Biotic formation of methylmercury: A bio–physico–chemical conundrum

Andrea G. Bravo 1,* Claudia Cosio 2,*

1Department of Marine Biology and Oceanography, Institute of Marine Sciences, Spanish National Research Council (CSIC), Barcelona, Spain
2Université de Reims Champagne Ardennes, UMR-I 02 INERIS-URCA-ULH SEBIO, Unité Stress Environnementaux et BIOsurveillance des milieux aquatiques, Reims, France

Abstract

Mercury (Hg) is a natural and widespread trace metal, but is considered a priority pollutant, particularly its organic form methylmercury (MMHg), because of human’s exposure to MMHg through fish consumption. Pioneering studies showed the methylation of divalent Hg (HgII) to MMHg to occur under oxygen-limited conditions and to depend on the activity of anaerobic microorganisms. Recent studies identified the hgcAB gene cluster in microorganisms with the capacity to methylate HgII and unveiled a much wider range of species and environmental conditions producing MMHg than previously expected. Here, we review the recent knowledge and approaches used to understand HgII-methylation, microbial biodiversity and activity involved in these processes, and we highlight the current limits for predicting MMHg concentrations in the environment. The available data unveil the fact that HgII methylation is a bio-physico-chemical conundrum in which the efficiency of biological HgII methylation appears to depend chiefly on HgII and nutrients availability, the abundance of electron acceptors such as sulfate or iron, the abundance and composition of organic matter as well as the activity and structure of the microbial community. An increased knowledge of the relationship between microbial community composition, physico-chemical conditions, MMHg production, and demethylation is necessary to predict variability in MMHg concentrations across environments.

The mercury problem

Mercury (Hg) is a natural and ubiquitous trace metal in the environment that might damage the central nervous system and causes tremors, distorted speech, kidney effects, respiratory failure, dizziness, blurred vision, hallucinations, and even death in severely exposed people (Clarkson and Magos 2006). This pollutant is naturally emitted during episodic events such as volcanic eruptions or ubiquitous weathering of Hg-containing rocks in the Earth’s crust and geothermal activity. Among anthropogenic Hg sources, artisanal and small-scale gold mining, coal combustion, production of nonferrous metals, cement production, and disposal of wastes containing Hg are of special concern (UNEP 2013). Hg is unique among transition metals due to its high volatility as gaseous elemental Hg (Hg0), with a residence time in the atmosphere of about 6–12 months, allowing for long-range transport of Hg. Although both Hg0 and inorganic divalent Hg (HgII) are released from many sources through a variety of natural and anthropogenic processes, the reported rise in Hg levels in the biosphere, and in terrestrial and marine systems is a consequence of anthropogenic emissions (Amos et al. 2013; Lamborg et al. 2014; Kocman et al. 2017). In contrast to Hg0 and HgII, direct anthropogenic sources of organic Hg, mono-methylmercury (MMHg, i.e., CH3Hg+) or dimethylmercury (DMHg, i.e., [CH3]2Hg) are scarce.

The chemical behaviors of the different chemical forms of Hg (i.e., Hg0, HgII, CH3Hg and (CH3)2Hg) play critical roles in the biogeochemical cycling of Hg. HgII allows for long-range transport (Jackson 1997; Pirrone et al. 2009), HgII is the dominant reservoir for Hg in soils and aquatic systems (Fleck et al. 2015; Eklöf et al. 2018), and MMHg is bioconcentrated and biomagnified in aquatic food webs, reaching up to 80–100% of the total-Hg (THg) measured in fish muscle (Bloom 1992; Mason et al. 2012; Bravo et al. 2014). As a consequence, MMHg exposure through fish consumption is of special concern for human health. A recent study performed in 175 countries, showed that 38% of studied populations (mainly insular and developing nations) were exposed to doses of MMHg above governmental thresholds (Lavoie et al. 2018). Indeed, concentration of Hg in fish is known to repeatedly overpass environmental quality guidelines even in

---

*Correspondence: andrea.bravo@icm.csic.es (A.G.B.); claudia.cosio@univ-reims.fr (C.C.)

