This is a preprint. This manuscript has been submitted for publication in FRONTIERS IN MARINE SCIENCES. Please note that, despite having undergone peer-review, the manuscript has yet to be formally accepted for publication. Subsequent versions of this manuscript may have slightly different content. The final version of this manuscript will be available via a DOI link provided at the moment of acceptance. Please feel free to contact any of the authors. We welcome feedback.

Author

Juanjo Rodríguez, Department of Ecology and Environmental Sciences, Umeå University, Umeå, Sweden

Corresponding author: Juanjo Rodríguez; jj.rserrano@gmail.com

Mercury methylation in boreal aquatic ecosystems under oxic conditions and climate change: a review

Juanjo Rodríguez

Department of Ecology and Environmental Sciences, Umeå University, Umeå, Sweden

Corresponding author: Juanjo Rodríguez; jj.rserrano@gmail.com

Keywords: Mercury methylation, Oxic waters, Climate change, Omic technologies, microbial communities

Abstract

Methylmercury (MeHg) formation is a concerning environmental issue described in waters and sediments from multiple aquatic ecosystems. The genetic and metabolic bases of mercury (Hg) methylation have been well described in anoxic environments, but a number of factors seem to point towards alternative pathways potentially occurring in pelagic waters under oxic conditions. Boreal aquatic ecosystems are predicted to undergo increasing concentrations of dissolved organic matter (DOM) as a result of higher terrestrial runoff induced by climate change, which may have important implications in the formation of MeHg in the water column. In this review, different Hg methylation mechanisms postulated in the literature are discussed, with particular focus on potential pathways independent of the *hgcAB* gene pair and occurring under oxic conditions. Potential effects of DOM on Hg methylation and MeHg bioaccumulation are examined in the context of climate in boreal aquatic ecosystems. Furthermore, the implementation of meta-omic technologies and standardized methods into field measurements and incubation experiments is discussed as a valuable tool to determine taxonomic and functional aspects of Hg methylation in oxic waters and under climate change-induced conditions.

Introduction

Mercury (Hg) is a heavy metal with no physiological role for any known life form. It can be naturally found in geological deposits and coal, but human activities have greatly enhanced the mobilisation and transport of Hg into natural environments (Streets et al., 2011; Mason et al., 2012). Anthropogenic activities, such as coal combustion, mining, and technology industries, release large quantities of mercury into the atmosphere every year (~ 2000 metric tons), which is mainly transported as elemental mercury (Hg^0) by atmospheric currents (Zhang et al., 2016; Kuss et al., 2018; Ghimire et al., 2019). In the global biogeochemical cycle of mercury (**Figure 1**), Hg^0 is oxidized by different molecules present in the atmosphere (e.g., halogen compounds, nitrate radicals or ozone) and eventually converted into divalent inorganic mercury (Hg^{2+}), which is then deposited into aquatic and terrestrial environments (Ariya & Peterson 2005; Kuss et al., 2018; Bowman et al., 2020). Thereafter, part of the Hg^{2+} is reduced and recirculated back

to the atmosphere as Hg^0, but, due to its high reactivity, Hg^{2+} tends to rapidly bind to more stable molecules present in waters and sediments, remaining in these ecosystems for long periods (Krabbenhoft & Sunderland 2013). Once in aquatic environments, mercury is transformed and uptaken by the resident biota as methylmercury (MeHg), which is bioaccumulated and biomagnified as it is transferred up to the food chain (Boening, 2000; Ullrich et al., 2001; Jonsson et al., 2014). This organic form of mercury is known to be a potent neurotoxin with severe effects for ecosystem health. On humans, MeHg can cause multiple adverse effects, such as neurological and endocrine disorders, cardiovascular problems, or fetal death (Tsubaki & Irukayama 1977; Grandjean et al. 2004; Tan et al. 2009).

MeHg problematic and early studies

The problematic of MeHg as environmental pollutant dates back to 1960s, where Minamata Disease was postulated to be caused by MeHg poisoning (Minamata Disease Research Group, 1966). Similarly, Westöö (1967) reported abnormally high concentrations of mercury in fish and other foodstuff in Sweden, where 80% to 100% of the mercury was present as MeHg. Considering these and other similar major public health outbreaks worldwide, the microbial participation in MeHg formation in natural aquatic ecosystems was postulated for the first time in a series of studies by Jernelöv et al. (1969), where mercury chloride was added into bottom lake sediments and MeHg was observed to be formed after 5-10 days. MeHg was not detected when the sediments were previously sterilized, which led to the conclusion that this process must involve microbial activity, although no microbial species or genes were postulated at the time (Jensen & Jernelöv, 1969; Jernelöv 1969). In addition, one year earlier, Wood et al. (1968) had shown that MeHg could be formed from methylcobalamin when an extract of a methanogenic bacterium (isolated from a canal mud) was incubated with ATP and Hg^{+2} under a hydrogen atmosphere (anoxic conditions).

In light of the emerging knowledge of biotic methylation as the primary source of MeHg, important progress was made by further studies identifying microbial species capable of Hg methylation, particularly in anoxic conditions. Initially, several studies reported microbial methylators under culture-based conditions, isolates and cell extracts (Wood et al., 1968; Taira, 1975; Ridley et al., 1977; Robinson & Tuovinen, 1984), which left yet unresolved the methylation of Hg by complex communities under natural conditions. Compeau & Bartha (1985) was the first comprehensive study identifying and isolating a particular bacterial species capable of Hg methylation in natural aquatic environments. They demonstrated *Desulfovibrio desulfuricans* capability to methylate Hg^{2+} in low-salinity anoxic estuarine sediments, postulating, for the first time, sulfate-reducing bacteria as major methylators in anoxic aquatic environments. Multiple later studies continued reporting new bacterial species and entire functional groups responsible for MeHg formation in natural environments, from sulfate- and iron-reducing bacteria (e.g., *Geobacter sulfurreducens*, *Desulfuromonas palmitatis*, *Desulfosporosinus acidiphilus*) to methanogenic *Archaea* (e.g., *Methanolobus tindarius*, *Methanobrevibacter smithii*, *Methanoculleus bourgensis*) (Kerin et al, 2006; Ranchou-Peyruse et al., 2009; Gilmour et al., 2013; Podar et al., 2015; Bravo et al., 2018; Jones et al., 2019; Capo et al., 2020; Gionfriddo et al., 2020). However, the molecular bases (genes and enzymes) behind Hg methylation remained unclear, which implied a substantial gap towards a full understanding of this process. This gap would be in turn fulfilled with the discovery of the hgcAB gene cluster.

126 ## HgcAB pathway and the emerging paradigm shift

127 Since its discovery in 2013 (Parks et al., 2013), the hgcAB gene cluster has captured most of
128 the attention in studies on Hg methylation, as *hgc*A and *hgc*B genes have been proven to be
129 involved in the formation of the bulk of MeHg found in aquatic environments. In a series of
130 experiments, Parks et al. (2013) demonstrated that the biotic formation of MeHg requires the
131 presence of both *hgc*A and *hgc*B genes in the methylator's genome. The deletion of either of
132 these genes supresses the ability to methylate Hg through the reductive acetyl–coenzyme A
133 pathway, but it does not hamper cell growth, which suggests that *hgc*A and *hgc*B are not
134 essential for survival (Parks et al., 2013). *Hgc*A has been described to encode the putative
135 protein HgcA, which is responsible for the transfer of methyl groups to Hg^{2+}. On the other
136 hand, *hgc*B encodes a 2[4Fe-4S] ferredoxin (HgcB protein) capable of reducing a corrinoid
137 cofactor present in HgcA, which enables the acceptance of a methyl group by HgcA. Therefore,
138 the redox potential is an important physicochemical factor that can control Hg uptake and
139 MeHg production by regulating the enzymatic activity of the methylators (Beckers et al. 2019,
140 Wang et al. 2021, Regnell & Watras 2019).

141 After a decade of numerous studies on the mechanism of Hg methylation, hgcAB gene cluster
142 has been found in every known anaerobic Hg methylator and in metagenomes from multiple
143 anoxic sediments and waters containing substantial MeHg concentrations (Paranjape & Hall
144 2017; Regnell & Watras, 2019; Gionfriddo et al., 2019; Ma et al., 2019; Capo et al., 2020).
145 This fact has led to the use of *hgcAB* genes as indicators of potential Hg methylation occurring
146 in anoxic aquatic environments. However, recent studies have reported environmentally high
147 concentrations of MeHg in surface waters (Lehnherr et al., 2011; Kirk et al., 2012; Paranjape
148 and Hall, 2017; Villar et al. 2019), where the concentration of oxygen would not allow Hg
149 methylators to perform through the acetyl-CoA/hgcAB pathway. These observations were
150 hypothesised by early studies to be caused by MeHg production in anoxic coastal sediments,
151 which would be then transported to the overlying water column and open waters (Kraepiel et
152 al., 2003; Hammerschmidt & Fitzgerald, 2006). However, this hypothesis conflicts with results
153 from more recent studies showing high demethylation rates and short MeHg lifetime
154 (Monperrus et al., 2007a; Whalin et al., 2007; Lehnherr et al., 2011; Ortiz et al., 2015; Wang
155 et al., 2020), which would prevent MeHg to remain in substantial quantities throughout such
156 journey (Cossa et al., 2017). Furthermore, this hypothesis also contrasts with MeHg profiles
157 reported by numerous studies, where the highest concentrations are found in subsurface layers,
158 while deeper waters show lower concentrations (Mason & Fitzgerald, 1993; Sunderland et al.,
159 2009; Cossa et al., 2009; Heimbürger et al., 2015; Munson et al., 2015). External sources, such
160 as atmospheric MeHg deposition and riverine transport, could also be regarded as significant
161 factors, but they are currently considered to be minor sources for the MeHg concentrations
162 found in most marine pelagic environments (Mason et al., 2012; Liu et al., 2021; Wang et al.,
163 2022).

164 Alternatively, *in situ* methylation of inorganic Hg has been suggested by numerous studies to
165 be the main source of MeHg in the water column. In an incubation experiment, Lehnherr et al.
166 (2011) reported water-column Hg methylation as a significant source of monomethylmercury
167 in Arctic pelagic marine food webs, estimating this process to account for up to 47% of the
168 total monomethylmercury found in these seawaters. Similar studies have also reported MeHg
169 formation in oxygenated marine waters where no known anaerobic methylators or *hgc*AB
170 genes were detected (Malcolm et al. 2010; Podar et al. 2015; Bowman et al. 2019; Rodríguez
171 et al. 2022). These observations pose an apparent paradox where MeHg is produced in non-
172 anoxic waters through a process where anaerobic methylators are considered the main players.
173 As a result, four main explanations can be postulated (**Figure 2**): 1) Hg methylation by abiotic
174 processes; 2) Biotic MeHg production within anoxic micro-environments; 3) Biotic MeHg

175 production under oxic conditions through metabolic pathways involving *hgcAB* (or *hgcAB*-
176 like) genes or 4) independent of *hgc*AB genes.

177 The abiotic methylation of Hg has been repeatedly addressed in numerous studies (Celo et al.,
178 2006; Li & Cai, 2013; Regnell & Watras, 2019; Chetelat et al., 2022). While photochemical
179 and non-photochemical methylation are partially responsible for the production of MeHg in
180 oxic waters, these abiotic mechanisms do not generally account for a substantial fraction of the
181 MeHg found in pelagic environment (Monperrus et al., 2007; Lehnherr et al., 2011; Munson et
182 al., 2018; Wang et al., 2022). Therefore, this review will mainly focus on the biotic mechanisms
183 potentially occurring in oxic waters.

184

185 Anoxic micro-environments as Hg methylation hotspots in oxic waters

186 The existence of settling particles in the pelagic zone, enriched in organic matter and showing
187 marked oxygen and pH gradients has been documented since the 1980s (Alldredge and Cohen
188 1987; Decho 1990; Shanks & Reeder, 1993). These particles can range in size from colloids (<
189 0.2 µm) to aggregates (> 300 µm). In particular, marine snow has been widely described as
190 aggregated mixtures of large particles (> 300 µm) mostly composed by organic matter from
191 plankton fecal pellets, living cells, detritus, and exudates (Alldredge & Silver, 1988; Turner,
192 2015). Alldredge and Cohen 1987 was one of the first studies reporting oxygen-depleted
193 microenvironments within and around such particles, where microbial processes requiring low
194 oxygen conditions, such as denitrification, may occur. Respiratory and photosynthetic
195 activities were measured in light and dark conditions. Their results showed significant oxygen
196 depletion (up to 45%) particularly during dark conditions, when respiration by the bacterial
197 communities inhabiting these particles overtakes the photosynthetic production of oxygen
198 carried out by phytoplankton communities. This respiratory activity is enhanced by the
199 presence of organic matter and essential nutrients present in these particles, which further
200 stimulates bacterial activity (Azam & Long, 2001). These observations were supported by later
201 studies (Ploug et al., 1997; Tang et al., 2011; Decho & Gutierrez; 2017; Bianchi et al., 2018).
202 In a combination of laboratory experiments and field observations, Shanks & Reeder 1993 also
203 found oxygen-depleted conditions within marine snow particles, where sulfide was detected in
204 concentrations far exceeding the values in the surrounding oxic waters. The production of
205 sulfide was also attributed to microbial activity supported by the organic matter within the
206 particles, and shed light to a similar paradox on the production of sulfide in oxygenated layers
207 of the water column.

208 Marine snow particles are not the only structures where anoxic microenvironments can be
209 formed within oxic waters. The interior of planktonic biota, particularly zooplankton, has also
210 been described to enclose anoxic conditions where anaerobic bacteria can thrive. Glud et al.
211 (2015) reported anoxic microenvironments within carcasses of the copepod *Calanus*
212 *finmarchicus* in fully oxygenated seawater from Godthåbsfjord (Greenland). They detected
213 denitrification activity by the bacterial communities naturally inhabiting the interior of these
214 copepods, suggesting sinking *C. finmarchicus* carcasses as hotspots of pelagic denitrification.
215 The gut of living zooplankton has also been reported to show anoxic conditions where different
216 bacterial processes take place (Proctor 1997; Braun et al. 1999). Tang et al. (2011) measured
217 oxygen levels inside the guts of the copepods *Calanus hyperboreusand* and *C. glacialis* from
218 arctic and subarctic waters. The recorded oxygen profiles indicated microbial respiration,
219 which can deplete oxygen levels inside the guts and maintain anoxic conditions.

