up to 55%. When both cooking and polyphenol-rich beverage treatments were
46 combined, only 1% of MeHg remained bioaccessible. These results call for *in vivo* validation, and
47 suggest that dietary practices should be considered when setting consumer guidelines for MeHg.
48 More realistic risk assessments could promote consumption of fish as a source of fatty acids,
49 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 (MeHgNO₃). 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

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

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

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

114

115

116

117 2. Methods

118

119 2.1. Food items, co-ingested foods and polyphenols

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

127

128 2.2. Food preparation methods

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

137 For co-ingestion experiments, fish samples were digested simultaneously with either
138 beverages or pure polyphenols. Beverages (tea, coffee, instant coffee) were prepared as per the
139 manufacturer's instructions, and lyophilized overnight into a powder (Freezone6, Labconco).
140 Powdered beverages were solubilized in 2 mL of MilliQ water, in two different doses: 40 mg or
141 120 mg, and were added to fish at the start of *in vitro* digestion experiments. In these experiments,
142 controls were amended with 2 mL of MilliQ water to adjust the volume. Pure polyphenols were
143 solubilized in 2 mL of dimethyl sulfoxide (DMSO), in amounts of 5 or 10 mg, and used in *in vitro*
144 digestions. Controls with no polyphenols were also amended with 2 mL of DMSO, to account for
145 volume increase.

146

147

148 2.3. Physiologically-based extraction test

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

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

162 Briefly, samples were placed in Falcon tubes with 40 mL of gastric solution, and were
163 incubated at 37 °C with agitation (100 rpm) for 1 hour. pH was then adjusted to 7 using 5 M NaOH.
164 Nine mL of intestinal solution were added to all samples, which were incubated at 37 °C with
165 agitation (100 rpm) for 2 hours. Following incubation, samples were centrifuged for 15 minutes at
166 3,000 g. The supernatant, considered to contain the bioaccessible (solubilized) fraction of MeHg,
167 was isolated and used for MeHg analyses.

168

169 2.4. Bioaccessibility

170 Bioaccessibility was calculated after PBET simulations with the following equation:

171

172 [Equation 1]

$$173 \quad \% \text{ bioaccessibility} = \frac{[\text{MeHg}] \text{ in PBET (ng/L)} \times \text{PBET volume (L)}}{[\text{MeHg}] \text{ in fish (ng/g)} \times \text{fish mass (g)}} \times 100$$

174

175 with $[\text{MeHg}] \text{ in PBET}$ being MeHg measured in the extract of the simulated digestion, PBET
176 volume being PBET digestive fluids volume, $[\text{MeHg}] \text{ in fish}$ is $[\text{MeHg}]$ in initial fish sample, and
177 fish mass is the mass of fresh fish used as input into the PBET simulation.

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

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

187

188 [Equation 2]

$$189 \quad \% \text{ bioaccessibility compared to control} = \frac{\text{average treated \%}}{\text{average control \%}} \times 100$$

190

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

194

195 2.5. MeHg analyses

196 MeHg in fish was measured in freeze-dried samples (Freezone6, Labconco), while MeHg
197 following simulated digestion was analyzed in PBET fluids. Prior to analysis, both dried fish
198 samples and PBET fluids were extracted overnight in 5 mL of 4 M HNO₃ (Fisher Scientific, ACS-
199 pur) at 60 °C. MeHg in fish and PBET solutions was measured by gas chromatography and cold-
200 vapor fluorescence spectrometry (CVAFS) (Tekran 2700, Tekran Instruments Corporation),
201 according to U.S. EPA method 1630 (detection limit of 0.01 ng L⁻¹, defined as three times the
202 standard deviation calculated on 10 ultrapure MilliQ blanks).

203

204 2.6. Fish matrix characterization

205 Lipids were quantified by gravimetry, using a method adapted from Folch et al., 1957.
206 Nitrogen was used as a proxy for protein content and was quantified with a CHN Element Analyzer
207 1108 (Thermo Fisher). Moisture content in fish muscle was quantified by subtracting sample dry
208 weight from wet weight after drying. Full methods on fish matrix characterization are presented in
209 Supplementary Information.

210

211 2.7. Polyphenol analyses

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

218

219 2.8. Risk assessment

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

222

223 [Equation 3]

$$224 PDI = \frac{[MeHg \text{ in fish }](\mu\text{g/g}) \times \text{average daily fish intake (g)}}{\text{average adult body weight (kg)}}$$

225

226 where *[MeHg in fish]* is the MeHg concentration measured in the fish sample tested; *average daily*
227 *fish intake* is based on fish consumption values from the Bureau of Chemical Safety of Canada
228 (22 g for an adult) (Bureau of Chemical Safety Health Canada, 2004); and the *average adult body*
229 *weight* is based on values from Nutrition Canada (60 kg for an adult) (Health Canada, 2004). PDIs
230 represent average exposure to MeHg, if an adult consumed each fish daily over a long period of
231 time. We then calculated a bioaccessibility-corrected PDI (PDI_{BA}):

232

233 [Equation 4]

$$234 PDI_{BA} = PDI \times \% \text{ bioaccessibility}$$

235

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

239

240 2.9. Statistical analyses

241 Statistical analyses were performed with R software (R Development Core Team) using
242 nonparametric methods, as normality and homoscedasticity were not respected (tested with
243 `shapiro.test()` and `bartlett.test()` functions). Differences in bioaccessibility across treatments were
244 compared with Kruskal-Wallis analysis of variance (`kruskal()` in the `agricolae` package) (De
245 Mendiburu, 2012), and Bonferonni corrections were used to correct for multiple hypothesis
246 testing. Linear regressions were used to model bioaccessibility and lipid content, and ordinations
247 was calculated on log-transformed data. Plots were prepared using the `ggplot2` (Wickham, 2009)
248 and `ggbiplot` (Vu, 2011) packages. Letters on plots denote significantly different treatments ($P <$
249 0.05), bars present averages from triplicate PBET digestions and error bars show standard
250 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).

283 When swordfish samples were frozen, decreasing freezing temperatures, from -20 to -80
284 °C and flash freezing did not lead to significantly different MeHg bioaccessibility levels ($P > 0.05$)
285 (Figure S3).

286 287 3.3. *Effects of co-ingested foods and polyphenols on MeHg bioaccessibility*

288 We tested the effects of co-ingested foods on MeHg bioaccessibility in swordfish and in
289 tuna compared to unamended raw controls (Figure 2). As previous reports have suggested that
290 polyphenols were responsible for altered MeHg bioaccessibility from fish (He and W.-X. Wang,
291 2011; Ouédraogo and Amyot, 2011; Shim et al., 2009), we selected several polyphenol-rich foods
292 and beverages, and one food not enriched in polyphenols (cornstarch). Our results show that
293 green tea led to the lowest MeHg bioaccessibility values in swordfish ($22.7 \pm 3.8\%$ when 120 mg
294 was used) (Figure 2A-B) and in tuna ($34.8 \pm 5.6\%$ with 120 mg of tea) ($P < 0.05$) (Figure 2C).
295 Black tea also significantly decreased MeHg bioaccessibility in swordfish ($28.6 \pm 13.9\%$ with 120
296 mg of tea) ($P < 0.05$). The effect of tea was greater when 120 mg of dried tea (equivalent to
297 approximately 375 mL of prepared tea) was used compared to 40 mg (approximately 125 mL).
298 As expected, cornstarch, which contained negligible amounts of polyphenols (Table S2) had no
299 effect on MeHg bioaccessibility (Figure 2B). Coffee and instant coffee had a slight, yet non-
300 significant effect, while blueberries had no detectable impact ($P > 0.05$) (Figure 2C). Therefore, it
301 appears only some polyphenol-rich beverages can alter MeHg bioaccessibility.

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

309 To verify the hypothetical role of these polyphenols on MeHg bioaccessibility, we repeated
310 PBET simulations using individual purified polyphenol compounds. First, we observed that the
311 effect of polyphenols increased with the amount added to digestion experiments, from 5 to 10 mg
312 (Figure 3). However, while catechin is cited in the literature as a hypothetical driver of reduced
313 MeHg bioaccessibility (He and W.-X. Wang, 2011), we found its purified form had no significant
314 effect ($P > 0.05$) (Figure 3A). Other forms of catechin, including cis-configuration epicatechin and
315 epigallocatechin gallate (EGCG) did significantly limit MeHg bioaccessibility to 61.7 ± 4.5 and 47.0
316 $\pm 15.5\%$ of unamended control, respectively ($P < 0.05$). Another flavon-3-ol (rutin) and a

317 hydroxycinnamic acid (cafeic acid) also significantly reduced bioaccessibility to $55.6 \pm 1.9\%$, and
318 $44.8 \pm 12.5\%$, respectively ($P < 0.05$) (Figure 3).

319

320 3.4. *Effects of multiple dietary practices on MeHg bioaccessibility*

321 Applied separately, cooking and polyphenols (as beverages or purified compounds) both
322 impacted MeHg bioaccessibility compared to raw, unamended controls (Figures 1-3). We tested
323 these treatments together within the same experiment, to assess their combined effects. Cooking
324 swordfish (by grilling, frying or boiling) and digesting it with 120 mg of green or black tea led to
325 less than 1% of MeHg remaining bioaccessible ($P < 0.05$, Figure 4A and B). Combining cooking
326 and 10 mg of purified gallic acid or catechin also significantly decreased MeHg bioaccessibility,
327 to 2-17% of bioaccessible MeHg compared to raw unamended controls (Figure 4C and D) ($P <$
328 0.05). The combined effects of cooking and co-ingested polyphenols (as beverages or purified
329 extracts) were also observed in grouper ($P < 0.05$, Figure S6) and tuna ($P < 0.05$, Figure S7). In
330 all cases, boiling combined with green or black tea were the most effective combination to reduce
331 MeHg bioaccessibility, decreasing bioaccessibility by 99% compared to raw, unamended controls
332 ($P < 0.05$, Figure 4, S6 and S7).

333

334 3.5. *Bioaccessibility and risk assessments*

335 Finally, we compared PDIs for an average adult calculated with the MeHg concentration
336 measured in tested fish, to corrected PDI_{BA} s considering bioaccessibility and the effect of dietary
337 practices. This is a theoretical exercise in the absence of *in vivo*-validated results, and is
338 presented here only to compare the impact of different treatments, rather than to estimate realistic
339 PDIs. Table S4 shows that the estimated daily intake for an adult is greatly reduced when
340 bioaccessibility is considered. All values calculated from MeHg concentrations were below the
341 provisional tolerable daily intake (the maximum amount of MeHg that can be ingested daily over
342 a lifetime without increasing risk of health effects) established by the World Health Organization
343 ($0.23 \text{ ug kg}^{-1} \text{ d}^{-1}$) (Joint FAO/WHO Expert Committee on Food Additives, 2007).

344

345 4. Discussion

346

347 4.1. *Cooking reduces MeHg bioaccessibility from fish; freezing has no effect*

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

350 to weight loss due to moisture and fat loss during cooking (Morgan et al., 1997). When considering
351 bioaccessibility however, studies consistently show a reduction of solubilized Hg (Ouédraogo and
352 Amyot, 2011; Torres-Escribano et al., 2011) and MeHg (He and W.-X. Wang, 2011) from cooked
353 fish muscle. Our results on MeHg bioaccessibility were consistent with these earlier findings
354 (Figure 1). Here we expanded on these studies by comparing multiple temperatures and cooking
355 times for each cooking treatment, and we found no significant differences between 100 and 150
356 °C for grilling, 100 and 160 °C and frying, nor for 5 and 10 minutes for boiling (Figure 1). All
357 temperatures tested were greater than 70 °C, the safe internal cooking temperature
358 recommended by Health Canada (Health Canada First Nations Branch, Government of Canada,
359 2016). However, while fish cooked by consumers may reach this safe internal threshold, higher
360 temperatures are typically used when preparing meals. Indeed, temperatures greater than 100
361 °C are typically used in studies measuring the effects of cooking on metals (Devesa et al., 2001;
362 Ersoy et al., 2006) and heterocyclic amines (Oz et al., 2007). It is likely that the impact of heat on
363 MeHg solubilization increases until a given temperature (below 100 °C), and further increases
364 have no effect on MeHg bioaccessibility within the range of temperatures commonly used in
365 cooking. Protein aggregation induced by high temperatures, leading to lowered pepsin
366 digestibility, may explain these results. Cooking has also been found to induce the formation of
367 disulfide bonds in proteins, which may further limit digestibility (Duodu et al., 2002; He et al., 2010;
368 Kulp et al., 2003). This is supported by observations that heat induces structural changes to meat:
369 at temperatures greater than 100 °C, oxidation can cause protein aggregation, slowing enzymatic
370 digestion by pepsin (Bax et al., 2012).