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Author Contribution Statement: The two authors have equally contributed to this work.
Bravo and Cosio Biotic formation of methylmercury

Comprehensive evaluations of the chemical and physical processes that govern Hg distribution and fate among the major environmental compartments can be found in the literature (Chételat et al. 2015; Sundsæth et al. 2015; Kim et al. 2016; Björklund et al. 2017; Dranguet et al. 2017; Paranjape and Hall 2017; Klapstein and Driscoll 2018). Briefly, in the water column HgII can (1) be reduced to Hg0 and re-emitted to the atmosphere, (2) methylated to the organic form MMHg, or (3) bind to organic matter (OM) as well as inorganic particles and directly deposit to bottom sediments. MMHg formed in aquatic ecosystems can also deposit to sediments, be methylated and/or form DMHg. Part of DMHg might be re-emitted to the atmosphere or again degraded to MMHg, which can also be biotically (Barkay et al. 2003) or abiotically demethylated (Fernández-Gómez et al. 2013). Although some of the MMHg found in aquatic systems might come from the degradation of DMHg to MMHg, i.e., in oceans (Mason et al. 2012), several studies concluded that most of the MMHg measured in ecosystems was formed in situ or in the surrounding catchment (e.g., soils, wetlands, etc.) and subsequently transported into rivers, lakes (Louis et al. 1996; Eklöf et al. 2012; Bravo et al. 2017), and oceans (Schartup et al. 2015). Abiotic methylation of HgII is possible if suitable methyl donors are present (Celo et al. 2006; Munson et al. 2018). Nevertheless, recent studies have shown that biological HgII methylation is in most environments performed by a variety of microorganisms, carrying the hgcA and hgcB gene cluster (Gilmour et al. 2013, 2018; Parks et al. 2013; Yu et al. 2018). An increasing number of recent studies detailed below have intended to evaluate the biotic HgII methylation by studying the biodiversity and activity of hgcAB+ microorganisms (Gionfriddo et al. 2016; Bravo et al. 2018b,a; Bowman et al. 2019; Jones et al. 2019; Villar et al. 2019). Nevertheless, it is established that net MMHg production also depends on other concomitant processes, including (1) the composition and activity of the whole microbial community that in turn modulate the activity of hgcAB+ microorganisms (Bravo et al. 2018a), (2) physico-chemistry that controls HgII bioavailability (Schaefer and Morel 2009; Jonsson et al. 2012; Chiasson-Gould et al. 2014), and uptake in microorganisms (Schaefer et al. 2011), and (3) biotic and abiotic MMHg demethylation (Du et al. 2019).

Recent reviews critically summarized HgII uptake and MMHg efflux in methylating anaerobes, methods and equations to analyze Hg methylation rates (Regnell and Watras 2019) as well as chemotrophic and biotic Hg methylation and demethylation processes by anaerobes and phototrophs (Grégoire and Poulin 2018; Du et al. 2019). In this review, we aim to summarize the main findings on mechanisms responsible for the formation of MMHg, focusing in detail on the new knowledge recently gained on microorganisms involved in MMHg formation. We also aim to highlight pitfalls and limitations impeding the progress in the current understanding, and we propose a road map to overcome these limitations. Indeed, to improve our ability to predict MMHg generation in the environment, a current research priority is to better understand the distribution of methylating populations in the context of the physico-chemical constraints known to affect MMHg production.