220 Considering previous studies reporting the existence of these anoxic microenvironments, the
221 hypothesis of such microenvironments being responsible for MeHg production in oxic pelagic

222 waters started to gain importance as an explanation for the MeHg paradox (Monperrus et al.,
223 2007; Cossa et al., 2009; Sunderland et al., 2009; Lehnherr et al., 2011; Sonke et al., 2013;
224 Schartup et al., 2015). Ortiz et al. (2015) was one of the first studies directly measuring the
225 production of MeHg within settling marine particles. In a microcosm experiment, marine
226 aggregates ranging in size (0.2 µm to > 300 µm; including marine snow) were produced from
227 sieved estuarine seawater and spiked with MeHg ($CH_3{}^{199}Hg$) and inorganic Hg ($^{200}Hg^{+2}$).
228 Methylation and demethylation rates were measured based on changes in isotopic composition
229 of ^{199}Hg and ^{200}Hg. The results pointed to a net Hg methylation particularly in larger particles
230 such as marine snow, which was comparable to rates found in benthic sediments. Gascón Díez
231 et al. (2016) carried out a similar study in the largest freshwater lake in Western Europe (Lake
232 Geneva). Over a period of two years, sediments and settling particles from this lake were
233 collected monthly. Total Hg/MeHg concentrations and methylation rates were measured in the
234 upper first cm of the sediments and in settling particles. Interestingly, MeHg concentrations
235 were 10-fold higher in settling particles compared to sediments, whereas total Hg
236 concentrations were similar in both compartments. Furthermore, while demethylation rates
237 were similar in sediments and particles, methylation rate constants (k_m) were 2-fold greater in
238 settling particles, which suggests a net MeHg formation in settling particles 10 orders of
239 magnitudes higher than sediments. In order to determine the biological origin of this MeHg
240 production, they amended the sediments and particles with molybdate, a known inhibitor of
241 sulfate reducing metabolism, which resulted in a reduction of MeHg production by 80%. In a
242 more recent study, Capo et al. (2020) addressed this question by a genetic and phylogenetic
243 approach. They analysed 81 metagenomes collected from Baltic Sea waters ranging in oxygen
244 levels from normoxic (2 mL O_2 L^{-1}) to hypoxic (< 2 mL O_2 L^{-1}) and anoxic (no detectable O_2).
245 The *hgcAB* genes were mainly detected in settling particles (marine snow) from anoxic waters,
246 with lower presence under hypoxic and normoxic conditions. However, higher *hgcAB*
247 abundance was found in marine snow compared to filtered water with no aggregates,
248 confirming that settling particles can be hotspots of MeHg production. In addition, a
249 phylogenetic study revealed a Hg methylator community predominantly composed by sulfate
250 reducing bacteria affiliated with *Deltaproteobacteria*.

251

252 **Hg methylation under oxic conditions: is there an alternative methylation**
253 **pathway?**

254 The production of MeHg in oxic waters by anaerobic methylators (microenvironments) and, to
255 a lesser extent, by abiotic processes may represent a substantial fraction of the total MeHg pool
256 in certain pelagic environments, but it does not satisfactorily resolve the MeHg paradox in oxic
257 waters where no *hgcAB* genes or anaerobic Hg methylator are detected. Are there Hg
258 methylation mechanisms other than the hgcAB pathway? Or do we need higher-resolution
259 molecular tools to detect *hgcAB* abundances below the current detection thresholds?

260 Podar et al. (2015) carried out the first comprehensive study on the distribution and prevalence
261 of *hgcAB* genes and unknown Hg methylators across different environments on Earth (>3500
262 microbial metagenomes), including oxic pelagic waters. The *hgcAB* gene pair was found in
263 every anoxic environment, whereas these genes were only detected in 7 of the 138
264 metagenomes from pelagic marine water columns. These observations are supported by a more
265 recent study (Bowman et al., 2019) where *hgcAB* genes were screened but not detected in the
266 upper water column (< 800 m) from Arctic Ocean seawater. These findings support the
267 hypothesis of alternatives Hg methylation pathways carried out under true oxic conditions. On
268 the contrary, other studies have reported the presence of hgcAB-like genes in surface waters.
269 Gionfriddo et al. (2016) identified for the first time hgcAB-like genes in two *Nitrospina*

genomes from waters of the East Antarctic Sea. These microaerophilic bacteria were therefore proposed to play an important role as Hg methylators in pelagic waters, although the authors still referred to microenvironments, such as brine pockets and periphytic biofilms associated with settling organic matter, as the most probable niches where Hg methylation may occur. A more recent study on the distribution of the *hgcAB* genes and Hg methylators in the global ocean (Villar et al., 2020) detected the presence and expression of *hgcAB* homologues in seawaters from most of ocean basins worldwide (except in the Arctic Ocean). These genes were linked to taxonomic relatives of known Hg methylators belonging to *Deltaproteobacteria*, *Firmicutes, Chloroflexi*, and particularly *Nitrospina*, which was suggested to be the predominant and widespread bacteria carrying and expressing *hgcAB*-like genes. However, as in Gionfriddo et al. (2016), the methylating activity of Nitrospina was not tested under true oxic conditions, and the authors attributed its methylating capacity to be likely perform in oxygen-deficient microenvironments within sinking marine particles. Moreover, although hgcAB-like genes are predicted to encode corrinoid iron-sulphur and transmembrane domains distinctive of HgcA and a 4Fe-4S ferredoxin motif characteristic of HgcB, to date organisms carrying these genes have not been proven to produce MeHg (Podar et al. 2015; Gilmour et al. 2018).

To address this controversy, Rodríguez et al. (2022) carry out a microcosm experiment (37 L aquaria) where surface water from the Baltic Sea was filtered through 0.7 µm to remove sinking particles potentially creating anoxic microenvironments, while preserving the natural prokaryotic communities. The water was exposed to increased concentration of inorganic Hg (250 pM Hg^{+2}) and an air-bubbling system ensured continuous oxygen saturation. Interestingly, MeHg production was detected above ambient levels after addition of inorganic Hg. Using 16S amplification and shotgun metagenomics, neither *hgcAB* genes nor known Hg methylators were detected in this study (including *Nitrospina*). Concentration of MeHg was highly correlated with bacterial activity, which strongly suggest the existence of an alternative Hg methylation pathway under truly oxic conditions where *hgcAB* gene pair is not involved.

MeHg formation and climate change

The potential magnification in MeHg formation and bioaccumulation as a consequence of climate change is currently of great concern for national and international environmental authorities (Stern et al., 2012; Krabbenhoft & Sunderland, 2013; Marnane, EEA Report 11/2018; Chetelat et al., 2022). But what is the link between climate change and MeHg formation in boreal aquatic systems? The key factor is organic matter. Prediction models point to an increase of precipitation regimes in the Northern hemisphere as a result of climate change, which in turn will lead to higher terrestrial runoff and river inflows into boreal aquatic ecosystems (Meier et al. 2011; Andersson et al., 2015). These terrestrial inflows contain high concentrations of dissolved organic matter (DOM) swept from the catchment area, thus increasing the overall concentration of DOM in the water column. The reactive nature of organic matter to interact with different environmental pollutants has been object of study for decades, and Hg has been particularly targeted by numerous studies (Hsu-Kim et al., 2013; Schartup et al., 2015; Ripszam et al., 2015b; Alava et al., 2017; Rodríguez et al., 2018; Jiang et al., 2018). Thus, evidence of the role of DOM on Hg methylation and bioaccumulation has exponentially increased over the last years, pointing to several levels at which DOM can interact with Hg (**Figure 3**): 1) As a complexing agent on Hg^{+2} speciation (Hsu-Kim et al., 2013; Chiasson-Gould et al. 2014; Jiang et al., 2018) and providing methyl groups required for methylation (Nagase et al., 1982, 1984; Weber et al., 1985; from Wang 2022); 2) by enhancing microbial metabolism (Hall et al., 2004; Paranjape and Hall, 2017); and 3) by promoting

318 changes in the physiology and permeability of the bacterial cell membrane (Campbell et al.,
319 1997; Vigneault et al., 2000).

320

321 ***DOM-Hg chemical interactions***

322 The chemical interactions between DOM and Hg largely depend on both DOM concentration
323 and composition. Different ligands within the DOM molecular pool strongly bind to Hg, which
324 can have a dual effect on Hg bioavailability and MeHg production. On the one hand, Hg^{+2} can
325 rapidly bind to biologically labile DOM (LDOM), forming LDOM-Hg aggregates that are
326 highly bioavailable for prokaryotic uptake (Hsu-Kim et al., 2013; Chiasson-Gould et al. 2014;
327 Jiang et al., 2018). This higher bioavailability increases the internalization of Hg into the
328 cytoplasm, where it is transformed into MeHg. A typical example of LDOM is the
329 autochthonous DOM produced by phytoplankton, also known as exudates (Seymour et al.,
330 2017; Mühlenbruch et al., 2018; Eigemann et al., 2022). These molecules can be easily
331 degraded or directly uptaken by prokaryotes, and consequently they have been linked to high
332 MeHg production rates (Kim et al., 2011; Lázaro et al., 2013; Bravo et al., 2017). On the other
333 hand, the bioavailability of Hg can be reduced when refractory compounds (RDOM) act as the
334 main ligands for Hg binding, forming RDOM-Hg aggregates. These molecules are more
335 recalcitrant for biodegradation and, therefore, Hg uptake and MeHg formation are reduced in
336 these situations (Chiasson-Gould et al., 2014; French et al., 2014; Bravo et al., 2017).
337 Furthermore, more complex interactions have been suggested to occur between LDOM-Hg and
338 RDOM-Hg aggregates, where Hg^{+2} can be dissociated from RDOM under photochemical
339 oxidation or microbial degradation and bind to LDOM, thus becoming more bioavailable
340 (Chiasson-Gould et al., 2014). The opposite situation has also been described, where Hg^{+2}
341 bioavailability is reduced due to the progressive degradation of the most labile fraction of DOM
342 by heterotrophic activity, which leads to higher RDOM:LDOM ratios and thus more abundant
343 RDOM-Hg aggregates. These complex interactions between different fractions of DOM and
344 Hg were hypothesized in two companion studies by Chiasson-Gould et al. (2014) and French
345 et al. (2014). In both studies, a bell-shaped relationship between DOM concentration and Hg
346 bioavailability was observed, where Hg bioavailability increased with increasing DOM
347 concentration until a threshold (8.5 – 10 mg C L^{-1}), from which Hg bioavailability started to
348 progressively decrease with increasing DOM concentration. Each of these studies focused on
349 different aspects of the Hg pathway. Whereas French et al. (2014) examined MeHg
350 bioaccumulation in littoral amphipods collected from 26 Arctic lakes (Canada), Chiasson-
351 Gould et al. (2014) used a modified strain of *Escherichia coli* as bioreporter under oxic
352 conditions. This bacterium contains a *merlux* construct that emits bioluminescence when Hg^{+2}
353 is actively transported through the cell membrane, which is used to quantify intracellular Hg^{+2}
354 levels. Interestingly, both studies observed similar patterns in DOM-Hg interactions.

355 In addition, Chiasson-Gould et al. (2014) also studied Hg^{+2} bioavailability under two
356 conditions: pseudoequilibrium and nonequilibrium at different DOM concentrations. They
357 observed a decrease in bioavailability when Hg^{+2} was pre-incubated with DOM over 24 h (i.e.,
358 DOM-Hg pseudoequilibrium), which was explained by Hg^{+2} being mostly bound to strong
359 binding sites in humic and fluvic acids. However, bioavailability increased when Hg^{+2} was
360 freshly added (i.e., DOM-Hg nonequilibrium) to solutions already containing cells and
361 humic/fluvic acids at increasing concentrations (0 to 10 mg C L^{-1}), after which bioavailability
362 started to decrease (bell-shaped pattern).

363 These observations suggest that the formation of DOM-Hg aggregates is a slow process
364 involving competitive ligand exchange dynamics with multiple functional groups within the
365 DOM pool, which may have important implications in a changing climate where DOM is

366 expected to increase in the water column of boreal aquatic systems. DOM chemical structure
367 and content should be considered of capital importance when studying effects on Hg
368 bioavailability, MeHg production and bioaccumulation, particularly considering that organic
369 matter in aquatic ecosystems is a heterogeneous mixture of molecules derived from terrestrial
370 (allochthonous) and internal (autochthonous) sources, as well as the fact that DOM present in
371 aquatic systems is usually more diverse in molecular structure and primary sources than in
372 terrestrial environments (Jafé et al., 2008; Schartup et al., 2015).

373

374 ***MeHg production enhanced by increasing microbial metabolism***

375 As discussed above, Hg bioavailability and MeHg formation can be increased through chemical
376 interactions with different DOM molecules, which ultimately interact with the Hg methylators.
377 However, since Hg bio-methylation is an enzymatic process, it can also be enhanced by direct
378 stimulation of the microbial metabolic activity, which has been observed to take place in
379 pelagic waters (French et al., 2014; Paranjape & Hall, 2017; Rodríguez et al. 2022). Numerous
380 studies have provided large evidence of the effects of DOM on the overall increase of microbial
381 metabolism and the importance of organic matter remineralization in the methylation of Hg
382 (Hall et al., 2004; Heimbürger et al., 2010; Bowman et al., 2016; Bravo et al., 2017; Kim et al.,
383 2017; Regnell & Watras, 2018; Herrero Ortega et al., 2018). In fact, the role of settling particles
384 on Hg methylation can be understood to operate at two different levels: 1) By providing low-
385 oxygen micro-environments; 2) By providing organic matter as fuel to increase microbial
386 metabolism. In line with this, Lehnherr et al. (2011) found significant correlations between
387 POC remineralization and MeHg concentrations in the Arctic Sea, but referred to poor POC
388 availability as probable cause for the low methylation rates detected in their experiments, which
389 may have been higher if the water samples had been collected during the seasonal
390 phytoplankton blooms. This relationship between phytoplankton production and Hg
391 methylation is also supported by other studies where peak MeHg concentrations were detected
392 in oxic subsurface euphotic zones during chlorophyll maximum (Bowman et al., 2015; Wang
393 et al., 2018).