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

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

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

391

392 4.2. *Plant polyphenols found in tea can limit MeHg solubilization*

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

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

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

426
427 Tea polyphenols may also have other impacts on the fate of MeHg in the body: *in vivo*
428 assays have suggested that tea could accelerate enterohepatic cycling of MeHg in the body
429 (Canuel et al., 2006b) or that tea extracts may limit oxidative stress induced by MeHg in rats and
430 alter its pharmacokinetics (Black et al., 2011). Green tea has also been found to increase Hg load
431 in blood after consumption of MeHg-contaminated fish (Janle et al., 2015). These mechanisms
432 should be further investigated through controlled *in vivo* experiments.

433
434 **4.3. Bioaccessibility studies can be used to inform current guidelines**

435 Studies performed on Hg and MeHg bioaccessibility in fish report values that range from
436 9-100%, with large variations often seen within a single species (Table 1). However, over half of
437 the values reported in Table 1 are below 50%, suggesting that only a fraction of mercury is
438 solubilized (bioaccessible) during simulated digestion, and thus potentially available for
439 absorption and metabolism in the intestine and liver (bioavailable) (Afonso et al., 2015a). Current
440 guidelines and recommendations assume that 100% of MeHg in fish is readily absorbed by the
441 gastrointestinal tract (Committee on Toxicological Effects of Methylmercury, National Research
442 Council of the United States, 2010; Ha et al., 2016). This value is based on early work on the
443 excretion of an orally administered radio-labelled MeHg nitrate solution from the human body
444 (Aberg et al., 1969). However, mercury in fish is more typically bound to sulfur-rich groups such
445 as thiols (Clarkson and Magos, 2008; Harris, 2003), and may behave differently. This suggests
446 that the form of Hg and its complexation to food or other elements may reduce its absorption, and
447 that the 100% MeHg absorption rate likely overestimates what is bioavailable from fish. When
448 considering raw tuna, our PDI estimate which accounts for incomplete bioaccessibility (PDI_{BA}) is
449 71.7% lesser than PDI (Table S4).

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

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

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

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

482

483 4.4. Other factors may alter MeHg bioaccessibility

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

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

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

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

518

519 4.5. *In vitro findings must be validated to improve current risk assessments*

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

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

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

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

559

References

- 561 Abdelouahab, N., Vanier, C., Baldwin, M., Garceau, S., Lucotte, M., Mergler, D., 2008.
562 Ecosystem matters: Fish consumption, mercury intake and exposure among fluvial lake
563 fish-eaters. *Science of the Total Environment* 407, 154–164.
564 doi:10.1016/j.scitotenv.2008.09.004
- 565 Aberg, B., Ekman, L., Falk, R., Greitz, U., Persson, G., Snihs, J.O., 1969. Metabolism of methyl
566 mercury (^{203}Hg) compounds in man. *Archives of Environmental Health: An International*
567 *Journal* 19, 478–484. doi:10.1080/00039896.1969.10666872
- 568 Afonso, C., Costa, S., Cardoso, C., Bandarra, N.M., Batista, I., Coelho, I., Castanheira, I.,
569 Nunes, M.L., 2015a. Evaluation of the risk/benefit associated to the consumption of raw and
570 cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid
571 and docosahexaenoic acid, total mercury, and methylmercury determined by an *in vitro*
572 digestion model. *Food Chemistry* 170, 249–256. doi:10.1016/j.foodchem.2014.08.044
- 573 Afonso, C., Costa, S., Cardoso, C., Oliveira, R., Lourenço, H.M., Viula, A., Batista, I., Coelho, I.,
574 Nunes, M.L., 2015b. Benefits and risks associated with consumption of raw, cooked, and
575 canned tuna (*Thunnus* spp.) based on the bioaccessibility of selenium and methylmercury.
576 *Environmental Research* 1–8. doi:10.1016/j.envres.2015.04.019
- 577 Ángeles García, M., Núñez, R., Alonso, J., Melgar, M.J., 2016. Total mercury in fresh and
578 processed tuna marketed in Galicia (NW Spain) in relation to dietary exposure. *Environ Sci*
579 *Pollut Res* 1–10. doi:10.1007/s11356-016-7634-9
- 580 Bax, M.-L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., Santé-Lhoutellier,
581 V., 2012. Cooking temperature is a key determinant of *in vitro* meat protein digestion rate:
582 investigation of underlying mechanisms. *J. Agric. Food Chem.* 60, 2569–2576.
583 doi:10.1021/jf205280y
- 584 Baynes, R.D., Bothwell, T.H., 1990. Iron Deficiency. *Annu. Rev. Nutr.* 10, 133–148.
585 doi:10.1146/annurev.nu.10.070190.001025
- 586 Black, P., Niu, L., Sachdeva, M., Lean, D., Poon, R., Bowers, W.J., Chan, H.M., Arnason, J.T.,
587 Pelletier, G., 2011. Modulation of the effects of methylmercury on rat neurodevelopment by
588 co-exposure with Labrador Tea (*Rhododendron tomentosum* ssp. *subarcticum*). *Food and*
589 *Chemical Toxicology* 49, 2336–2342. doi:10.1016/j.fct.2011.06.035
- 590 Brandon, E.F.A., Oomen, A.G., Rompelberg, C.J.M., Versantvoort, C.H.M., van Engelen,
591 J.G.M., Sips, A.J.A.M., 2006. Consumer product *in vitro* digestion model: Bioaccessibility of
592 contaminants and its application in risk assessment. *Regulatory Toxicology and*
593 *Pharmacology* 44, 161–171. doi:10.1016/j.yrtph.2005.10.002
- 594 Bravo, L., 2009. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional
595 significance. *Nutrition Reviews* 56, 317–333. doi:10.1111/j.1753-4887.1998.tb01670.x
- 596 Bureau of Chemical Safety Health Canada, 2007. Human health risk assessment of mercury in
597 fish and health benefits of fish consumption.
- 598 Bureau of Chemical Safety Health Canada, 2004. Fish consumption: Review and
599 recommendation of current intake figures for Canadian consumers.
- 600 Burger, J., Dixon, C., Boring, S., Gochfeld, M., 2003. Effect of deep-frying fish on risk from
601 mercury. *Journal of Toxicology and Environmental Health, Part A* 66, 817–828.
602 doi:10.1080/15287390306382
- 603 Cabañero, A.I., Madrid, Y., Cámara, C., 2006. Selenium long-term administration and its effect
604 on mercury toxicity. *J. Agric. Food Chem.* 54, 4461–4468. doi:10.1021/jf0603230
- 605 Cabañero, A.I., Madrid, Y., Cámara, C., 2004. Selenium and mercury bioaccessibility in fish
606 samples: an *in vitro* digestion method. *Analytica Chimica Acta* 526, 51–61.
607 doi:10.1016/j.aca.2004.09.039
- 608 Calatayud, M., Devesa, V., Virseda, J.R., Barberá, R., Montoro, R., Vélez, D., 2012. Mercury
609 and selenium in fish and shellfish: Occurrence, bioaccessibility and uptake by Caco-2 cells.

610 Food and Chemical Toxicology 50, 2696–2702. doi:10.1016/j.fct.2012.05.028
611 Canuel, R., de Grosbois, S.B., Atikessé, L., Lucotte, M., Arp, P., Ritchie, C., Mergler, D., Chan,
612 H.M., Amyot, M., Anderson, R., 2006a. New Evidence on Variations of Human Body Burden
613 of Methylmercury from Fish Consumption. Environ. Health Perspect. 114, 302–306.
614 doi:10.1289/ehp.7857
615 Canuel, R., de Grosbois, S.B., Lucotte, M., Atikessé, L., Larose, C., Rheault, I., 2006b. New
616 evidence on the effects of tea on mercury metabolism in humans. Archives of
617 Environmental & Occupational Health 61, 232–238. doi:10.3200/AEOH.61.5.232-238
618 Cardoso, C., Afonso, C., Lourenço, H., Costa, S., Nunes, M.L., 2014. Bioaccessibility
619 assessment methodologies and their consequences for the risk-benefit evaluation of food.
620 Trends in Food Science & Technology 1–19. doi:10.1016/j.tifs.2014.08.008
621 Chapman, L., Chan, H.M., 2000. The influence of nutrition on methyl mercury intoxication.
622 Environ. Health Perspect. 108, 29–56.
623 Chun, O.K., Chung, S.J., Song, W.O., 2007. Estimated dietary flavonoid intake and major food
624 sources of U.S. adults. The Journal of Nutrition 137, 1244–1252.
625 Cisneros-Montemayor, A.M., Pauly, D., Weatherdon, L.V., Ota, Y., 2016. A global estimate of
626 seafood consumption by coastal Indigenous peoples. PLoS ONE 11, e0166681–16.
627 doi:10.1371/journal.pone.0166681
628 Clarkson, T.W., Magos, L., 2008. The toxicology of mercury and its chemical compounds.
629 Critical Reviews in Toxicology 36, 609–662. doi:10.1080/10408440600845619
630 Cole, D.C., Kearney, J., Sanin, L.H., LeBlanc, A., Weber, J.-P., 2004. Blood mercury levels
631 among Ontario anglers and sport-fish eaters. Environmental Research 95, 305–314.
632 doi:10.1016/j.envres.2003.08.012
633 Committee on Toxicological Effects of Methylmercury, National Research Council of the United
634 States, 2010. Toxicological effects of methylmercury. National Academies of Science.,
635 Washington.
636 De Mendiburu, F., 2012. R package agricolae: Statistical Procedures for Agricultural Research.
637 Devesa, V., Martínez, A., Súnier, M.A., Vélez, D., Almela, C., Montoro, R., 2001. Effect of
638 cooking temperatures on chemical changes in species of organic arsenic in seafood. J.
639 Agric. Food Chem. 49, 2272–2276. doi:10.1021/jf0013297
640 Diaz-Bone, R.A., Van de Wiele, T., 2010. Biotransformation of metal(loid)s by intestinal
641 microorganisms. Pure and Applied Chemistry 82, 1–19. doi:10.1351/PAC-CON-09-06-08
642 Dong, Z., Yan, K., Liu, Y., Naidu, R., Duan, L., Wijayawardena, A., Semple, K.T., Rahman,
643 M.M., 2016. A meta-analysis to correlate lead bioavailability and bioaccessibility and predict
644 lead bioavailability. Environment International 92-93, 139–145.
645 doi:10.1016/j.envint.2016.04.009
646 Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M.L., Mills, E.N.C., Belton, P.S., Taylor, J.R.N.,
647 2002. Effect of grain structure and cooking on sorghum and maize *in vitro* protein
648 digestibility. Journal of Cereal Science 35, 161–174. doi:10.1006/jcrs.2001.0411
649 Ersoy, B., Yanar, Y., Küçükgülmez, A., Çelik, M., 2006. Effects of four cooking methods on the
650 heavy metal concentrations of sea bass fillets (*Dicentrarchus labrax* Linne, 1785). Food
651 Chemistry 99, 748–751. doi:10.1016/j.foodchem.2005.08.055
652 Farouk, M.M., Wieliczko, K.J., Merts, I., 2004. Ultra-fast freezing and low storage temperatures
653 are not necessary to maintain the functional properties of manufacturing beef. Meat Science
654 66, 171–179. doi:10.1016/S0309-1740(03)00081-0
655 Fernández-García, E., Carvajal-Lérida, I., Pérez-Gálvez, A., 2009. *In vitro* bioaccessibility
656 assessment as a prediction tool of nutritional efficiency. Nutrition Research 29, 751–760.
657 doi:10.1016/j.nutres.2009.09.016
658 Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and
659 purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–
660 509.