**Methylmercury formation is widespread in the environment**

Recent advances in the methodology to (1) determine in situ HgII methylation (Jonsson et al. 2014) and (2) identify the organisms involved in this process (Parks et al. 2013; Christensen et al. 2016) have revealed that MMHg can be formed in a wider range of environments than previously identified. Indeed, 30 yr ago, first studies showed that HgII methylation in aquatic systems occurred mainly in sediments and under anaerobic conditions (Compeau and Bartha 1984; Korthals and Winfrey 1987; Pak and Bartha 1998). In general, sediments and sinking particles are a complex matrix of solid phases including clays, quartz, metal oxides (FeOOH, MnO2, AlO3) carbonates, sulfides and a number of other minerals and OM. They provide various microenvironments and habitats to organism populations notably bacteria, archaea, algae, diverse invertebrates, and so forth. To date, biological HgII-methylation is known to be mediated by species carrying the hgcAB gene cluster (Parks et al. 2013). Because all the identified microorganisms with HgII-methylating capacity were anaerobes, it was assumed for a long time that MMHg formation was occurring in strictly anoxic environments, i.e., sediments. Nonetheless, several studies revealed that HgII-methylation can occur in oxygen deficient zones of water column (Eckley et al. 2005; Malcolm et al. 2010), sediments (Drott et al. 2008; Hines et al. 2012; Bouchet et al. 2013; Jonsson et al. 2014; Bravo et al. 2015; Liem- Nguyen et al. 2016) flooded soils, e.g., wetlands (Louis et al. 1996; Tjemgren et al. 2012; Windham-Myers et al. 2014) and ponds (Lehnerr et al. 2012; MacMillan et al. 2015; Herrero Ortega et al. 2018). In recent years, HgII-methylation processes were in addition observed in microenvironments such as periphyton, growing on macrophytes (Cleckner et al. 1999; Mauro et al. 2002; Guimaraës et al. 2006; Achá et al. 2011; Hamelin et al. 2011; Bouchet et al. 2018) and settling particles of oxic water columns, including pelagic ocean waters (Monperrus et al. 2007; Cossa et al. 2009; Sunderland et al. 2009; Lehnerr et al. 2011; Gascón Diez et al. 2016). Initially, MMHg formation in oxic waters was considered negligible due to high redox and low concentrations of bacteria and nutrients, but studies demonstrated that about 20–40% of the MMHg measured below the surface mixed layer originates from the surface and enters deeper ocean waters (Blum et al. 2013). Similarly, a query of more than 3500 publicly available microbial metagenomes performed by Podar et al. (2015) unveiled the presence of hgcAB-like genes in sediments and in previously unsuspected environments, including invertebrate digestive tracts, thawing permafrost soils, coastal “dead zones,” soils and...
extreme environments. Moreover, a recent study assessing 243 metagenomes from the Tara Oceans expedition reported high abundances of hgcAB genes in 77 samples across all oceans (Villar et al. 2019). The progress in genetics (Gilmour et al. 2013; Parks et al. 2013; Podar et al. 2015; Bravo et al. 2018b; Liu et al. 2018b; Jones et al. 2019) combined with recent advances in the use of stable isotopes to determine HgII methylation rate constants in sediments (Monperrus et al. 2007; Jonsson et al. 2012; Bravo et al. 2014, 2015), lakes (Eckley and Hintelmann 2006), water columns and oceans (Munson et al. 2018) as well as in sinking particles of marine and lake waters (Lehnerr et al. 2011; Gascon Diez et al. 2016) have demonstrated that the potential for MMHg formation in the environment is widespread across ecosystems.

Toward a better understanding of microbial methylmercury formation

The discovery of hgcAB

Fifty years ago, a decade after the first observation of the Minamata disease in Japan, pioneering research pointed to surface sediments, and bacteria activity as responsible of HgII-methylation (Jensen and Jernelöv 1969). One of the first studies targeting MMHg and bacteria, evaluated the HgII-methylation capacity of Pseudomonas fluorescens, Mycobacterium phlei, Escherichia coli, Aerobacter aerogenes, and Bacillus megaterium over a 7-day period in pure cultures (Vonk and Sijpesteijn 1973). In the presence of sublethal amounts of HgCl2, tested bacteria produced 49 to 169 ng L−1 d−1 of MMHg in aerobic conditions (Vonk and Sijpesteijn 1973). Another decade later, sulfate-reducing bacteria (SRB) were eventually identified as major HgII-methylator in saltmarsh through inhibition of their activity with sodium molybdate and isolation of Desulfovibrio desulfuricans from sediments (Compeau and Bartha 1985).