394 Oxygen deficient zones (ODZ) have been suggested as major sources of MeHg linked to
395 organic matter remineralization (Wang et al., 2012; Bowman et al., 2015; Cossa et al., 2017;
396 Kim et al., 2017; Gallorini & Loizeau, 2021). A common ground hypothesis is that POC (such
397 as phytoplankton exudates) and DOM originated in surface and subsurface waters gradually
398 sink to deeper layers, scavenging Hg in its path and forming aggregates that are later
399 metabolized by prokaryotic communities under anoxic or hypoxic conditions. However, while
400 ODZ may be the main source of MeHg in the water column, MeHg production has also be
401 linked to DOM degradation under fully oxygenated waters. Rodríguez et al. (2022) found
402 strong positive correlations between bacterial production and MeHg formation in oxygenated
403 coastal waters from the Baltic Sea under increasing DOM concentrations, where micro-
404 particles larger than 0.7 μm were discarded. Considering projected climate change scenarios
405 for the Northern hemisphere (Meier et al. 2011; Andersson et al. 2015), the addition of
406 terrestrial DOM led to an increase of the overall bacterial activity and diversity, as well as the
407 relative abundance of genes related to enzymatic activity and energy generation. Furthermore,
408 MeHg formation was detected shortly after the addition of Hg^{+2} (13 h), pointing to a rapid
409 response of bacterial communities to methylate Hg. This quick metabolic response was also
410 suggested by the rapid increase in relative abundance of genes related to cellular activity, with
411 no significant changes in taxonomic composition. These observations may indicate that Hg
412 methylation under oxic conditions just responds to housekeeping genes which increase their

413 overall activity when carbon, nutrients or temperature are provided, but further studies are
414 required to determine the exact mechanisms.

415 The rapid response of microbial communities to methylate Hg^{+2} may have important
416 implications under a changing climate where DOM concentration in the water column is
417 expected to increase. Although terrestrial DOM flushed from the catchment area in boreal
418 systems is usually rich in refractory organic carbon (Asmala et al. 2013; Bravo et al. 2017),
419 MeHg formation could potentially be increased through a rapid methylation of newly deposited
420 Hg by the local prokaryotic communities boosted by high DOM concentrations, before Hg^{+2}
421 becomes complexed with recalcitrant compounds in the DOM pool (Chiasson-Gould et al.,
422 2014; Rodríguez et al., 2022). In addition, the overall MeHg concentration may be further
423 increased by inputs of allochthonous MeHg derived from runoff (Krabbenhoft and Sunderland,
424 2013; Jonsson et al., 2012; Jonsson et al., 2014), as well as by reducing MeHg photo-
425 demethylation due to water brownification and subsequent light attenuation typically induced
426 by tDOM (Poste et al., 2015; Wu et al., 2021).

427

428 ***<u>Impacts of DOM on cell membrane permeability</u>***

429 Hg bioavailability can also increase by changes in the physiology of the bacterial cell
430 membrane resulting in higher permeability. Humic and fluvic acids from natural DOM have
431 been shown to act as surfactants, affecting the chemical properties of biological membranes
432 and particularly the level of permeability to neutrally charged chemical species (Vigneault et
433 al., 2000; Slaveykova et al., 2003; Ojwang' & Cook, 2013; Graham et al., 2017), which could
434 constitute a potential pathway for Hg^{+2} internalization (Benoit et al., 1999; Benoit et al., 2001).
435 Chiasson-Gould et al. (2014) studied changes in the bacterial membrane permeability under
436 different DOM concentrations and oxic conditions using bile salts, which reduce cell growth
437 due to increased membrane permeability. They observed no significant increase in membrane
438 permeability, and attributed the higher internalization of Hg^{+2} to interactions between DOM
439 and the bacterial cell wall that resulted in the destabilization of the lipopolysaccharide layer,
440 which could make Hg transport sites more accessible. Nonetheless, these conclusions should
441 be taken with caution as other studies have reported decreased uptake and toxicity of charged
442 metals by humic substances (Koukal et. al., 2003; Kostić et al., 2013; Perelomov et al., 2018).

443

444 ***<u>Temperature is also a substantially important factor</u>***

445 The central aspect from which all climate change effects derive is global warming. The
446 increasing temperatures have multiple impacts on a variety of biogeochemical systems, from
447 ice melting in polar and alpine regions to changes in hydrological patterns, increased sea levels,
448 and frequency of extreme atmospheric events (i.e., droughts and deluges), among others (Stern
449 et al., 2012; Andersson et al., 2015; Stott, 2016). Therefore, raising temperatures can have
450 indirect and direct effects on Hg methylation. Permafrost thaw in polar and sub-polar regions
451 has been observed to mobilize significant amounts of Hg accumulated over decades, which can
452 be ultimately transported into aquatic systems (Schuster et al., 2018; Schaefer et al., 2020;
453 Dastoor et al., 2022). Particularly in boreal regions, increased precipitation regimes are
454 predicted to import higher organic matter from catchment areas, with effects on Hg methylation
455 already discussed in the above sections. This increased terrestrial runoff will also import higher
456 amounts of both Hg^{+2} and MeHg into aquatic ecosystems (Stern et al., 2012; Jonsson et al.,
457 2014). Together with nutrients and organic carbon from tDOM, prokaryotic communities have
458 all the necessary elements to potentially carry out Hg methylation (Rodríguez et al., 2022). In
459 addition, a more direct impact of increasing temperatures is the positive effects on microbial

460 activity, further stimulating the overall microbial metabolism, including the methylation
461 activity (Monperrus et al., 2007a; Johnson et al., 2016). On the other hand, negative effects on
462 Hg methylation should be also considered by Hg cycling prediction models. For instance,
463 higher rates of demethylation may be caused by the overall increase of microbial activity
464 (Paranjape and Hall, 2017; Lu et al., 2016; Lu et al., 2017), as well as by higher exposure to
465 solar radiation due to loss of sea ice (Stern et al., 2012).

466 Other indirect effects may account for a potential increase of Hg methylation as a result of
467 increasing temperatures. Thermal stratification of the water column is associated with warmer
468 air temperatures, and it has been shown to promote hypoxic conditions as oxygenated surface
469 waters are prevented from mixing with the bottom layers (Cloern, 2001; Altieri et al., 2015).
470 This may lead to higher Hg methylation rates under anoxic conditions. Furthermore, oxygen
471 levels can also be depleted by heterotrophic activity boosted by both higher temperatures and
472 higher DOM concentration, where the eutrophication levels may increase Hg methylation
473 (Soerensen et al., 2016; Ji et al., 2020; Yao et al., 2020).

474

475 Experimental approaches to study MeHg formation in oxic waters

476 With so many confounding factors affecting the Hg methylation process, how do we conduct
477 suitable experimental strategies and measurements? Studying biological systems is a
478 particularly complex task due to the outstanding complexity derived from numerous
479 interdependent metabolic pathways, number of biological and chemical species involved, and
480 bidirectional interactions with multiple physicochemical factors.

481 Field measurements and laboratory incubation experiments are the most employed approaches
482 in the study of Hg methylation (Paranjape and Hall, 2017; Regnell & Watras, 2019; Ma et al.,
483 2019; Gallorini & Loizeau, 2021). Since MeHg is a potent neurotoxin naturally bioaccumulated
484 in natural environments, field experiments within aquatic systems are very constrained due to
485 the exposure of Hg or MeHg to the natural biota. Furthermore, both field measurements and
486 incubation experiments are restricted to a limited framework of variables, as it is virtually
487 impossible to account for all the variables that operate in natural ecological systems. However,
488 a combination of field measurements and controlled experiments is a valuable approach that
489 has been used in a number of studies on Hg methylation in pelagic environments (Lehnherr et
490 al., 2011; French et al., 2014; Paranjape and Hall, 2017; Wang et al., 2022). In some instances,
491 either field measurements or controlled experiments may be sufficient to advance our
492 knowledge towards a particular research question, but both approaches are subjected to their
493 own limitations (Wang et al., 2020).

494

495 ***Field measurements***

496 Field measurements typically consist of the collection of water and sediments to determine
497 physical variables (e.g., temperature and light intensity), chemical variables (e.g., Hg/MeHg
498 concentrations, organic matter content, oxygen, pH, salinity, etc.) and/or biological variables
499 (e.g., metabolic activity, taxonomic composition, gene expression, etc.). Since Hg methylation
500 is primarily a biochemical process, comprehensive studies should include a combination of
501 such bio-physico-chemical variables. However, until recently, field measurements generally
502 considered only chemical factors such as organic matter, oxygen, or relevant chemical species
503 such as thiols (in addition to Hg/MeHg) (Monperrus et al., 2007a; Merritt & Amirbahman,
504 2009; Li & Cai, 2013). Following the breakthrough of meta-omic technologies, more recent
505 studies have introduced taxonomic and metabolic parameters principally through the

sequencing of environmental DNA (eDNA). Bowman et al. (2019) and Villar et al. (2020) are among the most recent studies that have greatly contributed to survey the occurrence of Hg/MeHg concentrations and relevant microbial genes in oxic waters from the global oceans. In Bowman et al. (2019), water samples (<800 m depth) were collected from multiple areas ranging from Arctic to equatorial Pacific oceans, where detailed Hg speciation measurements were conducted, as well as targeted and shotgun metagenomics to evaluate the presence of Hg-cycling genes (*hgcAB* and *mer*) and other relevant genes potentially involved in Hg transportation and methylation. On the other hand, Villar et al. (2020) carried out a compilation of metagenomic and metatranscriptomic data obtained from locations covering most of the global ocean basins, with the purpose of addressing the paradox between MeHg production in the upper water column and absence of known anaerobic Hg methylating prokaryotes. Similarly, Podar et al. (2015) also conducted a compilation study using data generated by several metagenome sequencing projects. The compiled sequences were assembled and annotated to study the occurrence of *hgcAB* genes and Hg methylators across different environments, including boreal aquatic systems. Interestingly, these studies reached similar conclusions, where no *hgcAB* genes were generally detected in the oxic water column. Although hgcAB-like genes were found in Bowman et al. (2019) and Villar et al. (2020), to date these genes have not been proven to be involved in the methylation of Hg.

Incubation experiments

Incubation experiments have traditionally relied on experimental models consisting of one or few microbial species (Benoit et al., 2001; Kerin et al., 2006; Parks et al., 2013; Gilmour et al., 2013). Microbial isolates have been used to target specific aspects of the Hg methylation mechanism by simplifying the bio-physico-chemical system. Although this approach has been extensively used under anoxic conditions, only a few experiments have been conducted to investigate Hg methylation under oxic conditions. Chiasson-Gould et al. (2014) is one of the few examples, where the experimental system was reduced to a single bacterial strain (*E. coli* HMS174) genetically modified to study Hg^{+2} bioavailability. A natural DOM extract was used for incubation at different DOM concentrations, which allowed the identification of biochemical interactions between DOM, Hg^{+2} and the bacterial membrane that determined Hg^{+2} bioavailability and methylation. In a similar study, Golding et al. (2002) carried out an incubation experiment where the same *E. coli* strain (HMS174), as well as *Vibrio anguillarum* (a natural aquatic species), were exposed to both anaerobic and aerobic conditions under increased Hg^{+2} concentrations. By using this simplified experimental system, they observed higher uptake of Hg^{+2} under aerobic conditions, mediated by facilitated uptake mechanisms. In Cao et al. (2021), the aerobic methylation of Hg by two γ-proteobacteria strains (*P. fluorescens* TGR-B2 and *P. putida* TGR-B4) was evaluated under different oxygen concentrations, where they detected MeHg formation under both conditions, although Hg methylation under anaerobic conditions appeared to be more efficient.

As new molecular tools have been progressively developed in the field of Microbial Ecology, more integrated experimental designs have shown to be more valuable to study the complex interactions among the vast consortium of microbial species. Fundamental ecological functions are typically carried out by entire microbial communities, where syntrophic interactions between different members provide the necessary physicochemical conditions (Morris et al., 2013; Kato & Watanabe, 2010; Kouzuma et al., 2015). Therefore, whereas highly simplified experimental systems have the potential to unravel specific aspects of the genetic and metabolic bases of Hg methylation, the absence of certain microbial members can significantly alter the natural functioning of this process (Kerin et al. 2006; Ranchou-Peyruse et al. 2009; Bravo &

Cosio 2019). Mesocosm model ecosystems have been used in an attempt to reproduce natural conditions while isolating the system to apply controlled conditions. Combined with recently developed Hg isotope tracer methodologies (Jonsson et al. 2012; Jonsson et al. 2014) and high-throughput sequencing techniques, these experimental designs constitute powerful tools for comprehensive studies of how Hg methylation works in natural environments, and particularly under climate change-induced conditions. In a series of recent studies, Jonsson et al. (2017; 2022) used a 2000 L mesocosm setup to determine MeHg production and bioaccumulation in brackish waters and sediments collected from the Bothnian Sea, where multiple variables were included: increasing concentrations of DOM, nutrients, different Hg isotope tracers, a pelagic food web model (native heterotrophic bacteria, phytoplankton, protozoa, and mesozooplankton), light conditions, and controlled temperature. In a similar incubation experiment, Rodríguez et al. (2022) combined shotgun metagenomics with Hg/MeHg measurements to study Hg methylation by natural bacterial communities in oxygen-saturated pelagic waters, where variables such as DOM, Hg^{+2} concentration, oxygen level, and light intensity were considered.

In order to further improve our experimental models, it is important to point out potential biases and limitations from incubation studies based on the addition of isotopically enriched Hg species. In a recent critique article, Wang et al. (2020) evaluated the validity of this type of experimental approach as a method to study Hg methylation and demethylation rates in seawater. Based on field measurements and incubation experiments conducted along the Canadian Arctic Archipelago, they drew attention to potential reliability issues derived from methylation and demethylation at time zero, as well as the widely accepted assumption of first-order kinetics to calculate methylation and demethylation constants. Although these observations may respond to particular experimental procedures (Tsui et al. 2020; Zhang et al. 2021), and therefore generalizations should be taken with caution, future holistic studies should rely on an integration of field measurements and incubation experiments that allows the identification of potential experimental biases. Thus, field surveys can be highly valuable to guide subsequent incubation experiments where environmental parameters can be tuned to investigate hidden and emergent properties within the complex biological systems, as well as to generate prediction models for future scenarios under a changing climate.