661 Food and Agriculture Organization, 2016. The State of World Fisheries and Aquaculture
662 (SOFA). Department of Food and Agriculture Organization of the United Nations.
663 Food Directorate Bureau of Chemical Safety, Health Canada, n.d. Canadian standards
664 (maximum levels) for various chemical contaminants in foods.
665 George, R.M., 1993. Freezing processes used in the food industry. Trends in Food Science &
666 Technology 4, 134–138. doi:10.1016/0924-2244(93)90032-6
667 Gilmour, C.C., Podar, M., Bullock, A.L., Graham, A.M., Brown, S.D., Somenahally, A.C., Johs,
668 A., Hurt, R.A., Jr., Bailey, K.L., Elias, D.A., 2013. Mercury methylation by novel
669 microorganisms from new environments. Environ. Sci. Technol. 47, 11810–11820.
670 doi:10.1021/es403075t
671 Graham, H.N., 1992. Green tea composition, consumption, and polyphenol chemistry.
672 Preventive Medicine 21, 334–350. doi:10.1016/0091-7435(92)90041-F
673 Ha, E., Basu, N., Bose-O'Reilly, S., Dórea, J.G., McSorley, E., Sakamoto, M., Chan, H.M.,
674 2016. Current progress on understanding the impact of mercury on human health.
675 Environmental Research 152, 419–433. doi:10.1016/j.envres.2016.06.042
676 Harris, H.H., 2003. The chemical form of mercury in fish. Science 301, 1203–1203.
677 doi:10.1126/science.1085941
678 He, M., Ke, C.-H., Wang, W.-X., 2010. Effects of cooking and subcellular distribution on the
679 bioaccessibility of trace elements in two marine fish species. J. Agric. Food Chem. 58,
680 3517–3523. doi:10.1021/jf100227n
681 He, M., Wang, W.-X., 2011. Factors affecting the bioaccessibility of methylmercury in several
682 marine fish species. J. Agric. Food Chem. 59, 7155–7162. doi:10.1021/jf201424g
683 Health Canada, 2004. Canadian Community Health Survey, cycle 2.2.
684 Health Canada First Nations Branch, Government of Canada, 2016. Fish and Shellfish Safety -
685 Food Safety: Information for First Nations.
686 Hider, R.C., Liu, Z.D., Khodr, H.H., 2001. Metal chelation of polyphenols, in: Flavonoids and
687 Other Polyphenols, Methods in Enzymology. Elsevier, pp. 190–203. doi:10.1016/S0076-
688 6879(01)35243-6
689 Hur, S.J., Lim, B.O., Decker, E.A., McClements, D.J., 2011. *In vitro* human digestion models for
690 food applications. Food Chemistry 125, 1–12. doi:10.1016/j.foodchem.2010.08.036
691 Jacobs, S., Sioen, I., Jacxsens, L., Domingo, J.L., Sloth, J.J., Marques, A., Verbeke, W., 2016.
692 Risk assessment of methylmercury in five European countries considering the national
693 seafood consumption patterns. Food and Chemical Toxicology 1–9.
694 doi:10.1016/j.fct.2016.10.026
695 Jadán Piedra, C., Sánchez, V., Vélez, D., Devesa, V., 2016. Reduction of mercury
696 bioaccessibility using dietary strategies. LWT - Food Science and Technology 71, 10–16.
697 doi:10.1016/j.lwt.2016.03.015
698 Jadán-Piedra, C., Alcantara, C., Monedero, V., Zuniga, M., Vélez, D., Devesa, V., 2017a. The
699 use of lactic acid bacteria to reduce mercury bioaccessibility. Food Chemistry 228, 158–
700 166. doi:10.1016/j.foodchem.2017.01.157
701 Jadán-Piedra, C., Baquedano, M., Puig, S., Vélez, D., Devesa, V., 2017b. Use of
702 *Saccharomyces cerevisiae* to reduce the bioaccessibility of mercury from food. J. Agric.
703 Food Chem. acs.jafc.7b00285–33. doi:10.1021/acs.jafc.7b00285
704 Janle, E.M., Freiser, H., Manganais, C., Chen, T.-Y., Craig, B.A., Santerre, C.R., 2015. Green
705 tea increases the concentration of total mercury in the blood of rats following an oral fish
706 tissue bolus. BioMed research international 2015, 1–6. doi:10.1155/2015/320936
707 Joint FAO/WHO Expert Committee on Food Additives, 2007. Evaluation of certain food
708 additives and contaminants, WHO technical report series no. Rome, Italy.
709 Kosatsky, T., Przybysz, R., Armstrong, B., 2000. Mercury exposure in Montrealers who eat St.
710 Lawrence River sportfish. Environmental Research 84, 36–43. doi:10.1006/enrs.2000.4073
711 Kulp, K.S., Fortson, S.L., Knize, M.G., Felton, J.S., 2003. An *in vitro* model system to predict the

712 bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food and Chemical*
713 *Toxicology* 41, 1701–1710. doi:10.1016/S0278-6915(03)00197-2

714 Laird, B.D., Chan, H.M., 2013. Bioaccessibility of metals in fish, shellfish, wild game, and
715 seaweed harvested in British Columbia, Canada. *Food and Chemical Toxicology* 1–31.
716 doi:10.1016/j.fct.2013.04.033

717 Laird, B.D., Goncharov, A.B., Egeland, G.M., Man Chan, H., 2013. Dietary advice on Inuit
718 traditional food use needs to balance benefits and risks of mercury, selenium, and n3 fatty
719 acids. *Journal of Nutrition* 143, 923–930. doi:10.3945/jn.112.173351

720 Laird, B.D., Shade, C., Gantner, N., Chan, H.M., Siciliano, S.D., 2009a. Bioaccessibility of
721 mercury from traditional northern country foods measured using an *in vitro* gastrointestinal
722 model is independent of mercury concentration. *Science of the Total Environment* 407,
723 6003–6008. doi:10.1016/j.scitotenv.2009.08.014

724 Laird, B.D., Yeung, J., Peak, D., Siciliano, S.D., 2009b. Nutritional status and gastrointestinal
725 microbes affect arsenic bioaccessibility from soils and mine tailings in the Simulator of the
726 Human Intestinal Microbial Ecosystem. *Environ. Sci. Technol.* 43, 8652–8657.
727 doi:10.1021/es900837y

728 Legrand, M., Arp, P., Ritchie, C., Chan, H.M., 2005. Mercury exposure in two coastal
729 communities of the Bay of Fundy, Canada. *Environmental Research* 98, 14–21.
730 doi:10.1016/j.envres.2004.07.006

731 Legrand, M., Feeley, M., Tikhonov, C., Schoen, D., Li-Muller, A., 2010. Methylmercury blood
732 guidance values for Canada. *Canadian Journal of Public Health* 101, 28–31.

733 Mahaffey, K.R., Mergler, D., 1998. Blood levels of total and organic mercury in residents of the
734 upper St. Lawrence River basin, Québec: Association with age, gender, and fish
735 consumption. *Environmental Research* 77, 104–114. doi:10.1006/enrs.1998.3834

736 Mahaffey, K.R., Sunderland, E.M., Chan, H.M., Choi, A.L., Grandjean, P., Mariën, K., Oken, E.,
737 Sakamoto, M., Schoeny, R., Weihe, P., Yan, C.-H., Yasutake, A., 2011. Balancing the
738 benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from
739 fish consumption. *Nutrition Reviews* 69, 493–508. doi:10.1111/j.1753-4887.2011.00415.x

740 Martín-Doimeadios, R.C.R., Mateo, R., Jiménez-Moreno, M., 2017. Is gastrointestinal
741 microbiota relevant for endogenous mercury methylation in terrestrial animals?
742 *Environmental Research* 152, 454–461. doi:10.1016/j.envres.2016.06.018

743 Mergler, D., Anderson, H.A., Chan, L.H.M., Mahaffey, K.R., Murray, M., Sakamoto, M., Stern,
744 A.H., 2007. Methylmercury exposure and health effects in humans: A worldwide concern. *A*
745 *Journal of the Human Environment* 36, 3–11. doi:10.1579/0044-
746 7447(2007)36%5B3:MEAHEI%5D2.0.CO;2

747 Miettinen, J., Rahola, T., Hattula, T., Rissanen, K., Tillander, M., 1971. Elimination of ²⁰³Hg-
748 methylmercury in man. *Ann. Clin. Res.* 3, 116–122.

749 Minekus, M., Alvinger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou,
750 R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B.,
751 Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D.J.,
752 Ménard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J.,
753 Weitschies, W., Brodkorb, A., 2014. A standardised static *in vitro* digestion method suitable
754 for food – an international consensus. *Food Funct.* 5, 1113–1124. doi:10.1039/C3FO60702J

755 Molan, A.L., Flanagan, J., Wei, W., Moughan, P.J., 2009. Selenium-containing green tea has
756 higher antioxidant and prebiotic activities than regular green tea. *Food Chemistry* 114, 829–
757 835. doi:10.1016/j.foodchem.2008.10.028

758 Moreda-Piñeiro, J., Moreda-Piñeiro, A., Romarís-Hortas, V., Moscoso-Pérez, C., López-Mahía,
759 P., Muniategui-Lorenzo, S., Bermejo-Barrera, P., Prada-Rodríguez, D., 2011. *In-vivo* and *in-*
760 *vitro* testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and
761 mercury species in food samples. *TrAC Trends in Analytical Chemistry* 30, 324–345.
762 doi:10.1016/j.trac.2010.09.008

763 Morgan, J.N., Berry, M.R., Graves, R.L., 1997. Effects of commonly used cooking practices on
764 total mercury concentration in fish and their impact on exposure assessments. *Journal of*
765 *Exposure Analysis and Environmental Epidemiology* 7, 119–133.

766 Ouédraogo, O., Amyot, M., 2011. Effects of various cooking methods and food components on
767 bioaccessibility of mercury from fish. *Environmental Research* 111, 1064–1069.
768 doi:10.1016/j.envres.2011.09.018

769 Oz, F., Kaban, G., Kaya, M., 2007. Effects of cooking methods on the formation of heterocyclic
770 aromatic amines of two different species trout. *Food Chemistry* 104, 67–72.
771 doi:10.1016/j.foodchem.2006.10.075

772 Perelló, G., Martí-Cid, R., Llobet, J.M., Domingo, J.L., 2008. Effects of Various Cooking
773 Processes on the Concentrations of Arsenic, Cadmium, Mercury, and Lead in Foods. *J.*
774 *Agric. Food Chem.* 56, 11262–11269. doi:10.1021/jf802411q

775 R Development Core Team, R: a language and environment for statistical computing. R
776 Foundation for Statistical Computing.