Meta-omic technologies as tools to address the MeHg formation problematic

Until recently, simplified experimental systems were the main source of knowledge to study complex environmental microbial processes such as Hg methylation. After the discovery of the microbial bases of Hg methylation (Parks et al. 2013), early studies essentially relied on culture-dependent approaches to further advance our knowledge on the metabolic pathways, genes and species involved (Benoit et al., 2001; Kerin et al., 2006; Gilmour et al., 2013). The irruption of sequencing-based meta-omic technologies gave rise to a new kind of approach based on the generation of large quantities of sequencing data from entire microbial assemblages. Being culture-independent, this approach has allowed the implementation of more sophisticated experimental designs to disentangle the complex interactions between Hg methylators, their syntrophs, and relevant environmental variables (such as DOM) under natural or modelled conditions (Quince et al., 2017; Pérez-Cobas et al., 2020; Cho, 2021). In particular, metagenetics, metagenomics and metatranscriptomics (sequencing-based meta-omics) have been the meta-omic approaches most frequently used in the field of Microbial Ecology (**Table 1**), mainly due to: 1) cost-time effectiveness (simultaneous analyses of multiple samples); 2) analytical depth to cover even rare members of microbial communities; and 3) direct applicability in both field surveys and controlled experiments.

602 Metagenetics (also known as amplicon sequencing, marker-gene metagenomics, or targeted
603 metagenomics) is a targeted approach where specific genes (called marker genes) present in
604 the community gene pool are amplified by PCR, sequenced and aligned against an existing
605 reference database for taxonomic and/or functional characterization of the community. The
606 most frequently used gene marker for taxonomic characterization of bacterial communities is
607 the 16S rRNA gene, which is universally found in bacteria with enough variability (nine
608 hypervariable regions) as to allow taxonomic classification and biodiversity profiling
609 (Větrovský et al. 2013). As discussed in previous sections, the marker genes in Hg methylation
610 studies have consistently been the *hgcAB* gene pair. In a recent study, Capo et al. (2022)
611 presented a *hgc* gene database (Hg-MATE) covering Hg-cycling microorganisms from
612 terrestrial and aquatic ecosystems. This catalogue compiles isolated, single-cell and
613 metagenome-reconstructed genomes, which can be used as a reference database to identify
614 *hgcAB* genes from complex meta-omic datasets. Due to the lack of knowledge about alternative
615 Hg methylation pathways potentially operating under truly oxic conditions, studies only relying
616 on *hgcAB* genes may be limiting our ability to detect important Hg methylators in pelagic
617 waters (Podar et al. 2015; Jones et al. 2019; Lin et al. 2020).

618 Shotgun metagenomics (or simply metagenomics) is a non-targeted approach where fragments
619 of the metagenome (i.e., collection of all genomes present in a community) are randomly
620 sequenced and, therefore, taxonomically relevant genes (such as 16S rRNA) as well as
621 functional genes (i.e., involved in physiological processes) can be represented in the dataset,
622 including bacteria, archaea, eukaryotes, and viruses (Quince et al. 2017). One advantage of
623 shotgun metagenomics is the possibility to create biodiversity and functional profiles
624 simultaneously from the very same environmental sample, which can be a valuable tool to
625 detect intercorrelations between the presence of Hg-cycling genes and abundance of potential
626 methylators, as well as other functional genes potentially involved in the Hg methylation
627 process (Rodríguez et al. 2022). Another advantage is the recovery of metagenome-assembled
628 genomes (MAGs), which can provide taxonomic and functional information on specific taxa
629 (down to strain-level diversity) potentially involved in Hg methylation (Bowers et al. 2017;
630 Gionfriddo et al., 2019; McDaniel et al., 2020; Peterson et al., 2020; Lin et al., 2021; Capo et
631 al. 2022). On the other hand, some drawback must be also pointed out, such as the lower
632 sequencing depth compared to specific targeted approaches, where the latter can direct the
633 totality of the sequencing effort towards detecting marker genes such as 16S rRNA or *hgcAB*.
634 In addition, although this technique can offer valuable insights about the functional potential
635 of microbial communities (i.e., presence of genes involved in different metabolic pathways), it
636 does not reflect the actual metabolic activity, as genes need to be transcribed to carry out their
637 biological functions. Other meta-omic techniques are employed to address these aspects, such
638 as metatranscriptomics or metaproteomics (Yap et al. 2022). Overall, shotgun metagenomics
639 represents a cost-time-effective approach to obtain an integral picture of complex microbial
640 communities where detailed resolution is not required.

641 Metatranscriptomics refers to the non-targeted sequencing of the mRNA pool from biological
642 samples, which offers a higher level of functional resolution as compared to shotgun
643 metagenomics. Multiple studies on Hg methylation have used this approach, particularly to
644 determine levels of *hgcAB* gene expression and thus estimate Hg methylation activity
645 (Vishnivetskaya et al. 2018; Christensen et al. 2019; Lin et al., 2021). However, the association
646 between these two variables must be taken with caution, as increases in *hgcAB* transcripts may
647 be caused by higher abundance of Hg methylators, and not necessarily by higher cell-specific
648 *hgcAB* expression (Lin et al. 2021). In addition, the expression of *hgcAB* does not confer the
649 capacity for Hg methylation on its own, as the cellular uptake of Hg (i.e., Hg bioavailability)
650 is a necessary requirement. In fact, a number of studies have shown lack of correlation between

651 *hgcAB* expression and concentration of MeHg or Hg methylation rates (Goñi-Urriza et al. 2015;
652 Bravo et al. 2016; Vishnivetskaya et al. 2018; Christensen et al. 2019), although demethylation
653 activity must be considered as a potential confounding factor for the lack of correlation. Despite
654 these drawbacks, metatranscriptomics is one of the most promising tools in our repertory to
655 potentially elucidate novel Hg methylation pathways occurring in oxic waters. In addition, the
656 analysis of RNA is a measure of the active microbial fraction, allowing the distinction between
657 viable and nonviable cells (Villar et al. 2020; Lin et al. 2021). DNA-based meta-omics (i.e.,
658 metagenetics and metagenomics) are still significantly more employed in Hg methylation
659 studies, mainly due to more simplified and cost/time-effective sample preparation procedures.
660 As new methods are developed to improve technical aspects of mRNA isolation, library
661 preparation and sequencing, metatranscriptomics is becoming an extremely useful approach to
662 disentangle complex microbial processes in natural environments.

663 Considering the advantages and disadvantages of the above-discussed sequencing-based meta-
664 omic techniques, an increasingly popular approach is the combination of metagenomics and
665 metatranscriptomics (multi-omics) to obtain a more comprehensive overview of natural
666 microbial communities (Lin et al. 2021). This approach can facilitate the study of how relevant
667 climate change-related variables (e.g., DOM and temperature) may affect the interconnections
668 between community structure (i.e., occurrence of Hg methylators and syntrophs) and
669 functioning (i.e., Hg methylation activity and overall metabolism). Functional redundancy is a
670 typical property of natural prokaryotic communities that confers high levels of resistance and
671 resilience under environmental disturbance, which can lead to the decoupling of community
672 composition, functional potential, and the actual metabolic activity (Allison & Martiny 2008;
673 Bissett et al. 2013; Rodríguez et al. 2022). In this context, multi-omics offers an excellent tool
674 for incubation experiments aiming the study of how the metabolic potential to perform Hg
675 methylation and, more importantly, the actual ability to methylate Hg, may be affected under
676 climate change scenarios.

677

678 Need for standardized methods and current challenges

679 A common issue frequently reported is the lack of standardization among different Hg
680 methylation studies employing meta-omic approaches, which may lead to rather disparate
681 results even from samples from the same sampling areas (Yap et al. 2022; Capo et al. 2022).
682 Previous studies have addressed the standardization of analytical methods related to Hg and
683 MeHg concentration and characterization (Creswell et al., 2013; Wen et al., 2017). This review
684 will briefly address potential ways to standardize the analysis of microbial communities, from
685 sample collection to bioinformatic data processing.

686 Meta-omic studies on aquatic environments usually begin with the collection of water, which
687 is commonly carried out by filtration methods where the cellular fraction is captured onto a
688 fine-pored matrix such as membrane filters (e.g., Lin et al., 2021; Rodríguez et al., 2022). The
689 preservation of the matrix is a crucial step to maintain the integrity of the DNA and, especially,
690 the RNA molecules. Inadequate preservation methods can easily result in loss of genetic
691 material and hence can reduce the accuracy and resolution of the sequencing techniques. In
692 addition, the degradation rate of DNA/RNA molecules can be related to their molecular size
693 and nucleotide composition, which can vary among different taxonomic groups (Mitchell &
694 Takacs-Vesbach, 2008; Kumar et al., 2020; Pavlovska et al., 2021). Therefore, deficient
695 preservation can lead to dissimilar degradation of different DNA/RNA species, resulting in an
696 artificial selection towards certain taxonomic groups over others, and potential loss of low-
697 abundant members of the communities. Similar to *hgcAB*$^+$ Hg methylators, other potential
698 methylators using different pathways in oxic waters may be rare members of the communities

699 and, therefore, a proper sample preservation is required to ensure detection and sufficient
700 representation for subsequent analyses. Although preservation buffers have been frequently
701 used (e.g., DNA/RNA Shield™ and RNAlater®), the most prevalent method in meta-omic
702 studies is the use of deep freezing, which is usually achieved by liquid nitrogen (Anchordoquy
703 & Molina, 2007; Pavlovska et al., 2021). The use of pre-fixative solutions can also improve
704 the preservation of the original DNA/RNA pool, particularly when the filtration process takes
705 prolonged periods of times (i.e., up to hours). In these cases, microbial communities may
706 experience changes in community composition and gene expression while enclosed in
707 collection bottles, which can be partially prevented by the addition of fixative alcoholic
708 solutions (such as phenol-ethanol solution) to stop all biological activity while maintaining the
709 original taxonomic and functional composition (Charvet et al., 2019; Rodríguez et al. 2022).
710 Furthermore, the processing of samples immediately after collection is an emerging approach
711 promoted by the development of the portable sequencers MinION (Oxford Nanopore
712 Technologies), which eliminates the need for preservation and thus ensures a greater integrity
713 of DNA and RNA molecules (Tyler et al., 2018; Runtuwene et al., 2019).

714 The detection of Hg methylators using *hgcAB* primers is also a controversial aspect widely
715 discussed in Hg methylation studies (Gionfriddo et al., 2020; Capo et al., 2020; McDaniel et
716 al., 2020). The characterization of *hgcAB* genes can be biased by primer design, sequence
717 length, or by the use of different classification methods. Since the first characterization of the
718 *hgcAB* gene pair by Parks et al. 2013, a number of *hgcAB* variants have been described for
719 different Hg methylators (Jones et al., 2019; Villar et al., 2020; Capo et al., 2022). While the
720 design of hgcAB primers in early studies was based on the limited knowledge of a reduced
721 number of Hg methylators (Liu et al., 2014; Bae et al., 2014; Schaefer et al., 2014), newly
722 developed primers increasingly capture a wider diversity (Christensen et al., 2019; Bravo and
723 Cosio, 2019; Jones et al., 2019 Gionfriddo et al. 2020). More efficient primers may help to
724 detect Hg methylators in oxic pelagic environments, where *hgcAB* genes are scarce and
725 sequencing depths can be insufficient for the detection of low-abundant genes. On the other
726 hand, due to the lack of knowledge of the genetic bases and species involved, the study of
727 alternative Hg methylation pathways potentially occurring under oxic conditions will require
728 the design of more universal 16S rRNA primers to cover a wider range of prokaryotic diversity,
729 as well as the use of non-targeted metagenomic approaches (such as MAGs by shotgun
730 metagenomics) for a comprehensive functional characterization of specific taxa potentially
731 involved.

732 Bioinformatic analysis of sequencing data is another aspect with little consensus among the
733 literature. The existence of multiple bioinformatic pipelines to analyse metagenomic and
734 metranscriptomic data has been shown to cause contrasting results (Cho, 2021; Yap et al.,
735 2022). Bioinformatic analyses typically include a series of steps such as cleaning, assembly,
736 read mapping, gene prediction, gene identification, and gene counting. However, within every
737 step, multiple bioinformatic programs have been developed over the years (Pérez-Cobas et al.
738 2020). In particular, the use of common, standardized reference databases (either for taxonomic
739 identification or for *hgcAB* genes) has been reiterated in the literature as an important step to
740 facilitate comparisons across different studies (Gallorini & Loizeau, 2021; Wang et al., 2022;
741 Capo et al., 2022). Normalization of gene counts is another key aspect to determine the
742 prevalence of different members of the microbial communities, as well as to allow comparative
743 analyses with similar studies. For instance, in meta-omic studies considering *hgcAB* genes,
744 number of mapped reads and expression levels of housekeeping genes (such as *rpoB*, *gyrB*,
745 and *recA*) are frequently used to normalize *hgcAB* read counts (Tada et al. 2020; Vigneron et
746 al. 202; Lin et al. 2021; Capo et al. 2022). Rarefaction to a common library size and
747 normalization to gene length and metatranscriptomic sequencing depth are also normalization

approaches frequently used in microbial community studies (Rodríguez et al., 2018; Pérez-Cobas et al., 2020). Considering that different normalization approaches can cope with different analytical biases, it is recommended to employ several normalization methods when reporting meta-omic data, which may facilitate data interpretation and inter-study comparisons.

Summary and concluding remarks

The study of Hg methylation in the water column under oxic conditions is still in its infancy. The formation of MeHg in pelagic areas where no anaerobic methylators are detected poses an apparent paradox that may be explained by different processes: 1) Abiotic Hg methylation; 2) Biotic MeHg production within anoxic micro-environments; 3) Biotic MeHg production under oxic conditions through metabolic pathways involving *hgc*AB genes or 4) independent of *hgc*AB genes. Anoxic microenvironments within settling particles have been frequently postulated as the main source of MeHg in the oxic water column, but recent studies suggest that Hg methylation can take place in fully oxygenated waters where anoxic microenvironments are not present. Given the current controversy, more studies on Hg methylation under truly oxic conditions are required to clarify whether Hg methylation is also performed through metabolic pathways different to the reductive acetyl–CoA pathway involving *hgcAB* genes.

Boreal aquatic ecosystems are predicted to be impacted by global warming through increases in temperatures and DOM concentration in the water column. In particular, DOM is known to affect Hg methylation at different levels: 1) Through chemical interactions affecting Hg^{+2} speciation and providing methyl groups; 2) Enhancing microbial metabolism; 3) Promoting changes in the bacterial cell membrane which affect Hg uptake. These aspects have been poorly studied in boreal aquatic systems under oxic conditions, and, therefore, comprehensive studies including the main biochemical factors involved are required to predict potential ecosystem effects under climate change scenarios.

Meta-omic approaches represent promising tools to accomplish this task. A combination of metagenetics, metagenomics, and metatranscriptomics is advised to simultaneously study taxonomic and functional aspects of Hg methylation, which is particularly valuable in the study of unknown pathways not involving *hgcAB* genes. Due to the wide variety of techniques for sample processing, DNA/RNA sequencing, and data analysis, standardization of meta-omic approaches may improve data interpretation and inter-study comparisons. In addition, an integration of field measurements and incubation experiments is recommended to improve our experimental models when studying how Hg methylation works in natural aquatic environments under oxic conditions, and particularly under climate change-induced conditions.

791 # References

792 Alava, J. J., Cheung, W. W., Ross, P. S., & Sumaila, U. R. (2017). Climate change–
793 contaminant interactions in marine food webs: Toward a conceptual framework. *Global*
794 *change biology*, *23*(10), 3984-4001.

795 Alldredge, Alice L., and Mary W. Silver. "Characteristics, dynamics and significance of
796 marine snow." *Progress in oceanography* 20.1 (1988): 41-82.

797 Alldredge, Alice L., and Yehuda Cohen. "Can microscale chemical patches persist in the sea?
798 Microelectrode study of marine snow, fecal pellets." *Science* 235.4789 (1987): 689-691.

799 Allison, S. D., and Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial
800 communities. *Proc. Natl. Acad. Sci.* 105, 11512–11519. doi: 10.1073/pnas.0801925105

801 Altieri, Andrew H., and Keryn B. Gedan. "Climate change and dead zones." *Global change*
802 *biology* 21.4 (2015): 1395-1406.

803 Anchordoquy, T. J., & Molina, M. C. (2007). Preservation of DNA. *Cell Preservation*
804 *Technology*, *5*(4), 180-188.

805 Andersson, A., Meier, H. M., Ripszam, M., Rowe, O., Wikner, J., Haglund, P., et al. (2015).
806 Projected future climate change and Baltic Sea ecosystem management. *Ambio* 44, 345–
807 356. doi: 10.1007/s13280-015-0654-8

808 Ariya, P. A., & Peterson, K. A. (2005). Chemical transformation of gaseous elemental Hg in
809 the atmosphere. In *Dynamics of Mercury Pollution on Regional and Global Scales:* (pp.
810 261-294). Springer, Boston, MA.

811 Azam, F. and Long, R.A., 2001. Sea snow microcosms. *Nature*, *414*(6863), pp.495-498.

812 Beckers, F., Mothes, S., Abrigata, J., Zhao, J., Gao, Y., & Rinklebe, J. (2019). Mobilization
813 of mercury species under dynamic laboratory redox conditions in a contaminated
814 floodplain soil as affected by biochar and sugar beet factory lime. The Science of the
815 Total Environment, 672, 604–617. https://doi.org/10.1016/j.scitotenv.2019.03.401

816 Benoit, J. M.; Gilmour, C. C.; Mason, R. P. The influence of sulfide on solid phase mercury
817 bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3).
818 Environ. Sci. Technol. 2001, 35 (1), 127−132.

819 Benoit, J. M.; Gilmour, C. C.; Mason, R. P.; Heyes, A. Sulfide controls on mercury speciation
820 and bioavailability to methylating bacteria in sediment pore waters. Environ. Sci.
821 Technol. 1999, 33 (6), 951−957.

822 Bianchi, D., Weber, T. S., Kiko, R., & Deutsch, C. (2018). Global niche of marine anaerobic
823 metabolisms expanded by particle microenvironments. *Nature Geoscience*, *11*(4), 263-
824 268

825 Bissett, A., Brown, M. V., Siciliano, S. D., and Thrall, P. H. (2013). Microbial community
826 responses to anthropogenically induced environmental change: towards a systems
827 approach. Ecol. Lett. 16, 128–139. doi: 10.1111/ele.12109

828 Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: a general review.
829 *Chemosphere* 40, 1335–1351. doi: 10.1016/ S0045-6535(99)00283-0

830 Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, et al. 2017. Minimum
831 information about a single amplified genome (MISAG) and a metagenome-assembled
832 genome (MIMAG) of bacteria and archaea. Nat. Biotechnol. 35(8):725–31

833 Bowman, K. L., Hammerschmidt, C. R., Lamborg, C. H., & Swarr, G. (2015). Mercury in the north atlantic ocean: The US GEOTRACES zonal and meridional sections. Deep Sea Research Part II: Topical Studies in Oceanography, 116, 251–261. https://doi.org/10.1016/j.dsr2.2014.07.004

837 Bowman, K. L., Hammerschmidt, C. R., Lamborg, C. H., Swarr, G. J., & Agather, A. M. (2016). Distribution of mercury species across a zonal section of the eastern tropical South Pacific Ocean (US GEOTRACES GP16). *Marine Chemistry*, *186*, 156-166.

840 Bowman, K.L., Collins, R.E., Agather, A.M., Lamborg, C.H., Hammerschmidt, C.R., Kaul, D., Dupont, C.L., Christensen, G.A. and Elias, D.A., 2020b. Distribution of mercury-cycling genes in the Arctic and equatorial Pacific Oceans and their relationship to mercury speciation. *Limnology and Oceanography*, *65*, pp.S310-S320.

844 BRAUN, S. T., L. M. PROCTOR,S.ZANI,M.T.MELLON,ANDJ. P.ZEHR. 1999. Molecular evidence for zooplankton-associatednitrogen-fixing anaerobes based on amplification of the nifHgene. FEMS Microbiol. Ecol.28:273–279.

847 Bravo, A. G., J. L. Loizeau, P. Dranguet, S. Makri, E. Björn, V. G. Ungureanu, V. I. Slaveykova, and C. Cosio. 2016. Per- sistent Hg contamination and occurrence of Hg-methylating transcript (hgcA) downstream of a chlor-alkali plant in the Olt River (Romania). Environ. Sci. Pollut. Res. 23: 10529–10541. doi:10.1007/s11356-015-5906-4

852 Bravo, A.G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J.K., Poté, J. and Cosio, C., 2018. Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases. *The ISME journal*, *12*(3), pp.802-812

856 Cao, D., Chen, W., Xiang, Y., Mi, Q., Liu, H., Feng, P., Shen, H., Zhang, C., Wang, Y. and Wang, D., 2021. The efficiencies of inorganic mercury bio-methylation by aerobic bacteria under different oxygen concentrations. *Ecotoxicology and Environmental Safety*, *207*, p.111538.

860 Capo, E., Bravo, A.G., Soerensen, A.L., Bertilsson, S., Pinhassi, J., Feng, C., Andersson, A.F., Buck, M. and Björn, E., 2020a. Marine snow as a habitat for microbial mercury methylators in the Baltic Sea. *bioRxiv*, pp.2020-03.

863 Capo, E., Bravo, A.G., Soerensen, A.L., Bertilsson, S., Pinhassi, J., Feng, C., Andersson, A.F., Buck, M. and Björn, E., 2020b. Deltaproteobacteria and spirochaetes-like bacteria are abundant putative mercury methylators in oxygen-deficient water and marine particles in the Baltic Sea. *Frontiers in microbiology*, *11*, p.574080.

867 Capo, E., Peterson, B.D., Kim, M., Jones, D.S., Acinas, S.G., Amyot, M., Bertilsson, S., Björn, E., Buck, M., Cosio, C. and Elias, D.A., 2022. A consensus protocol for the recovery of mercury methylation genes from metagenomes. *Molecular Ecology Resources*, *23*(1), pp.190-204.

871 Celo, V., Lean, D.R. and Scott, S.L., 2006. Abiotic methylation of mercury in the aquatic environment. *Science of the Total Environment*, *368*(1), pp.126-137.

873 Charvet, S., Riemann, L., Alneberg, J., Andersson, A. F., von Borries, J., Fischer, U., & Labrenz, M. (2019). AFISsys-An autonomous instrument for the preservation of brackish water samples for microbial metatranscriptome analysis. *Water research*, *149*, 351-361.

876 Chetelat, J., McKinney, M.A., Amyot, M., Dastoor, A., Douglas, T.A., Heimbürger-Boavida, L.E., Kirk, J., Kahilainen, K.K., Outridge, P.M., Pelletier, N. and Skov, H., 2022. Climate

878 change and mercury in the Arctic: Abiotic interactions. *Science of the Total*
879 *Environment*, *824*, p.153715.

880 Chiasson-Gould, S. A., Blais, J. M., and Poulain, A. J. (2014). Dissolved organic matter
881 kinetically controls mercury bioavailability to bacteria. *Environ. Sci. Technol.* 48, 3153–
882 3161. doi: 10.1021/es4038484

883 Cho, J. C. (2021). Omics-based microbiome analysis in microbial ecology: from sequences to
884 information. *Journal of Microbiology*, *59*, 229-232.

885 Christensen, G. A., and others. 2019. Determining the reliabil- ity of measuring mercury
886 cycling gene abundance with cor- relations with mercury and methylmercury
887 concentrations. Environ. Sci. Technol. 53: 8649–8663. doi:10.1021/acs.est. 8b06389

888 Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem.
889 Marine Ecology Progress Series, 210, 223–253.

890 Cossa, D., Averty, B., & Pirrone, N. (2009). The origin of methylmercury in open
891 Mediterranean waters. Limnology and Oceanography, 54(3), 837–844.
892 https://doi.org/10.4319/lo.2009.54.3.0837

893 Cossa, D., de Madron, X. D., Sch€afer, J., Lanceleur, L., Guedron, S., Buscail, R., Thomas,
894 B., Castelle, S., & Naudin, J.-J. (2017). The open sea as the main source of
895 methylmercury in the water column of the Gulf of Lions (Northwestern Mediterranean
896 margin). Geochimica et Cosmochimica Acta, 199, 222–237. https://doi.org/10.1016/
897 j.gca.2016.11.037

898 Creswell, J. E., Engel, V., Carter, A., & Davies, C. (2013, December). Results from three years
899 of the world's largest interlaboratory comparison for total mercury and methylmercury:
900 Method performance and best practices. In *AGU Fall Meeting Abstracts* (Vol. 2013, pp.
901 B41C-0405).

902 Dastoor, A., Angot, H., Bieser, J., Christensen, J.H., Douglas, T.A., Heimbürger-Boavida,
903 L.E., Jiskra, M., Mason, R.P., McLagan, D.S., Obrist, D. and Outridge, P.M., 2022.
904 Arctic mercury cycling. *Nature Reviews Earth & Environment*, *3*(4), pp.270-286.

905 Decho, A. W., & Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSs) in
906 ocean systems. *Frontiers in microbiology*, *8*, 922.

907 Decho, Alan W. "Microbial exopolymer secretions in ocean environments: their role (s) in
908 food webs and marine processes." *Oceanogr. Mar. Biol. Annu. Rev* 28.7 (1990): 73-153.

909 Eigemann, F., Rahav, E., Grossart, H.P., Aharonovich, D., Sher, D., Vogts, A. and Voss, M.,
910 2022. Phytoplankton exudates provide full nutrition to a subset of accompanying
911 heterotrophic bacteria via carbon, nitrogen and phosphorus allocation. *Environmental*
912 *Microbiology*, *24*(5), pp.2467-2483.

913 French, T. D., Houben, A. J., Desforges, J. P. W., Kimpe, L. E., Kokelj, S. V., Poulain, A. J.,
914 et al. (2014). Dissolved organic carbon thresholds affect mercury bioaccumulation in
915 Arctic lakes. *Environ. Sci. Technol.* 48, 3162–3168. doi: 10.1021/es403849d

916 Gallorini, A., & Loizeau, J. L. (2021). Mercury methylation in oxic aquatic macro-
917 environments: a review. *Journal of Limnology*, *80*(2).

918 Ghimire, P. S., Tripathee, L., Zhang, Q., Guo, J., Ram, K., Huang, J., ... & Kang, S. (2019).
919 Microbial mercury methylation in the cryosphere: Progress and prospects. Science of the
920 Total Environment, 697, 134150.

921 Gilmour, C.C., Podar, M., Bullock, A.L., Graham, A.M., Brown, S.D., Somenahally, A.C.,
922 Johs, A., Hurt Jr, R.A., Bailey, K.L. and Elias, D.A., 2013. Mercury methylation by novel
923 microorganisms from new environments. *Environmental science & technology*, *47*(20),
924 pp.11810-11820.

925 Gionfriddo, C., Podar, M., Gilmour, C., Pierce, E. and Elias, D., 2019. *ORNL compiled*
926 *mercury methylator database* (No. MCI533). ORNLCIFSFA (Critical Interfaces Science
927 Focus Area); Oak Ridge National Lab.(ORNL), Oak Ridge, TN (United States).

928 Gionfriddo, C.M., Wymore, A.M., Jones, D.S., Wilpiszeski, R.L., Lynes, M.M., Christensen,
929 G.A., Soren, A., Gilmour, C.C., Podar, M. and Elias, D.A., 2020. An improved hgcAB
930 primer set and direct high-throughput sequencing expand Hg-methylator diversity in
931 nature. *Frontiers in Microbiology*, *11*, p.541554.