777 Ragan, M.A., Smidsrød, O., Larsen, B., 1979. Chelation of divalent metal ions by brown algal
778 polyphenols. *Marine Chemistry* 7, 265–271. doi:10.1016/0304-4203(79)90043-4

779 Rideout, K., Kosatsky, T., 2017. Fish for dinner? Balancing risks, benefits, and values in
780 formulating food consumption advice. *Risk Analysis* 16, 1885–12. doi:10.1111/risa.12769

781 Rowland, I.R., 1988. Interactions of the gut microflora and the host in toxicology. *Toxicologic*
782 *Pathology* 16, 147–153. doi:10.1177/019262338801600207

783 Sanza, P.D., de Elvira, C., Martino, M., Zaritzky, N., Otero, L., Carrasco, J.A., 1999. Freezing
784 rate simulation as an aid to reducing crystallization damage in foods. *Meat Science* 52,
785 275–278. doi:10.1016/S0309-1740(99)00002-9

786 Sängler, A.M., Stoiber, W., 2001. 7. Muscle fiber diversity and plasticity, in: *Fish Physiology*. pp.
787 187–250.

788 Schottel, J., Mandal, A., Clark, D., Silver, S., Hedges, R.W., 1974. Volatilisation of mercury and
789 organomercurials determined by inducible R-factor systems in enteric bacteria. *Nature* 251,
790 335–337. doi:10.1038/251335a0

791 Shim, S.-M., Ferruzzi, M.G., Kim, Y.-C., Janle, E.M., Santerre, C.R., 2009. Impact of
792 phytochemical-rich foods on bioaccessibility of mercury from fish. *Food Chemistry* 112, 46–
793 50. doi:10.1016/j.foodchem.2008.05.030

794 Siedlikowski, M., Bradley, M., Kubow, S., Goodrich, J.M., Franzblau, A., Basu, N., 2016.
795 Bioaccessibility and bioavailability of methylmercury from seafood commonly consumed in
796 North America - *In vitro* and epidemiological studies. *Environmental Research* 149, 266–
797 273. doi:10.1016/j.envres.2016.02.013

798 Sunderland, E.M., 2007. Mercury exposure from domestic and imported estuarine and marine
799 fish in the U.S. seafood market. *Environ. Health Perspect.* 115, 235–242.
800 doi:10.1289/ehp.9377

801 Torres-Escribano, S., Ruiz, A., Barrios, L., Vélez, D., Montoro, R., 2011. Influence of mercury
802 bioaccessibility on exposure assessment associated with consumption of cooked predatory
803 fish in Spain. *J. Sci. Food Agric.* 91, 981–986. doi:10.1002/jsfa.4241

804 Torres-Escribano, S., Vélez, D., Montoro, R., 2010. Mercury and methylmercury bioaccessibility
805 in swordfish. *Food Additives and Contaminants* 27, 327–337.
806 doi:10.1080/19440040903365272

807 Toyos-Vargas, E., Robles-Romo, A., Méndez, L., Palacios, E., Civera, R., 2016. Changes in
808 fatty acids, sterols, pigments, lipid classes, and heavy metals of cooked or dried meals,
809 compared to fresh marine by-products. *Animal Feed Science and Technology* 221, 195–
810 205. doi:10.1016/j.anifeedsci.2016.09.004

811 Van de Wiele, T., Gallow, C.M., Kubachka, K.M., Creed, J.T., Basta, N., Dayton, E.A., Whitacre,
812 S., Laing, Du, G., Bradham, K., 2010. Arsenic metabolism by human gut microbiota upon *in*
813 *vitro* digestion of contaminated soils 1–7. doi:10.1289/ehp.0901794[Online26March

814 Van de Wiele, T.R., Oomen, A.G., Wragg, J., Cave, M., Minekus, M., Hack, A., Cornelis, C.,
815 Rompelberg, C.J.M., De Zwart, L.L., Klinck, B., Van Wijnen, J., Verstraete, W., Sips,
816 A.J.A.M., 2007. Comparison of five in vitro digestion models to in vivo experimental results:
817 Lead bioaccessibility in the human gastrointestinal tract. *Journal of Environmental Science*
818 *and Health, Part A* 42, 1203–1211. doi:10.1080/10934520701434919
819 Vu, V.Q., 2011. *qqv/ggbiplot*.
820 Wang, H.-S., Xu, W.-F., Chen, Z.-J., Cheng, Z., Ge, L.-C., Man, Y.-B., Giesy, J.P., Du, J.,
821 Wong, C.K.C., Wong, M.H., 2013. *In vitro* estimation of exposure of Hong Kong residents to
822 mercury and methylmercury via consumption of market fishes. *Journal of Hazardous*
823 *Materials* 248-249, 387–393. doi:10.1016/j.jhazmat.2012.12.060
824 Wang, T., Jónsdóttir, R., Ólafsdóttir, G., 2009. Total phenolic compounds, radical scavenging
825 and metal chelation of extracts from Icelandic seaweeds. *Food Chemistry* 116, 240–248.
826 doi:10.1016/j.foodchem.2009.02.041
827 Wickham, H., 2009. *ggplot2: elegant graphics for data analysis*. Springer Science & Business
828 Media, New York.
829 Yannai, S., Sachs, K.M., 1993. Absorption and accumulation of cadmium, lead and mercury
830 from foods by rats. *Food and Chemical Toxicology* 31, 351–355. doi:10.1016/0278-
831 6915(93)90190-A
832
833

834 **Table legends**

835

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

838

839 Figure legends

840

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

846

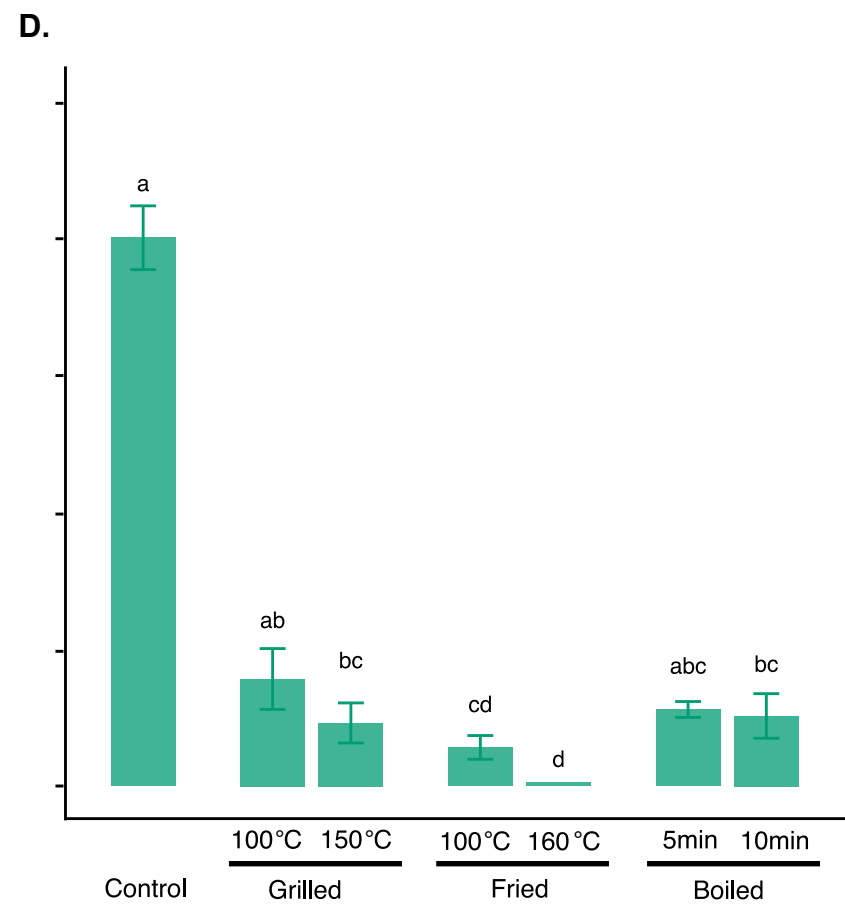
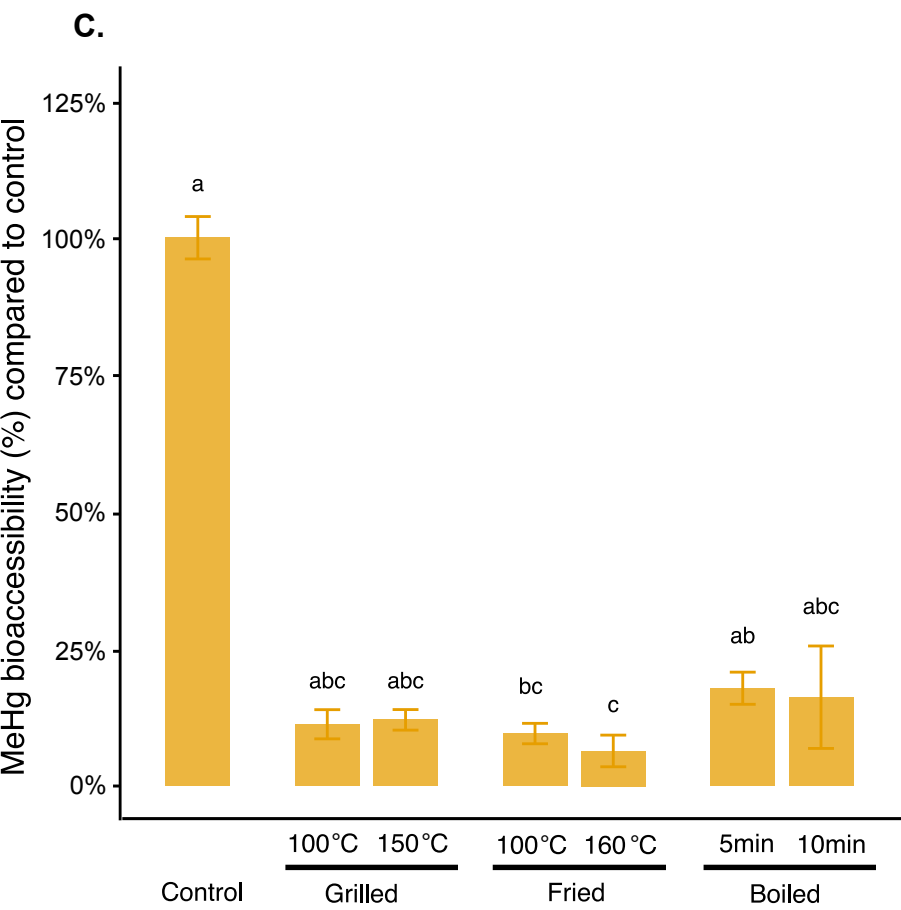
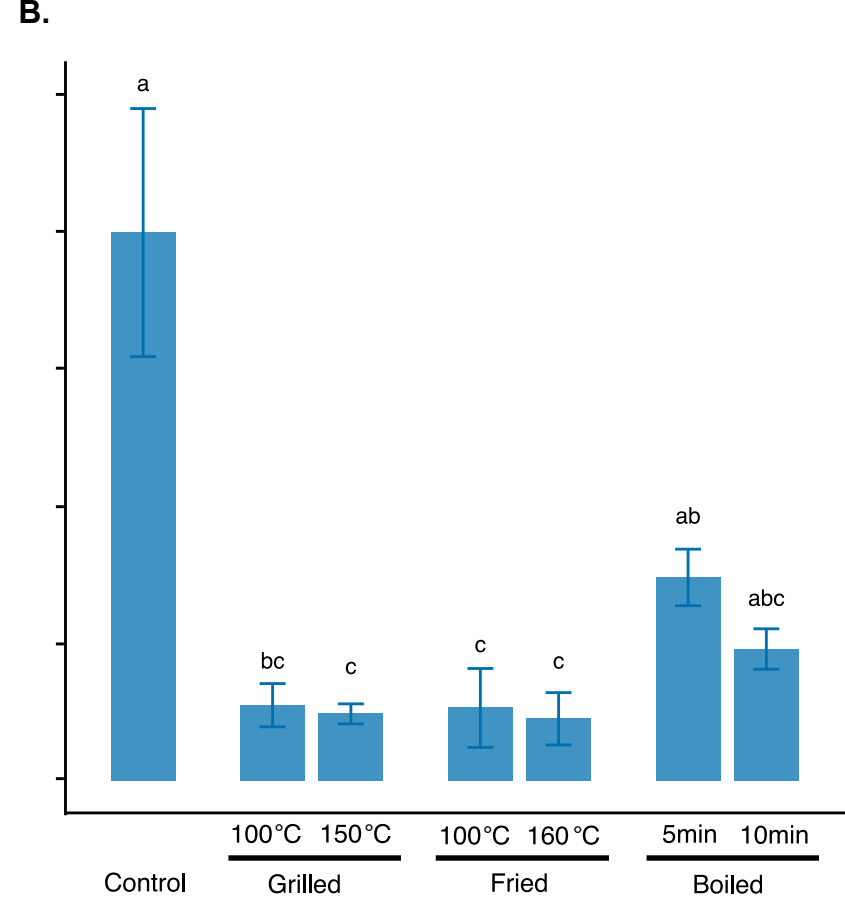
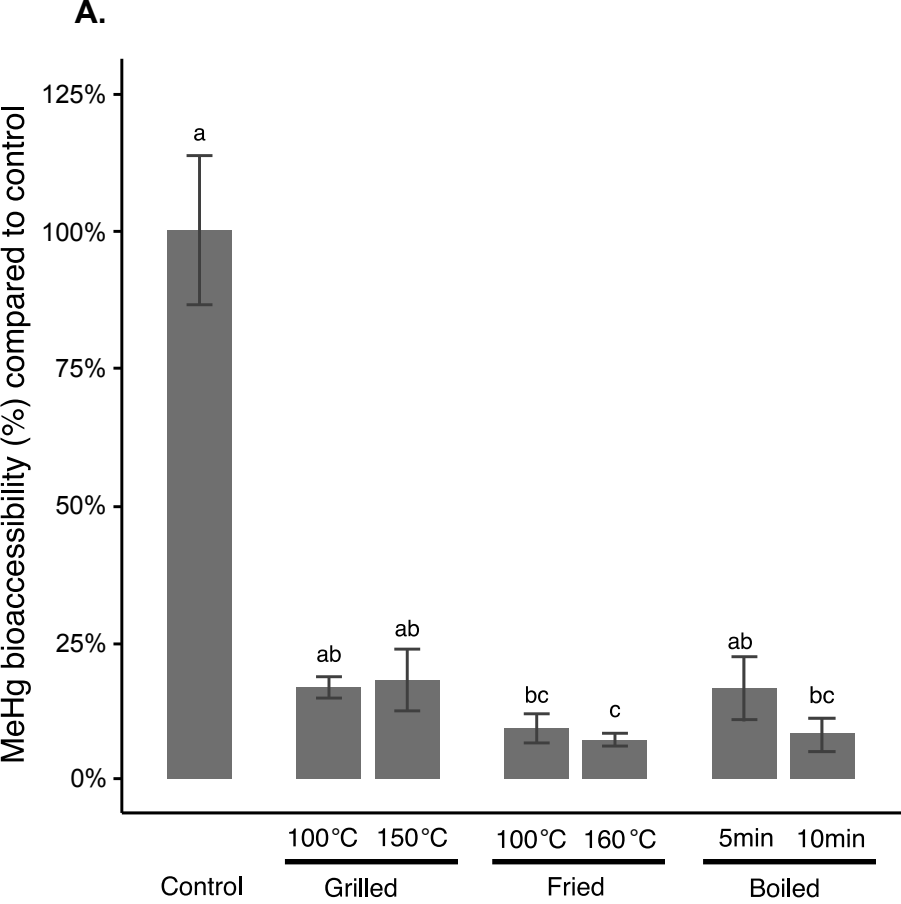
847 **Figure 2.** Effect of polyphenol-rich beverages and foods on MeHg bioaccessibility in **A. & B.**
848 swordfish and **C.** tuna. Results were normalized to controls at 100%, to allow comparison across
849 experiments (see Methods). Letters denote significantly different treatments (Kruskal-Wallis, $P <$
850 0.05) after Bonferonni multiple comparison correction, bars present averages from triplicate PBET
851 digestions and error bars show standard deviation of triplicates.