932 Glud, R. N., Grossart, H. P., Larsen, M., Tang, K. W., Arendt, K. E., Rysgaard, S., ... & Gissel
933 Nielsen, T. (2015). Copepod carcasses as microbial hot spots for pelagic
934 denitrification. *Limnology and Oceanography*, *60*(6), 2026-2036.

935 Golding, G.R., Kelly, C.A., Sparling, R., Loewen, P.C., Rudd, J.W. and Barkay, T., 2002.
936 Evidence for facilitated uptake of Hg (II) by Vibrio anguillarum and Escherichia coli
937 under anaerobic and aerobic conditions. *Limnology and Oceanography*, *47*(4), pp.967-
938 975.

939 Goñi-Urriza, M., Y. Corsellis, L. Lanceleur, E. Tessier, J. Gury, M. Monperrus, and R.
940 Guyoneaud. 2015. Relationships between bacterial energetic metabolism, mercury
941 methyla- tion potential, and hgcA/hgcB gene expression in Des- ulfovibrio
942 dechloroacetivorans BerOc1. Environ. Sci. Pollut. Res. 22: 13764–13771.
943 doi:10.1007/s11356-015- 4273-5

944 Graham, A.M., Cameron-Burr, K.T., Hajic, H.A., Lee, C., Msekela, D. and Gilmour, C.C.,
945 2017. Sulfurization of dissolved organic matter increases Hg–sulfide–dissolved organic
946 matter bioavailability to a Hg-methylating bacterium. *Environmental Science &*
947 *Technology*, *51*(16), pp.9080-9088.

948 Grandjean, P., Murata, K., Budtz-Jørgensen, E., & Weihe, P. (2004). Cardiac autonomic
949 activity in methylmercury neurotoxicity: 14-year follow-up of a Faroese birth cohort. The
950 Journal of pediatrics, 144(2), 169-176. doi: 10.1016/j.jpeds.2003.10.058

951 Hall, B. D., Louis, V. L. S., and Bodaly, R. D. (2004). The stimulation of methylmercury
952 production by decomposition of flooded birch leaves and jack pine needles.
953 *Biogeochemistry* 68, 107–129. doi: 10.1023/B:BIOG.0000025745.28447.8b

954 Hammerschmidt, C. R., & Fitzgerald, W. F. (2006). Methylmercury cycling in sediments on
955 the continental shelf of southern New England. Geochimica et Cosmochimica Acta,
956 70(4), 918–930. https://doi.org/10.1016/j.gca.2005.10. 020

957 Heimbürger, L.-E., Cossa, D., Marty, J.-C., Migon, C., Averty, B., Dufour, A., & Ras, J.
958 (2010). Methyl mercury dis- tributions in relation to the presence of nano-and
959 picophytoplankton in an oceanic water column (Ligurian Sea, north-western
960 Mediterranean). Geochimica et Cosmochimica Acta, 74(19), 5549–5559.
961 https://doi.org/10.1016/j. gca.2010.06.036

962 Heimbürger, L.-E., Sonke, J. E., Cossa, D., Point, D., Lagane, C., Laffont, L., Galfond, B. T.,
963 Nicolaus, M., Rabe, B., & van der Loeff, M. R. (2015). Shallow methylmercury
964 production in the marginal sea ice zone of the central Arctic Ocean. Scientific Reports,
965 5(1), 1–6. https://doi.org/10.1038/srep10318

966 Herrero Ortega, S., Catalán, N., Björn, E., Gröntoft, H., Hilmarsson, T. G., Bertilsson, S., et al. (2018). High methylmercury formation in ponds fueled by fresh humic and algal derived organic matter. *Limnol. Oceanogr.* 63, S44–S53. doi: 10.1002/lno.10722

969 Hsu-Kim, H., Kucharzyk, K. H., Zhang, T., and Deshusses, M. A. (2013). Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. *Environ. Sci. Technol.* 47, 2441–2456. doi: 10.1021/es304370g

973 Jaffe, R.; McKnight, D.; Maie, N.; Cory, R.; McDowell, W. H.;́ Campbell, J. L. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. J. Geophys. Res. 2008, 113, 1−15.

976 Jensen, Sören, and Arne Jernelöv. "Biological methylation of mercury in aquatic organisms." Nature 223.5207 (1969): 753-754. https://doi.org/10.1038/223753a0

978 Jernelov, A., 1969. Are there any differences between" biologically" and" chemically" synthesized methyl merc-ury. Vatten, 3, p.304.

980 Ji, X., Liu, C., Zhang, M., Yin, Y. and Pan, G., 2020. Mitigation of methylmercury production in eutrophic waters by interfacial oxygen nanobubbles. *Water Research*, *173*, p.115563.

982 Jiang, T., Bravo, A. G., Skyllberg, U., Björn, E., Wang, D., Yan, H., et al. (2018). Influence of dissolved organic matter (DOM) characteristics on dissolved mercury (Hg) species composition in sediment porewater of lakes from Southwest China. *Water Res.* 146, 146–158. doi: 10.1016/j. watres.2018.08.054

986 Johnson NW, Mitchell CPJ, Engstrom DR, Bailey LT, Coleman Wasik JK, and Berndt ME. 2016. Methylmercury production in a chronically sulfate-impacted sub-boreal wetland. Environmental Science: Processes and Impacts, 18(6): 725–734. doi:10.1039/c6em00138f.

990 Jones, D.S., Walker, G.M., Johnson, N.W., Mitchell, C.P., Coleman Wasik, J.K. and Bailey, J.V., 2019. Molecular evidence for novel mercury methylating microorganisms in sulfate-impacted lakes. The ISME journal, 13(7), pp.1659-1675.

993 Kato, S., & Watanabe, K. (2010). Ecological and evolutionary interactions in syntrophic methanogenic consortia. *Microbes and environments*, *25*(3), 145-151.

995 Kerin, E. J., C. C. Gilmour, E. Roden, M. T. Suzuki, J. D. Coates, and R. P. Mason. 2006. Mercury methylation by dissimilatory Iron-reducing bacteria. Appl. Environ. Microbiol. 72: 7919–7921. doi:10.1128/AEM.01602-06

998 Kim, H., Soerensen, A. L., Hur, J., Heimburger, L. E., Hahm, D., Rhee, T. S., ... & Han, S. (2017). Methylmercury mass budgets and distribution characteristics in the Western Pacific Ocean. *Environmental Science & Technology*, *51*(3), 1186-1194.

1001 Kim, M., Han, S., Gieskes, J., & Deheyn, D. D. (2011). Importance of organic matter lability for monomethylmercury production in sulfate-rich marine sediments. *Science of the Total Environment*, *409*(4), 778-784.

1004 Kirk, J. L., Lehnherr, I., Andersson, M., Braune, B. M., Chan, L., Dastoor, A. P., et al. (2012). Mercury in Arctic marine ecosystems: sources, pathways and exposure. *Environ. Res.* 119, 64–87. doi: 10.1016/j.envres.2012.08.012

1007 Kostić, I. S., Anđelković, T. D., Nikolić, R. S., Cvetković, T. P., Pavlović, D. D., & Bojić, A. L. (2013). Comparative study of binding strengths of heavy metals with humic acid. *Hemijska industrija*, *67*(5), 773-779.

1010 Koukal, B.; Gueguen, C.; Pardos, M.; Dominik, J. Influence of humic substances on the toxic
1011 effects of cadmium and zinc to the green alga Pseudokirchneriella subcapitata.
1012 Chemosphere 2003, 53 (8), 953−961.

1013 Kouzuma, A., Kato, S., & Watanabe, K. (2015). Microbial interspecies interactions: recent
1014 findings in syntrophic consortia. *Frontiers in microbiology*, *6*, 477.

1015 Krabbenhoft, D. P., & Sunderland, E. M. (2013). Global change and mercury. *Science*,
1016 *341*(6153), 1457-1458. doi: 10.1126/science.1242838

1017 Kraepiel, A. M., Keller, K., Chin, H. B., Malcolm, E. G., & Morel, F. M. (2003). Sources and
1018 variations of mercury in tuna. Environmental Science & Technology, 37(24), 5551–
1019 5558. https://doi.org/10.1021/es0340679

1020 Kumar, G., Eble, J. E., & Gaither, M. R. (2020). A practical guide to sample preservation and
1021 pre-PCR processing of aquatic environmental DNA. *Molecular ecology*
1022 *resources*, *20*(1), 29-39.

1023 Kuss, J., Krüger, S., Ruickoldt, J., & Wlost, K. P. (2018). High-resolution measurements of
1024 elemental mercury in surface water for an improved quantitative understanding of the
1025 Baltic Sea as a source of atmospheric mercury. *Atmospheric Chemistry and*
1026 *Physics*, *18*(6), 4361-4376.

1027 Lázaro, W. L., Guimarães, J. R. D., Ignácio, A. R., Da Silva, C. J., & Díez, S. (2013).
1028 Cyanobacteria enhance methylmercury production: a hypothesis tested in the periphyton
1029 of two lakes in the Pantanal floodplain, Brazil. *Science of the total environment*, *456*,
1030 231-238.

1031 Lehnherr, I., Louis, V. L. S., Hintelmann, H., and Kirk, J. L. (2011). Methylation of inorganic
1032 mercury in polar marine waters. *Nat. Geosci.* 4, 298–302. doi: 10.1038/ngeo1134

1033 Li, Y., & Cai, Y. (2013). Progress in the study of mercury methylation and demethylation in
1034 aquatic environments. *Chinese Science Bulletin*, *58*, 177-185.

1035 Lin, H., Ascher, D.B., Myung, Y., Lamborg, C.H., Hallam, S.J., Gionfriddo, C.M., Holt, K.E.
1036 and Moreau, J.W., 2021. Mercury methylation by metabolically versatile and
1037 cosmopolitan marine bacteria. The ISME journal, 15(6), pp.1810-1825.

1038 Liu, M., Zhang, Q., Maavara, T., Liu, S., Wang, X., & Raymond, P. A. (2021). Rivers as the
1039 largest source of mercury to coastal oceans worldwide. Nature Geoscience, 14(9), 672–
1040 677. https://doi.org/10.1038/s41561-021-00793-2

1041 Lu, X., Gu, W., Zhao, L., Farhan Ul Haque, M., DiSpirito, A. A., Semrau, J. D., & Gu, B.
1042 (2017). Methylmercury uptake and degradation by methanotrophs. *Science*
1043 *Advances*, *3*(5), e1700041.

1044 Lu, X., Liu, Y., Johs, A., Zhao, L., Wang, T., Yang, Z., ... & Gu, B. (2016). Anaerobic mercury
1045 methylation and demethylation by Geobacter bemidjiensis Bem. *Environmental science*
1046 *& technology*, *50*(8), 4366-4373.

1047 Ma, M., Du, H., & Wang, D. (2019). Mercury methylation by anaerobic microorganisms: A
1048 review. Critical Reviews in Environmental Science and Technology, 49(20), 1893–1936.
1049 https://doi.org/10.1080/10643389.2019. 1594517

1050 Marnane, I., 2018. Mercury in Europe's environment a priority for European and global
1051 action. *EEA Report*, (11/2018).

1052 Mason, R. P., & Fitzgerald, W. F. (1993). The distribution and biogeochemical cycling of
1053 mercury in the equatorial Pacific Ocean. Deep Sea Research Part I: Oceanographic
1054 Research Papers, 40(9), 1897–1924. https://doi.org/10. 1016/0967-0637(93)90037-4

1055 Mason, R. P., Choi, A. L., Fitzgerald, W. F., Hammerschmidt, C. R., Lamborg, C. H.,
1056 Soerensen, A. L., & Sunderland, E. M. (2012). Mercury biogeochemical cycling in the
1057 ocean and policy implications. Environmental Research, 119, 101–117.
1058 https://doi.org/10.1016/j.envres.2012.03.013

1059 McDaniel, E.A., Peterson, B.D., Stevens, S.L., Tran, P.Q., Anantharaman, K. and McMahon,
1060 K.D., 2020. Expanded phylogenetic diversity and metabolic flexibility of mercury-
1061 methylating microorganisms. *Msystems*, *5*(4), pp.e00299-20.

1062 Meier, M., Andersson, H., Dieterich, C., Eilola, K., Gustafsson, B., Höglund, A., &
1063 Schimanke, S. (2011). Transient scenario simulations for the Baltic Sea Region during
1064 the 21st century. DiVA: diva2:947527

1065 Merritt, K. A., & Amirbahman, A. (2009). Mercury methylation dynamics in estuarine and
1066 coastal marine environments—a critical review. Earth-Science Reviews, 96(1-2), 54-66.

1067 Minamata Disease Research Group. (1966) [Minamata Disease: Studies About Methyl-
1068 Mercury Poisoning] (in Japanese). Kumamoto, Japan: Kumamoto University School of
1069 Medicine.

1070 Mitchell, K. R., & Takacs-Vesbach, C. D. (2008). A comparison of methods for total
1071 community DNA preservation and extraction from various thermal
1072 environments. *Journal of Industrial Microbiology and Biotechnology*, *35*(10), 1139-
1073 1147.

1074 Monperrus M, Tessier E, Amouroux D, Leynaert A, Huonnie P, and Donald OFX. 2007a.
1075 Mercury methylation, demethylation and reduction rates in coastal and marine surface
1076 waters of the Mediterranean Sea. Marine Chemistry, 107: 49–63.
1077 doi:10.1016/j.marchem.2007.01.018

1078 Morris, B. E., Henneberger, R., Huber, H., & Moissl-Eichinger, C. (2013). Microbial
1079 syntrophy: interaction for the common good. *FEMS microbiology reviews*, *37*(3), 384-
1080 406.

1081 Mühlenbruch, M., Grossart, H.P., Eigemann, F. and Voss, M., 2018. Mini-review:
1082 Phytoplankton-derived polysaccharides in the marine environment and their interactions
1083 with heterotrophic bacteria. *Environmental microbiology*, *20*(8), pp.2671-2685

1084 Munson, K. M., Lamborg, C. H., Boiteau, R. M., & Saito, M. A. (2018). Dynamic mercury
1085 methylation and deme- thylation in oligotrophic marine water. Biogeosciences, 15(21),
1086 6451–6460. https://doi.org/10.5194/bg-15-6451- 2018

1087 Munson, K. M., Lamborg, C. H., Swarr, G. J., & Saito, M. A. (2015). Mercury species
1088 concentrations and fluxes in the central tropical Pacific Ocean. Global Biogeochemical
1089 Cycles, 29(5), 656–676. https://doi.org/10.1002/ 2015GB005120

1090 Ojwang', L.M. and Cook, R.L., 2013. Environmental conditions that influence the ability of
1091 humic acids to induce permeability in model biomembranes. *Environmental science &*
1092 *technology*, *47*(15), pp.8280-8287.