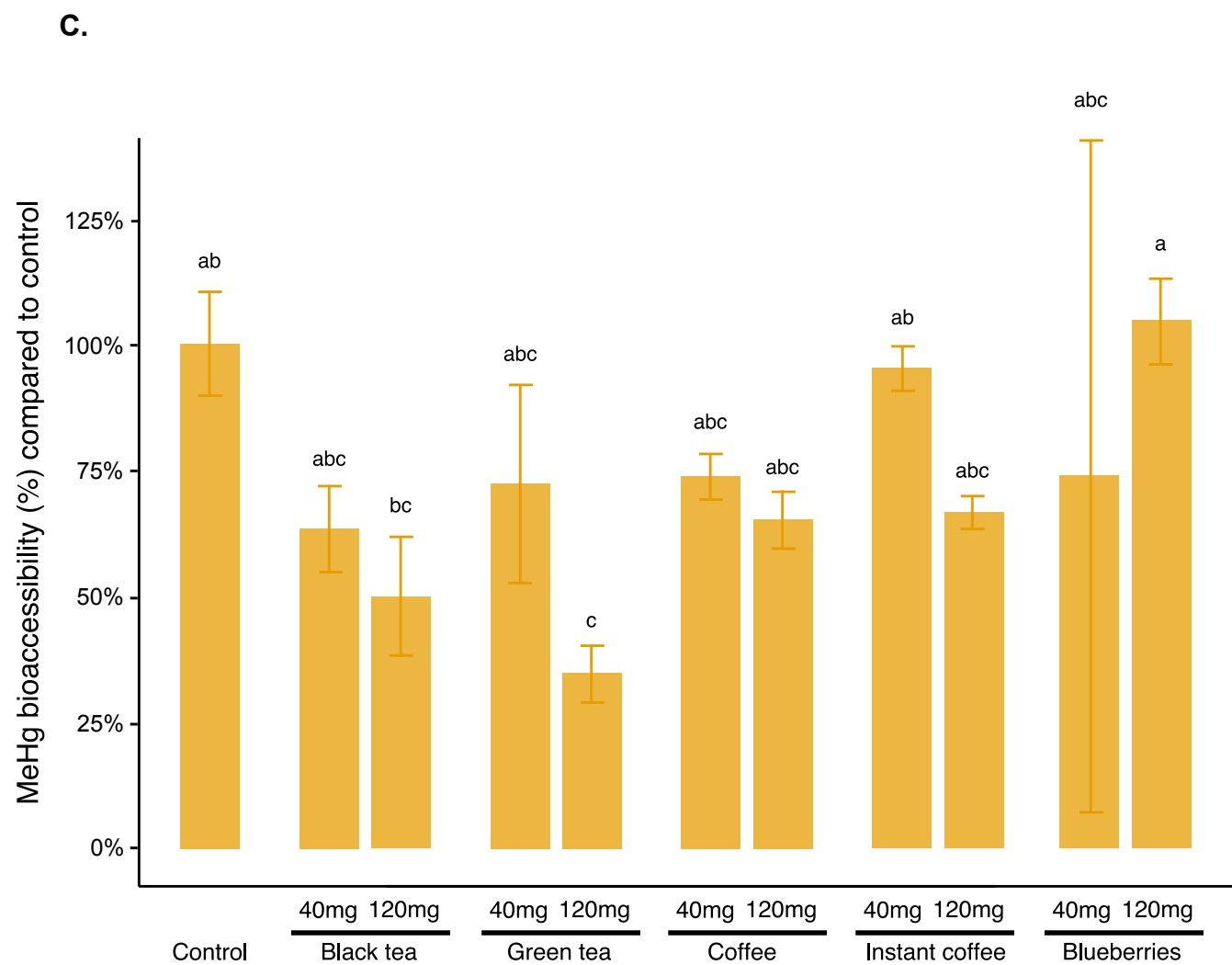
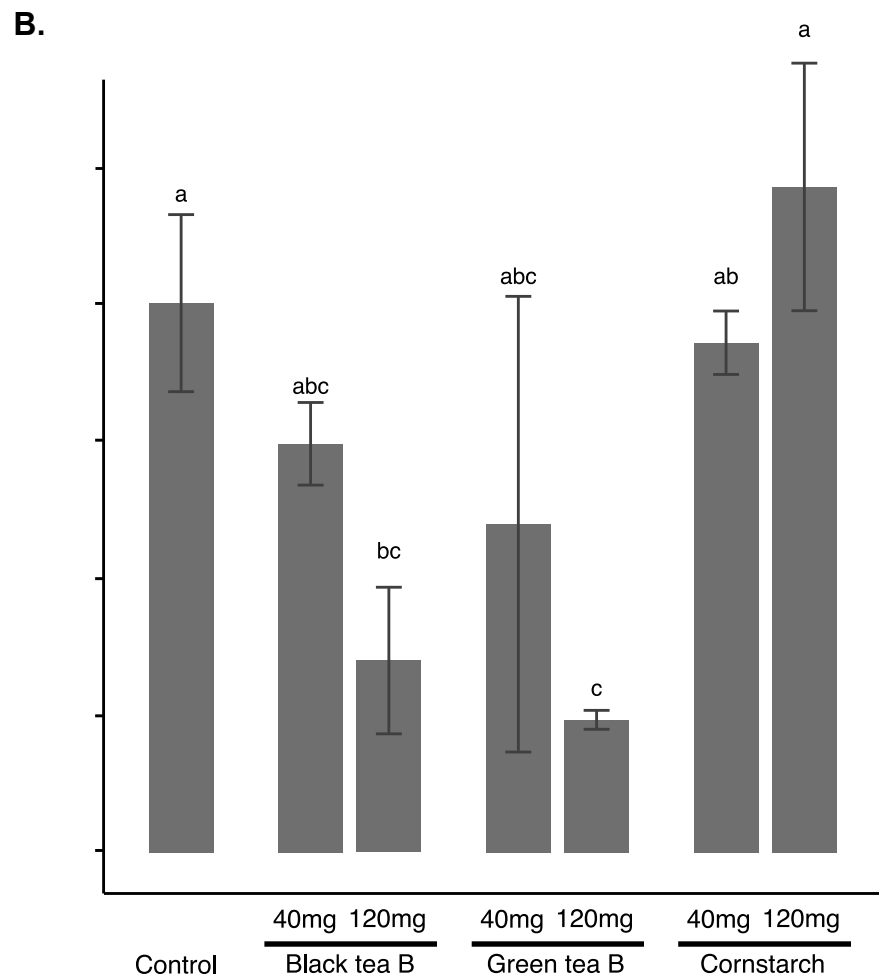
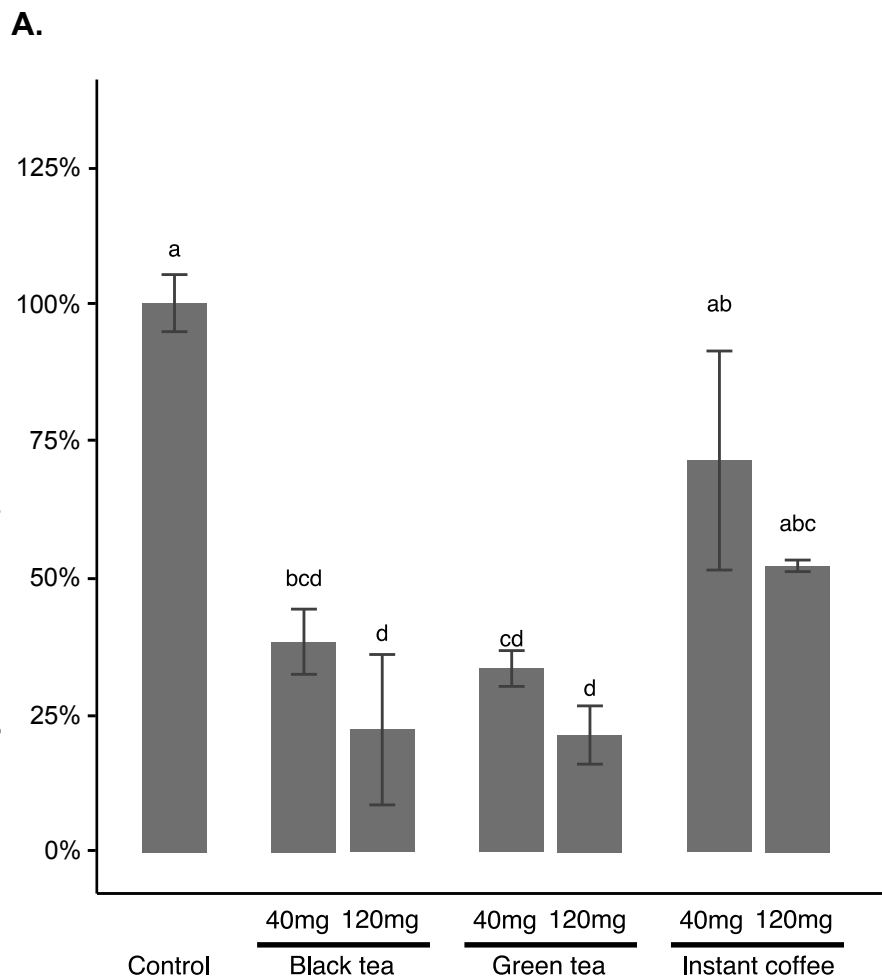
852