1093 Ortiz, V.L., Mason, R.P. and Ward, J.E., 2015. An examination of the factors influencing
1094 mercury and methylmercury particulate distributions, methylation and demethylation
1095 rates in laboratory-generated marine snow. *Marine chemistry*, *177*, pp.753-762.

1096 Paranjape, A. R., and Hall, B. D. (2017). Recent advances in the study of mercury methylation
1097 in aquatic systems. Facets 2, 85–119. doi: 10.1139/ facets-2016-0027

1098 Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian,
1099 Y., Brown, S. D., Brandt, C. C., Palumbo, A. V., Smith, J. C., Wall, J. D., Elias, D. A.,
1100 & Liang, L. (2013). The genetic basis for bacterial mercury methylation. Science (New
1101 York, N.Y.), 339(6125), 1332–1335. https://doi.org/10.1126/science. 1230667

1102 Pavlovska, M., Prekrasna, I., Parnikoza, I., & Dykyi, E. (2021). Soil sample preservation
1103 strategy affects the microbial community structure. *Microbes and environments*, *36*(1),
1104 ME20134.

1105 Perelomov, L. V., Sarkar, B., Sizova, O. I., Chilachava, K. B., Shvikin, A. Y., Perelomova, I.
1106 V., & Atroshchenko, Y. M. (2018). Zinc and lead detoxifying abilities of humic
1107 substances relevant to environmental bacterial species. *Ecotoxicology and environmental*
1108 *safety*, *151*, 178-183.

1109 Pérez-Cobas, A. E., Gomez-Valero, L., & Buchrieser, C. (2020). Metagenomic approaches in
1110 microbial ecology: an update on whole-genome and marker gene sequencing
1111 analyses. *Microbial genomics*, *6*(8).

1112 Peterson, B.D., McDaniel, E.A., Schmidt, A.G., Lepak, R.F., Janssen, S.E., Tran, P.Q.,
1113 Marick, R.A., Ogorek, J.M., DeWild, J.F., Krabbenhoft, D.P. and McMahon, K.D., 2020.
1114 Mercury methylation genes identified across diverse anaerobic microbial guilds in a
1115 eutrophic sulfate-enriched lake. *Environmental science & technology*, *54*(24), pp.15840-
1116 15851.

1117 Ploug, H., Ku€hl, M., Buchholz, B., & Jørgensen, B. (1997). Anoxic aggregates an ephemeral
1118 phenomenon in the ocean. Aquatic Microbial Ecology, 13, 285–294.
1119 https://doi.org/10.3354/ame013285

1120 Poste, A. E., Braaten, H. F. V., de Wit, H. A., Sørensen, K., & Larssen, T. (2015). Effects of
1121 photodemethylation on the methylmercury budget of boreal Norwegian
1122 lakes. *Environmental Toxicology and Chemistry*, *34*(6), 1213-1223.

1123 PROCTOR, L. M. 1997. Nitrogen-fixing, photosynthetic, anaerobicbacteria associated with
1124 pelagic copepods. Aquat. Microb.Ecol.12:105–113, doi:10.3354/ame012105

1125 Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. 2017. Shotgun metagenomics, from
1126 sampling to analysis. Nat. Biotechnol. 35(9):833–44

1127 Ranchou-Peyruse, M., M. Monperrus, R. Bridou, R. Duran, D. Amouroux, J. C. Salvado, and
1128 R. Guyoneaud. 2009. Over- view of mercury methylation capacities among anaerobic
1129 bacteria including representatives of the sulphate-reducers: implications for
1130 environmental studies. Geomicrobiol. J. 26: 1–8. doi:10.1080/01490450802599227

1131 Regnell, O., & Watras, C. J. (2019). Microbial mercury methylation in aquatic environments:
1132 A critical review of published field and laboratory studies. Environmental Science &
1133 Technology, 53(1), 4–19. https://doi.org/10.1021/ acs.est.8b02709

1134 Regnell, O., and Watras, C. J. (2018). Microbial mercury methylation in aquatic environments:
1135 a critical review of published field and laboratory studies. *Environ. Sci. Technol.* 53, 4–
1136 19. doi: 10.1021/acs.est.8b02709

1137 Ridley, W.P., Dizikes, L.J. and Wood, J.M., 1977. Biomethylation of toxic elements in the
1138 environment. Science, 197(4301), pp.329-332.

1139 Ripszam, M., Paczkowska, J., Figueira, J., Veenaas, C., and Haglund, P. (2015b). Dissolved
1140 organic carbon quality and sorption of organic pollutants in the Baltic Sea in light of
1141 future climate change. *Environ. Sci. Technol.* 49, 1445–1452. doi: 10.1021/es504437s

1142 Robinson, J.B. and Tuovinen, O., 1984. Mechanisms of microbial resistance and
1143 detoxification of mercury and organomercury compounds: physiological, biochemical,
1144 and genetic analyses. Microbiological reviews, 48(2), pp.95-124.

1145 Rodríguez, J., Gallampois, C. M., Timonen, S., Andersson, A., Sinkko, H., Haglund, P., ... &
1146 Rowe, O. (2018). Effects of organic pollutants on bacterial communities under future
1147 climate change scenarios. *Frontiers in microbiology*, *9*, 2926.

1148 Rodríguez, J., Andersson, A., Björn, E., Timonen, S., Brugel, S., Skrobonja, A., & Rowe, O.
1149 (2022). Inputs of Terrestrial Dissolved Organic Matter Enhance Bacterial Production and
1150 Methylmercury Formation in Oxic Coastal Water. *Frontiers in Microbiology*, *13*.

1151 Runtuwene, L. R., Tuda, J. S., Mongan, A. E., & Suzuki, Y. (2019). On-site MinION
1152 sequencing. *Single molecule and single cell sequencing*, 143-150.

1153 S. Jonsson, U. Skyllberg, M. B. Nilsson, E. Lundberg, A. Andersson, E. Björn, Differentiated
1154 availability of geochemical mercury pools controls methylmercury levels in estuarine
1155 sediment and biota. Nat. Commun. 5, 4624 (2014).

1156 S. Jonsson, U. Skyllberg, M. B. Nilsson, P.-O. Westlund, A. Shchukarev, E. Lundberg, E.
1157 Björn, Mercury methylation rates for geochemically relevant HgII species in sediments.
1158 Environ. Sci. Technol. 46, 11653–11659 (2012).

1159 Schaefer, K., Elshorbany, Y., Jafarov, E., Schuster, P.F., Striegl, R.G., Wickland, K.P. and
1160 Sunderland, E.M., 2020. Potential impacts of mercury released from thawing
1161 permafrost. *Nature communications*, *11*(1), p.4650.

1162 Schartup, A. T., Ndu, U., Balcom, P. H., Mason, R. P., & Sunderland, E. M. (2015).
1163 Contrasting effects of marine and terrestrially derived dissolved organic matter on
1164 mercury speciation and bioavailability in seawater. *Environmental science &*
1165 *technology*, *49*(10), 5965-5972.

1166 Schuster, P.F., Schaefer, K.M., Aiken, G.R., Antweiler, R.C., Dewild, J.F., Gryziec, J.D.,
1167 Gusmeroli, A., Hugelius, G., Jafarov, E., Krabbenhoft, D.P. and Liu, L., 2018. Permafrost
1168 stores a globally significant amount of mercury. *Geophysical Research Letters*, *45*(3),
1169 pp.1463-1471.

1170 Seymour, J.R., Amin, S.A., Raina, J.B. and Stocker, R., 2017. Zooming in on the phycosphere:
1171 the ecological interface for phytoplankton–bacteria relationships. *Nature*
1172 *microbiology*, *2*(7), pp.1-12

1173 Slaveykova, V.I., Wilkinson, K.J., Ceresa, A. and Pretsch, E., 2003. Role of fulvic acid on
1174 lead bioaccumulation by Chlorella kesslerii. *Environmental science & technology*, *37*(6),
1175 pp.1114-1121.

1176 Soerensen, A.L., Schartup, A.T., Gustafsson, E., Gustafsson, B.G., Undeman, E. and Björn,
1177 E., 2016. Eutrophication Increases Phytoplankton Methylmercury Concentrations in a
1178 Coastal Sea□ A Baltic Sea Case Study. *Environmental Science & Technology*, *50*(21),
1179 pp.11787-11796.

1180 Sonke JE, Heimbürger L-E, and Dommergue A. 2013. Mercury biogeochemistry: paradigm
1181 shifts, outstanding issues and research needs. Comptes Rendus Geoscience, 345: 213–
1182 224. doi:10.1016/j. crte.2013.05.002.

1183 Stern, G.A., Macdonald, R.W., Outridge, P.M., Wilson, S., Chetelat, J., Cole, A., Hintelmann,
1184 H., Loseto, L.L., Steffen, A., Wang, F. and Zdanowicz, C., 2012. How does climate
1185 change influence arctic mercury?. *Science of the total environment*, *414*, pp.22-42.

1186 Stott, P. (2016). How climate change affects extreme weather events. *Science*, *352*(6293),
1187 1517-1518.

1188 Streets, D. G., Devane, M. K., Lu, Z., Bond, T. C., Sunderland, E. M., & Jacob, D. J. (2011).
1189 All-time releases of mercury to the atmosphere from human activities. Environmental
1190 science & technology, 45(24), 10485-10491. doi: 10.1021/es202765m

1191 Sunderland, E. M., Krabbenhoft, D. P., Moreau, J. W., Strode, S. A., & Landing, W. M. (2009).
1192 Mercury sources, distribution, and bioavailability in the north Pacific Ocean: Insights
1193 from data and models. Global Biogeochemical Cycles, 23(2), n/a–14.
1194 https://doi.org/10.1029/2008GB003425

1195 Taira, M. (1975). Studies on microbial synthesis and decomposition of organomercury
1196 compounds. Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene), 30(4), 461-489.

1197 Tan, S. W., Meiller, J. C., & Mahaffey, K. R. (2009). The endocrine effects of mercury in
1198 humans and wildlife. Critical reviews in toxicology, 39(3), 228-269. doi:
1199 0.1080/10408440802233259

1200 Tang, K. W., Glud, R. N., Glud, A., Rysgaard, S., & Nielsen, T. G. (2011). Copepod guts as
1201 biogeochemical hot- spots in the sea: Evidence from microelectrode profiling of Calanus
1202 spp. Limnology and Oceanography, 56(2), 666–672.
1203 https://doi.org/10.4319/lo.2011.56.2.0666

1204 Tsubaki, T., & Irukayama, K. (1977). Minamata disease. Methylmercury poisoning in
1205 Minamata and Niigata, Japan. North-Holland Publishing Company, PO Box 211,
1206 Amsterdam, The Netherlands. ISBN : 0444998160

1207 Tsui, M.T.K., Blum, J.D. and Kwon, S.Y., 2020. Review of stable mercury isotopes in ecology
1208 and biogeochemistry. Science of the Total Environment, 716, p.135386.

1209 Turner, Jefferson T. "Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's
1210 biological pump." *Progress in Oceanography* 130 (2015): 205-248.

1211 Tyler, A. D., Mataseje, L., Urfano, C. J., Schmidt, L., Antonation, K. S., Mulvey, M. R., &
1212 Corbett, C. R. (2018). Evaluation of Oxford Nanopore's MinION sequencing device for
1213 microbial whole genome sequencing applications. *Scientific reports*, *8*(1), 1-12.

1214 Ullrich, S. M., Tanton, T. W., and Abdrashitova, S. A. (2001). Mercury in the aquatic
1215 environment: a review of factors affecting methylation. *Crit. Rev. Environ. Sci. Technol.*
1216 31, 241–293. doi: 10.1080/20016491089226

1217 Větrovský, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial
1218 genomes and its consequences for bacterial community analyses. *PloS one*, 8(2), e57923.
1219 doi: 10.1371/journal.pone.0057923

1220 Vigneault, B., Percot, A., Lafleur, M. and Campbell, P.G., 2000. Permeability changes in
1221 model and phytoplankton membranes in the presence of aquatic humic
1222 substances. *Environmental science & technology*, *34*(18), pp.3907-3913.

1223 Villar, E., L. Cabrol, and L.-E. Heimbürger-Boavida. 2020. Widespread microbial mercury
1224 methylation genes in the global ocean. bioRxiv. doi:10.1101/648329

1225 Vishnivetskaya, T. A., and others. 2018. Microbial community structure with trends in
1226 methylation gene diversity and abundance in mercury-contaminated rice paddy soils in
1227 Guizhou, China. Environ. Sci. Process. Impacts 20: 673–685. doi:10.1039/C7EM00558J

1228 Wang, F., Macdonald, R. W., Armstrong, D. A., & Stern, G. A. (2012). Total and methylated
1229 mercury in the Beaufort Sea: The role of local and recent organic
1230 remineralization. *Environmental science & technology*, *46*(21), 11821-11828.

1231 Wang, J., Shaheen, S. M., Jing, M., Anderson, C. W., Swertz, A.-C., Wang, S.-L., Feng, X.,
1232 & Rinklebe, J. r. (2021). Mobilization, methylation, and demethylation of mercury in a
1233 paddy soil under systematic redox changes. Environmental Science & Technology,
1234 55(14), 10133–10141. https://doi.org/10.1021/acs.est.0c07321

1235 Wang, K., Liu, G., & Cai, Y. (2022). Possible pathways for mercury methylation in oxic
1236 marine waters. *Critical Reviews in Environmental Science and Technology*, *52*(22),
1237 3997-4015.