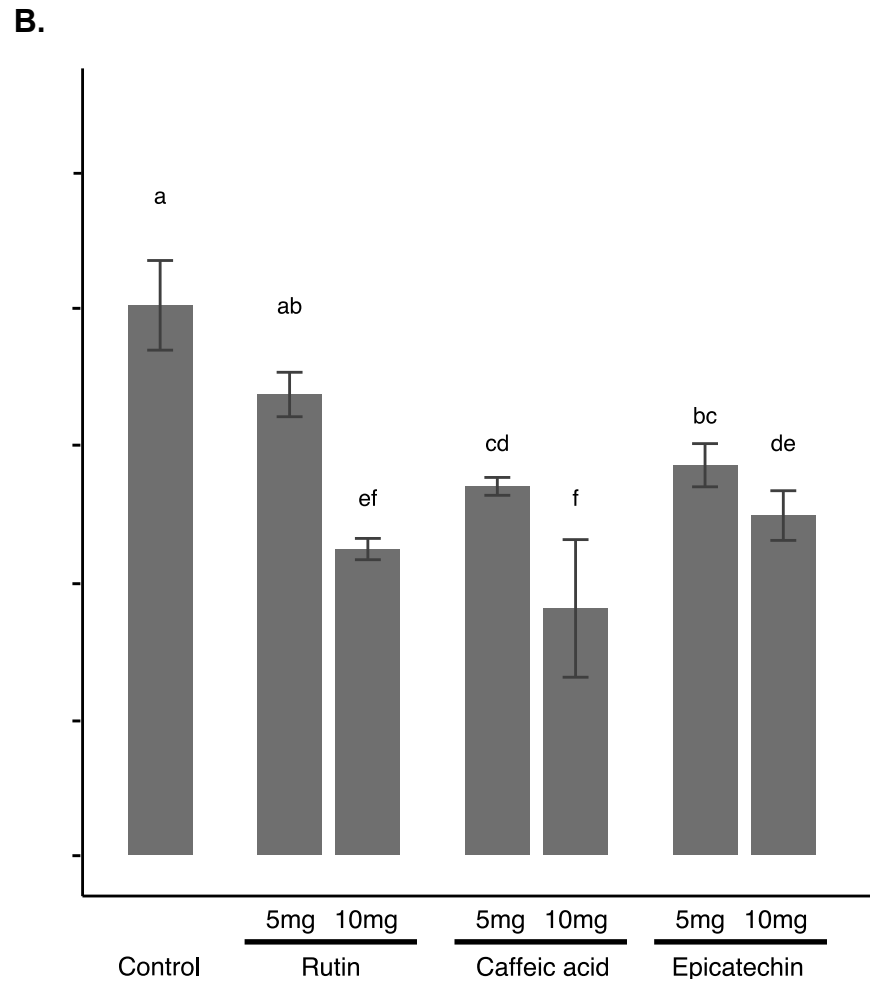
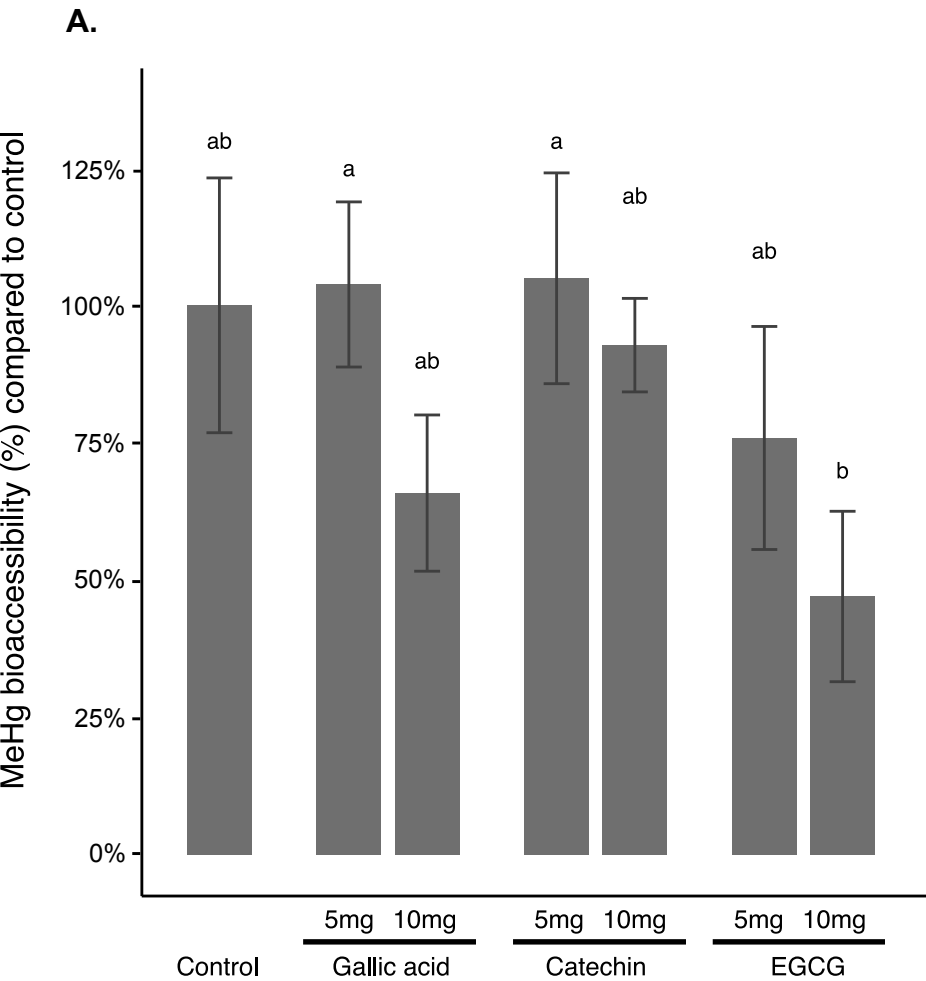
853 **Figure 3.** Effect of pure polyphenols on MeHg bioaccessibility in swordfish. **A & B.** Gallic acid,
854 epigallocatechin gallate, rutin and caffeic acid lead to significant decreases in MeHg
855 bioaccessibility (Kruskal-Wallis, $P < 0.05$). Results were normalized to controls at 100%, to allow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

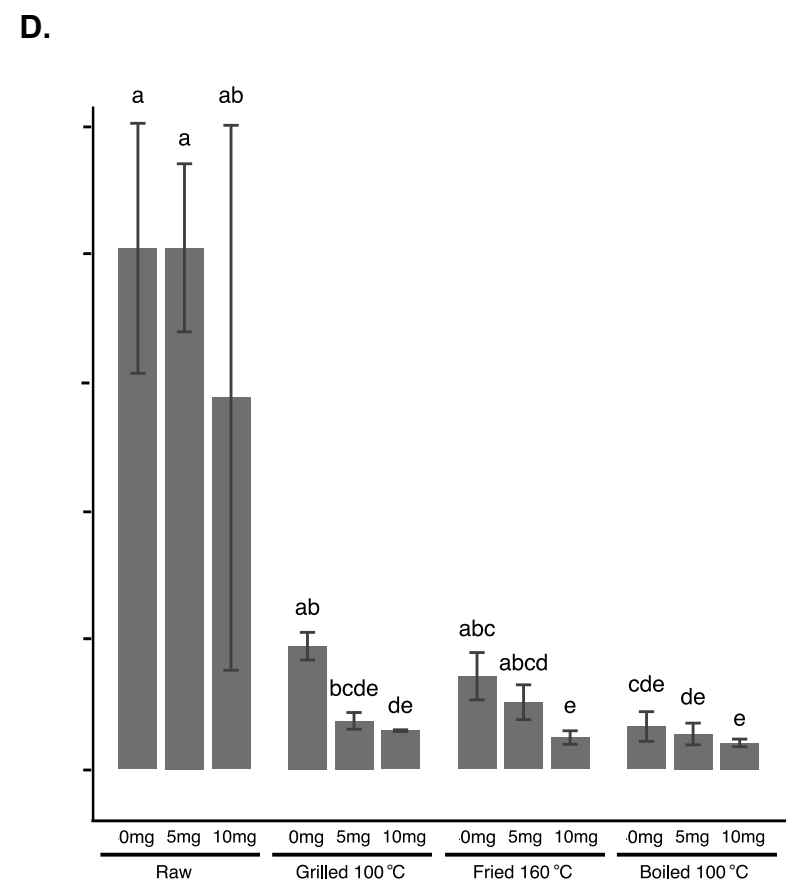
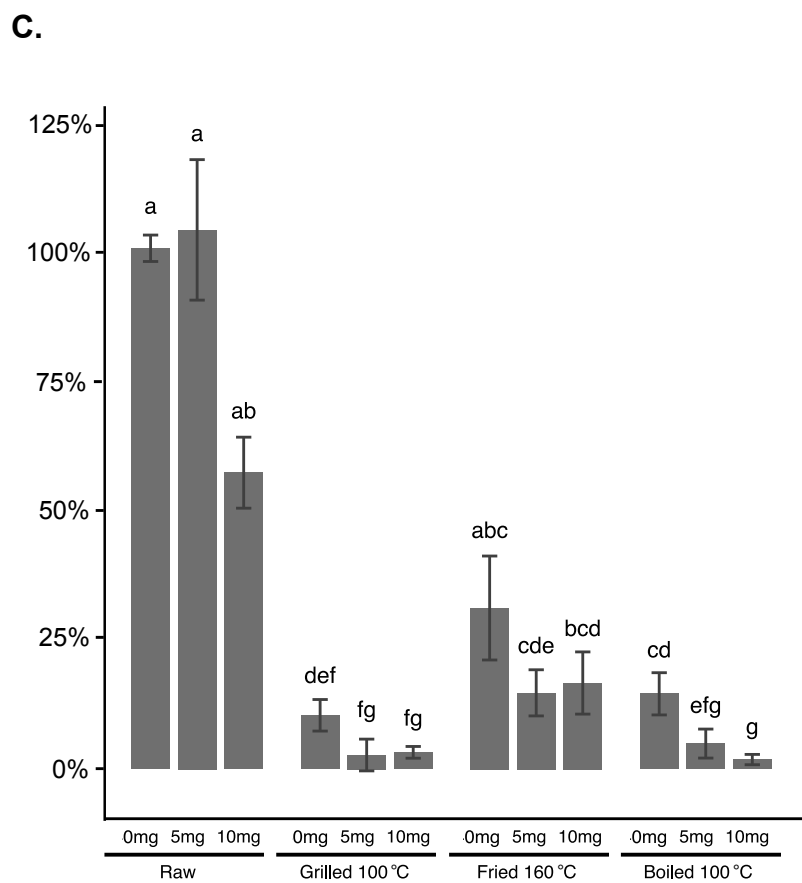
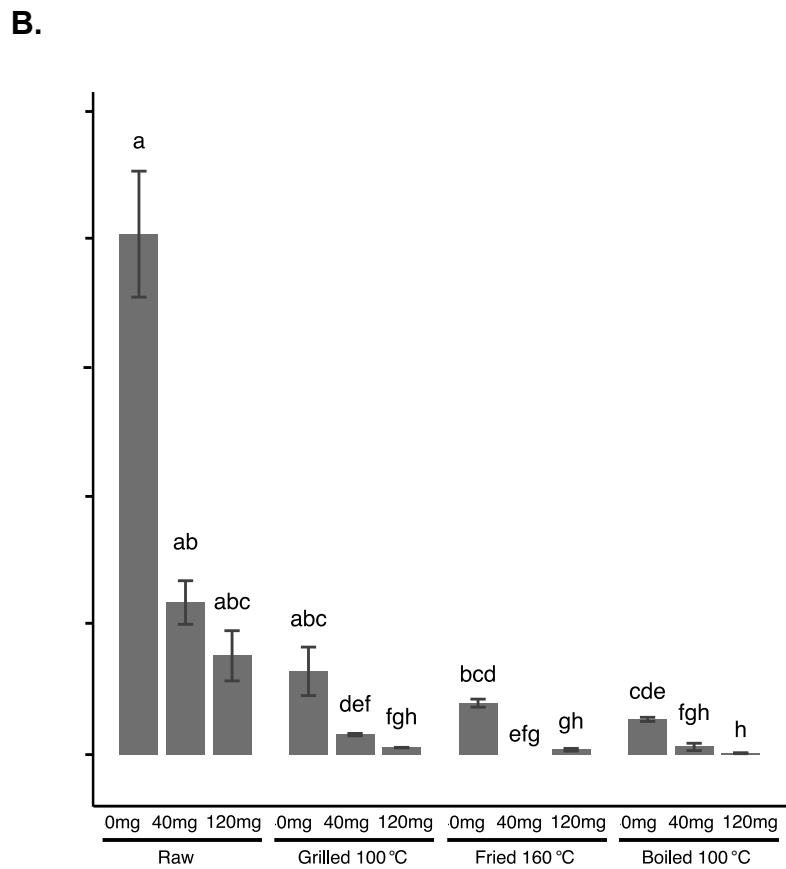
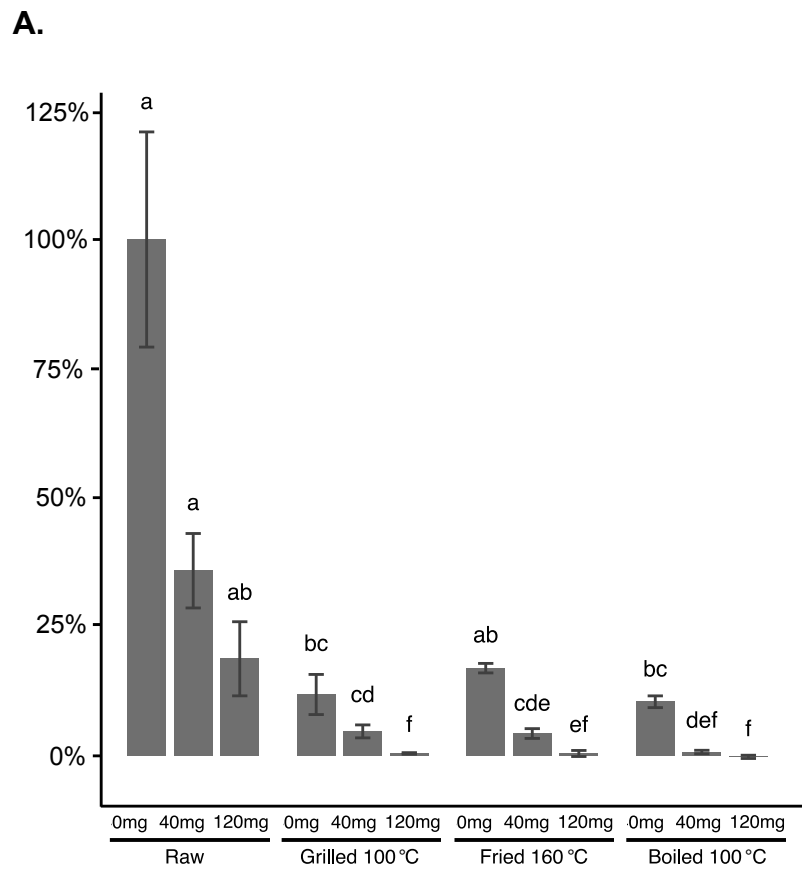
859

860 **Figure 4.** Mixed effect of cooking and polyphenols/polyphenol-rich beverages on MeHg
861 bioaccessibility in swordfish for polyphenol-rich beverages and foods (**A.** black tea, **B.** green tea)
862 and for pure polyphenols (**C.** gallic acid, **D.** catechin). Results were normalized to controls at
863 100%, to allow comparison across experiments (see Methods). Letters denote significantly
864 different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple comparison correction,
865 bars present averages from triplicate PBET digestions and error bars show standard deviation of
866 triplicates.









Supplemental Material

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

Catherine Girard (1), Tania Charette (2, 3), Maxime Leclerc (2, 3), B. Jesse Shapiro (3), Marc Amyot (1,2,3)

(1) Center for Northern Studies (CEN), Département de sciences biologiques, Université de Montréal, Montreal, Canada.

(2) ÉcoLac, Département de sciences biologiques, Université de Montréal, Montréal, Canada.

(3) Groupe de recherche interuniversitaire en limnologie et en environnement aquatique (GRIL), Département de sciences biologiques, Université de Montréal, Montreal, Canada.

#Address correspondence to Marc Amyot, m.amyot@umontreal.ca

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 (Fisher 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]

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

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 N₂. N₂ 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.

64

65 *Polyphenol analyses*

66 Polyphenols were quantified by UPLC-MSMS using a Waters Acquity Ultra-Performance
67 LC system with a quaternary pump system and an Acquity high-strength silica T3 column (120
68 mm x 2.1 mm, 1.8 mm particle size) (Waters). The stationary phase was 100% silica particles.
69 Compounds were separated with a mobile phase of 0.2% acetic acid (eluent A) and acetonitrile
70 (eluent B), with a flow rate of 0.4 mL min⁻¹. The gradient elution was as follows: initial 5% B, 5-
71 20% B (0 – 4.5 min), isocratic 20% B (4.50 – 6.45 min), 20-45% B (6.45 – 13.50 min), 45-100%
72 B (13.5 – 16.5 min), isocratic 100% B (16.5-19.5 min), 100-5% B (19.5 – 19.52 min), isocratic
73 5% B (19.52 – 22.5 min). MS analyses were performed on a TQD mass spectrometer (Waters)
74 with a Z-spray electrospray interface in negative mode, with data acquisition carried out by
75 multiple reactions monitoring (MRM). Ionization source parameters were as follow: capillary
76 voltage at 2.5 kV, source temperature at 140 °C, cone gas flow rate of 80 L h⁻¹, desolvation gas
77 flow rate of 900 L h⁻¹ and desolvation temperature of 350 °C. Nitrogen (>99%) was used as a
78 nebulizing gas, and argon (>99%) as a collision gas. Data acquisition was performed with
79 MassLynx 4.1 software. Results were quantified as gallic acid equivalents, and detection limits
80 were calculated at 0.9 ug g⁻¹ using standard gallic acid.

81

82 **Supplementary References**

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

85

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) <i>n</i> =4	86.35 ± 3.41% (13.25-14.33) <i>n</i> =3	11.85 ± 2.36% (8.79-14.89) <i>n</i> =4	70.06 ± 1.18% (69.13-72.23) <i>n</i> =4
Grouper	612.42 ± 315.32 ng g ⁻¹ (389.45-835.39) <i>n</i> =2	86.35 ± 0.36% (13.77-13.88) <i>n</i> =3	8.55 ± 1.20% (6.40-9.09) <i>n</i> =2	64.43 ± 20.35% (50.04-78.82) <i>n</i> =2
Tuna	694.00 ± 777.80 ng g ⁻¹ (144.01-1243.98) <i>n</i> =2	87.42 ± 0.34% (13.93-14.04) <i>n</i> =3	10.05 ± 0.13% (9.91-10.16) <i>n</i> =2	60.66 ± 11.88% (52.26-69.06) <i>n</i> =2
Salmon	20.16 ng g ⁻¹ (-) <i>n</i> =1	n/a	26.54% (-) <i>n</i> =1	70.00% (-) <i>n</i> =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

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

Treatment	Swordfish		Grouper		Tuna		Salmon	
	PDI ug kg ⁻¹	PDI _{BA} ug kg ⁻¹	PDI ug kg ⁻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		-

109
 110
 111

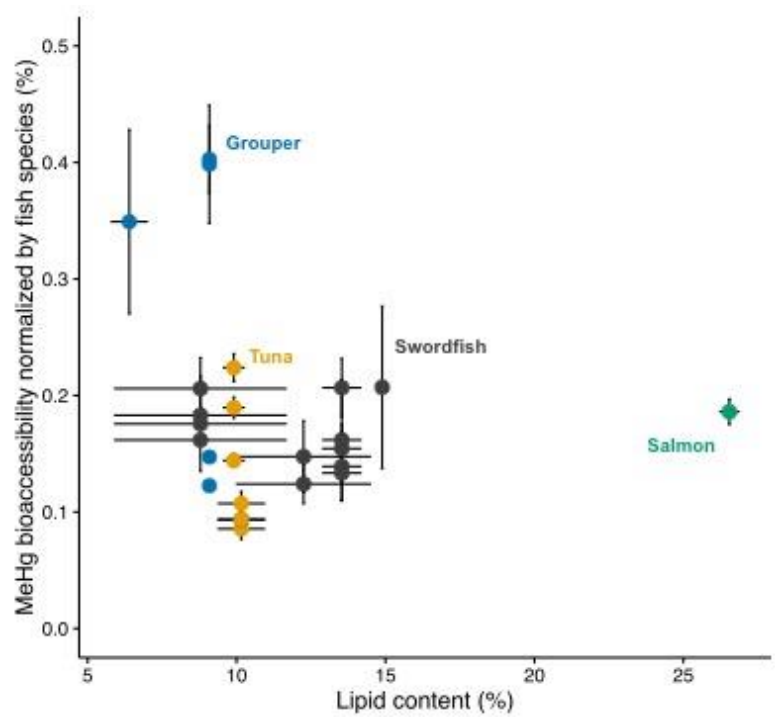
112

113 Supplementary Figures

114

115 Figure S1

116



117

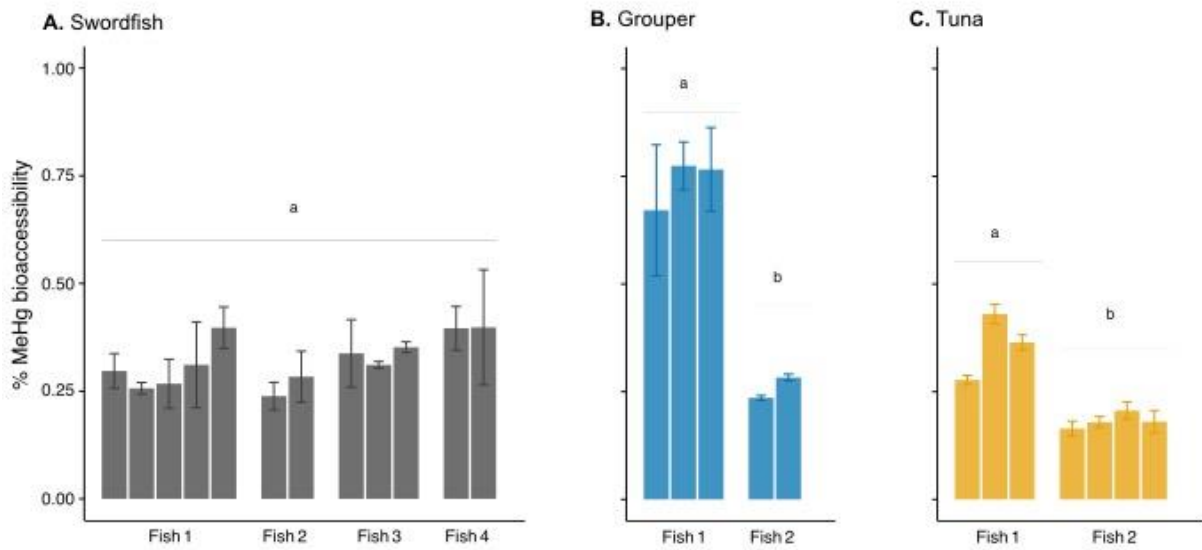
118 **Supplementary Figure S1.** MeHg bioaccessibility in relation to lipid content in different fish
119 species (linear regression, $P > 0.05$). Points present averages from individual fish, and error
120 bars show standard deviation for lipid content (x-axis) and MeHg bioaccessibility (y-axis).

121

122

123
124
125

Figure S2

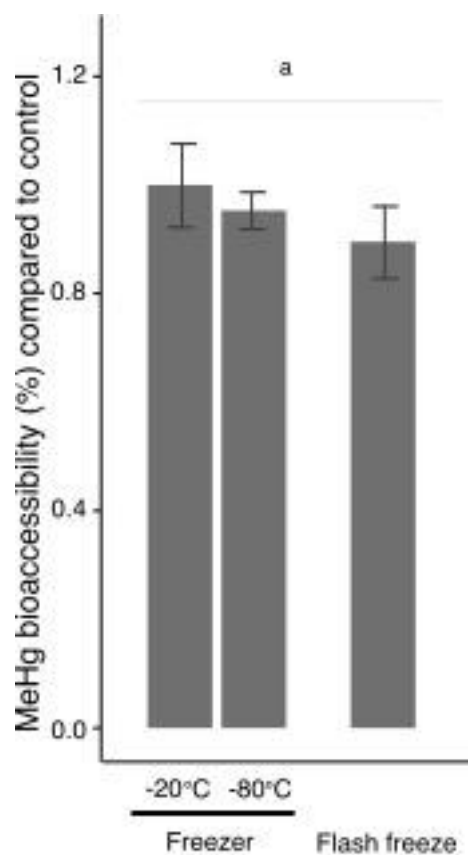


126
127
128
129
130
131
132
133

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 Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.