1238 Wang, K., Munson, K. M., Beaupre-Laperriere, A., Mucci, A., Macdonald, R. W., & Wang,
1239 F. (2018). Subsurface seawater methylmercury maximum explains biotic mercury
1240 concentrations in the canadian arctic. Scientific Reports, 8(1), 1–5.
1241 https://doi.org/10.1038/s41598-018-32760-0

1242 Wang, K., Munson, K.M., Armstrong, D.A., Macdonald, R.W. and Wang, F., 2020.
1243 Determining seawater mercury methylation and demethylation rates by the seawater
1244 incubation approach: A critique. *Marine Chemistry*, *219*, p.103753.

1245 Wen, Q., Su, Z., Cai, W., Huang, W., Fan, J., Huang, H., ... & Hu, S. (2017). Recent progress
1246 on detection methods of methyl mercury. *Journal of Food Safety and Quality*, *8*(3), 845-
1247 853.

1248 Westoo, G.: Determination of methylmercury compounds in foodstuffs, Acta. Chem. Scand.,
1249 21, 1790-1800 (1967).

1250 Whalin, L., Kim, E.-H., & Mason, R. (2007). Factors influencing the oxidation, reduction,
1251 methylation and deme- thylation of mercury species in coastal waters. Marine Chemistry,
1252 107(3), 278–294. https://doi.org/10.1016/j.mar- chem.2007.04.002

1253 Wu, P., Kainz, M. J., Valdés, F., Zheng, S., Winter, K., Wang, R., ... & Bishop, K. (2021).
1254 Elevated temperature and browning increase dietary methylmercury, but decrease
1255 essential fatty acids at the base of lake food webs. *Scientific Reports*, *11*(1), 16859.

1256 Yao, C., He, T., Xu, Y., Ran, S., Qian, X. and Long, S., 2020. Mercury bioaccumulation in
1257 zooplankton and its relationship with eutrophication in the waters in the karst region of
1258 Guizhou Province, Southwest China. *Environmental Science and Pollution*
1259 *Research*, *27*, pp.8596-8610.

1260 Yap, M., Ercolini, D., Álvarez-Ordóñez, A., O'Toole, P.W., O'Sullivan, O. and Cotter, P.D.,
1261 2022. Next-generation food research: use of meta-omic approaches for characterizing
1262 microbial communities along the food chain. *Annual Review of Food Science and*
1263 *Technology*, *13*, pp.361-384.

1264 Zhang Y, Jacob DJ, Horowitz HM, Chen L, Amos HM, Krabbenhoft DP, et al. 2016. Observed
1265 decrease in atmospheric mercury explained by global decline in anthropogenic
1266 emissions. Proceedings of the National Academy of Science, 113: 526–531.
1267 doi:10.1073/pnas.1516312113

1268 Zhang, L., Liang, X., Wang, Q., Zhang, Y., Yin, X., Lu, X., Pierce, E.M. and Gu, B., 2021.
1269 Isotope exchange between mercuric [Hg (II)] chloride and Hg (II) bound to minerals and
1270 thiolate ligands: Implications for enriched isotope tracer studies. Geochimica et
1271 Cosmochimica Acta, 292, pp.468-481.

1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300

1301 # Tables

Table 1. Description of the main sequencing-based meta-omic approaches.

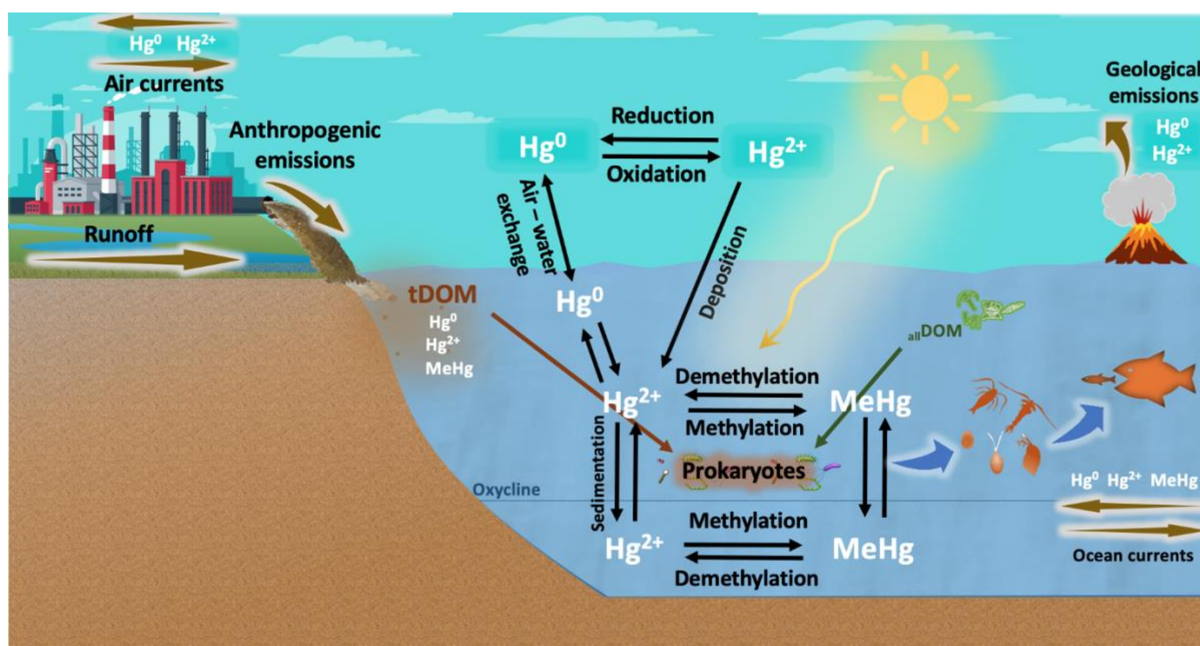
	Main applications	Advantages	Disadvantages
Metagenetics (targeted DNA sequencing)	• 16S/18S rRNA taxonomy. • Detection of hgcAB genes.	• Detailed taxonomic characterization. • High detection power for hgcAB genes. • Cost/time effective sample processing.	• Limitation to detect unknown genes potentially involved in Hg methylation in oxic waters. • Very limited information of functional potential.
Metagenomics (non-targeted DNA sequencing)	• 16S/18S rRNA taxonomy. • Detection of hgcAB and other functional genes.	• Overall screening of entire microbial metagenome. • Full characterization of the community functional potential, including hgcAB and other genes potentially involved. • Cost/time effective sample processing.	• Lower taxonomic resolution. • Lower power to detect hgcAB genes. • Limited information of the actual metabolic activity.
Metatranscriptomics (non-targeted mRNA sequencing)	• Characterization of gene expression levels, including hgcAB and other functional genes.	• Higher functional resolution. • Measure of the active microbial fraction. • Potential to detect unknown genes actively involved in Hg methylation in oxic waters.	• Potential non-correlation between hgcAB expression and concentration of MeHg or Hg methylation rates. • Cost/time demanding sample processing.

1302

1303

1304 # Figures

1305



1306 **Figure 1.** Geobiochemical cycle of mercury (Hg) in aquatic environments. Hg is released by anthropogenic activities and geological processes. Elemental mercury (Hg^0) is transported by atmospheric currents and oxidized to divalent inorganic mercury (Hg^{2+}), which is eventually deposited in aquatic ecosystems. Part of this Hg^{2+} is reduced to Hg^0 and recirculated back into the atmosphere, but a substantial portion of Hg^{2+} is bound to DOM molecules and transformed into methylmercury (MeHg) by abiotic processes (e.g., UV radiation) and, to a greater extent, by prokaryotic communities. MeHg formation can be enhanced by DOM from terrestrial origin (tDOM) and DOM freshly produced by phytoplankton communities (allochthonous DOM, $_{all}$DOM), and is then bioaccumulated up to the food web. Hg methylation has been well described to occur in anoxic layers of the water column through metabolic pathways involving *hgcAB* genes. However, the biotic methylation of Hg under oxic conditions is not well understood and is currently under debate.

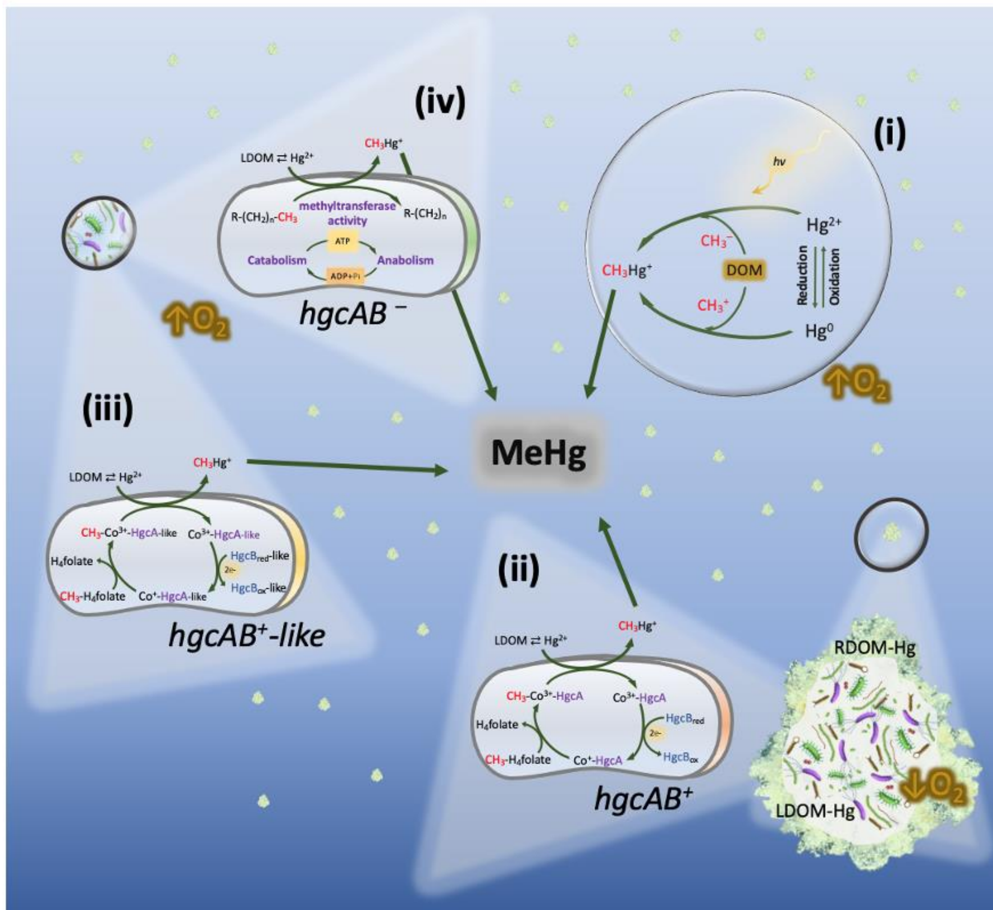


Figure 2. Hg methylation pathways potentially co-occurring in oxic layers of the water column. **(i)** Abiotic Hg methylation, with photochemical and non-photochemical (involving DOM molecules) processes taking place simultaneously. **(ii)** Biotic Hg methylation by hgcAB$^+$ anaerobes (e.g., *Shewenella*, *Desulfobacula*, or *Desulforhopalus*) inhabiting settling marine snow. The formation of methylmercury (CH_3Hg^+) is carried out under anoxic micro-environments through the acetyl-CoA/hgcAB pathway. **(iii)** Hg methylation potentially carried out through metabolic pathways similar to the acetyl-CoA/hgcAB pathway by aerobic prokaryotes carrying *hgcAB*-like genes, such as bacteria belonging to the genus *Nitrospina*. **(iv)** Hg methylation postulated to take place under truly oxic conditions by unknown aerobic methylators. The formation of MeHg may respond to housekeeping genes which increase their overall activity when carbon, nutrients and Hg^{2+} are present.

1307

1308

1309

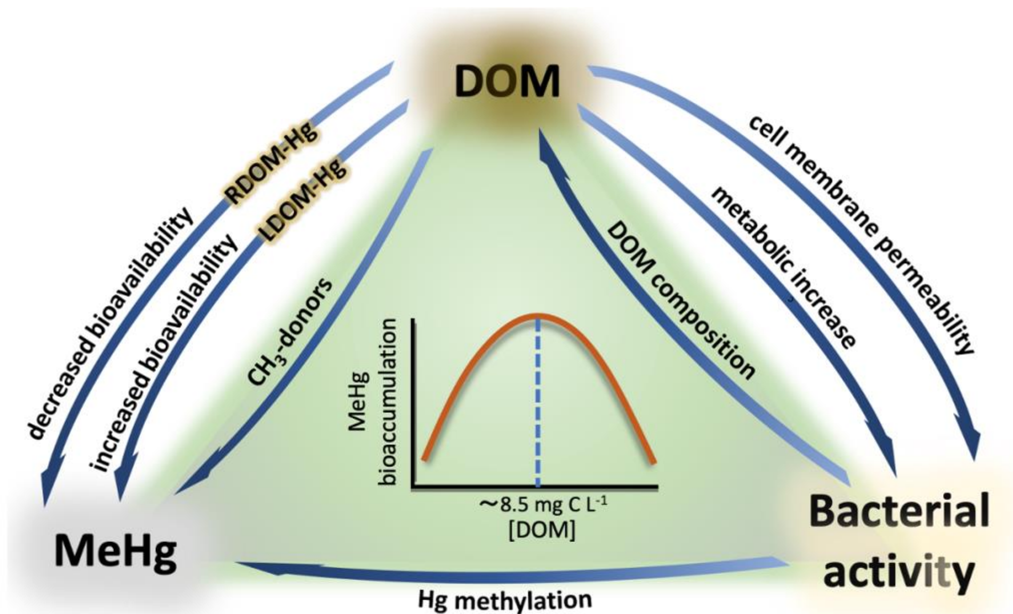


Figure 3. Interactions between DOM, MeHg, and bacterial activity. RDOM-Hg and LDOM-Hg refer to the aggregates formed by Hg and refractory compounds (RDOM) and labile compounds (LDOM). The relationship between MeHg bioaccumulation and DOM concentration is represented by a bell-shaped pattern, where MeHg bioaccumulation increases with increasing DOM concentration until a threshold (~8.5 C L^{-1}), from which Hg bioavailability starts to progressively decrease with increasing DOM concentration.

1310