134 **Figure S3**

135



136

137

138 **Supplementary Figure S3.** Effect of freezing on MeHg bioaccessibility in swordfish. Letters

139 denote significantly different treatments (Kruskal-Wallis, $P < 0.05$) after Bonferonni multiple

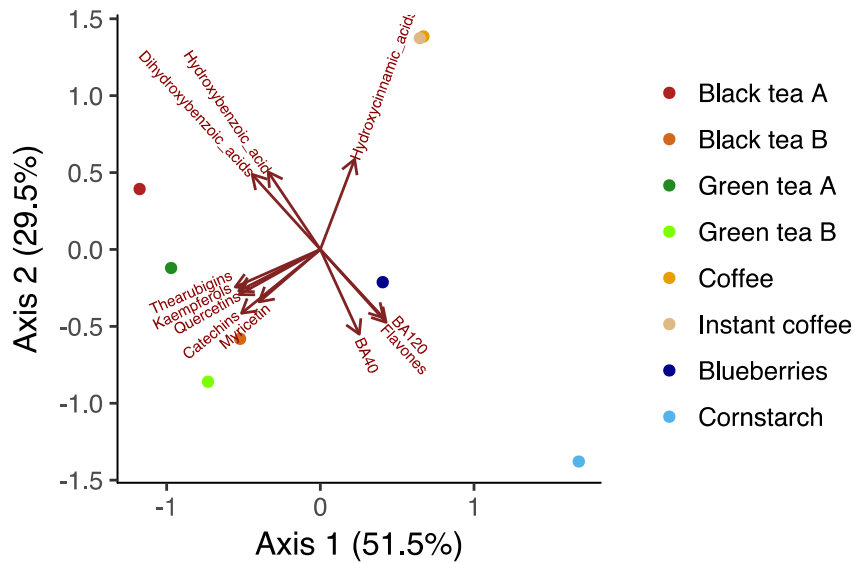
140 comparison correction, bars present averages from triplicate PBET digestions and error bars

141 show standard deviation of triplicates.

142

143
144

Figure S4

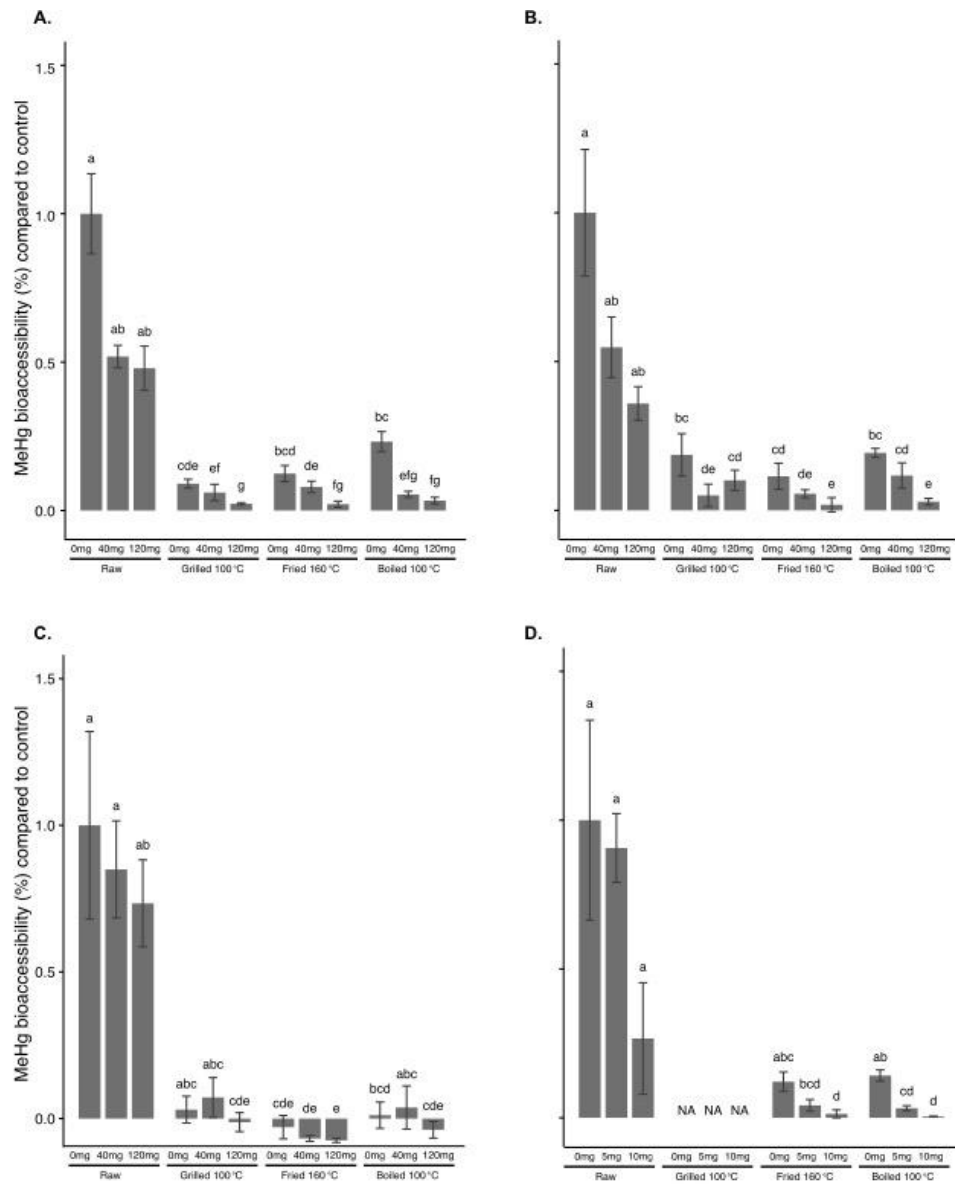


145
146
147
148
149
150
151

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.

152
153
154

Figure S5

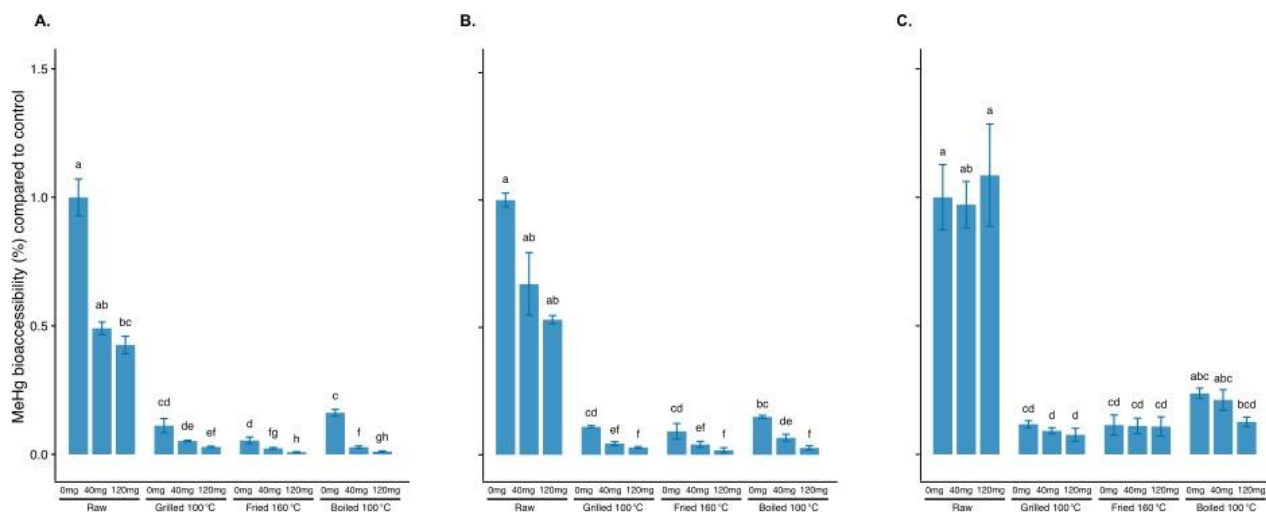


155
156

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

163
164

Figure S6

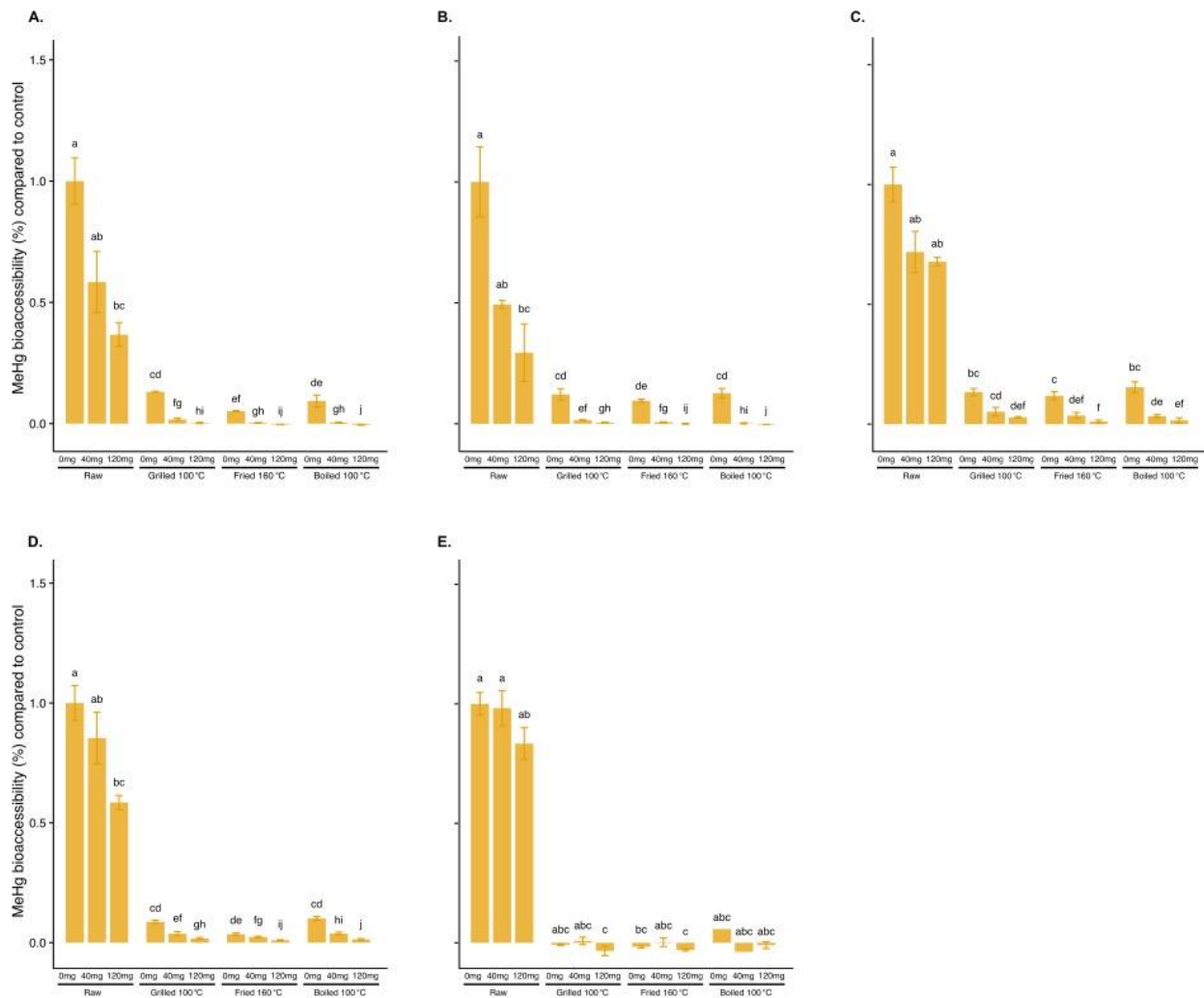


165
166
167
168
169

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.

170
171

Figure S7



172
173
174
175
176
177
178
179
180
181

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 Bonferroni multiple comparison correction, bars present averages from triplicate PBET digestions and error bars show standard deviation of triplicates.