The relationship between bacterial sulfate reduction and HgII-methylation was studied adding HgII to anoxic sediment slurries or lake water overlying intact sediment cores collected in Quabbin Reservoir, MA (Gilmour et al. 1992). Comparable profiles of sulfate reduction and HgII-methylation in sediment cores were reported, further suggesting that HgII-methylation was linked to this specific bacterial metabolism. Almost 20 yr ago, a correlation between the HgII-methylation and sulfate reduction rates in sediment of a saltmarsh was also shown (King et al. 2000, 2001). Sulfate reduction was then accepted as the main metabolic pathway related to HgII-methylation. In 2006, two studies revealed the role of iron-reducing bacteria (FeRB) on HgII-methylation in ferruginous conditions (Fleming et al. 2006; Kerin et al. 2006). In 2010, Hamelin et al. further identified methanogens as important HgII-methylators in lake periphyton. Two laboratory studies later confirmed the efficiency of methanogens in converting HgII to MMHg (Yu et al. 2013; Gilmour et al. 2018). By culturing and isolating HgII-methylating strains or by using inhibitors of known HgII-methylators such as molybdate for sulfate-reduction and BESA for methanogenesis, HgII-methylation has mainly been then attributed to the action of SRB (Devereux et al. 1996; Pak and Bartha 1998; Hylander et al. 2001; Achá et al. 2011, 2012; Yu et al. 2012; Bravo et al. 2016), and in some cases to FeRB (Fleming et al. 2006; Bravo et al. 2015, 2018b) as well as methanogens (Hamelin et al. 2011; Bravo et al. 2018a).

A recent breakthrough in the understanding of the biological HgII-methylation pathway was the identification of a two-gene cluster, hgcAB -involved in C1 metabolism and the acetyl-CoA pathway (Qian et al. 2016) required for HgII-methylation (Parks et al. 2013). The gene hgcA encodes a corrinoid protein that is essential for the biosynthesis of the folate branch of acetyl-CoA pathway, whereas the gene hgcB encodes a ferredoxin-like protein thought to be an electron donor to hgcA (Parks et al. 2013). Both provide methyl groups required for HgII methylation, although it is not clear whether MMHg production is a controlled or an accidental metabolic process (Qian et al. 2016). However, deletion of either gene eliminated HgII methylation in Desulfovibrio desulfuricans ND132 (Parks et al. 2013). By directly measuring HgII methylation in several bacterial and archaeal strains encoding hgcAB, Gilmour et al. (2013) confirmed that HgII-methylation capability could be predicted by the presence of hgcAB in the genome. For the first time, Gilmour et al. (2013) demonstrated HgII-methylation capability in previously completely unsuspected species including syntrophic, acetogenic, and fermentative Firmicutes.

In recent years, the biodiversity of hgcAB microorganisms was actively studied in contrasting environments. First biodiversity studies using this newly identified gene cluster were based on classical polymerase chain reaction (PCR) amplification with one pair of primers targeting hgcA developed based on the couple of available sequenced genomes of methylating strains at that time (Table 1), followed by cloning and sequencing (Bae et al. 2014; Liu et al. 2014; Schaefer et al. 2014; Smith et al. 2015). In soils of the Florida Everglades, the sequences identified were distributed in diverse phyla, including Deltaproteobacteria, Chloroflexi, Firmicutes, and Methanomicrobia; however, hgcA clone libraries from all sites were dominated by sequences clustering within the order Syntrophobacterales (Bae et al. 2014) (Table 2). By comparing the taxonomically identified hgcA sequences with the activity of SRB (mRNA of dsrB gene), Bae et al. concluded that Syntrophobacterales largely dominated the HgII-methylating microbial community of the Florida Everglades (Bae et al. 2014). In the Three Gorges Reservoir in China, PCR amplification and sequencing of hgcA gene resulted in the identification of δ-Proteobacteria, methanogens and a Clostridia group as putative HgII methylators in this ecosystem. Authors reported in addition a positive correlation between the abundance of hgcA and dsrB genes and MMHg concentrations, suggesting SRB as the main group responsible for HgII methylation in those systems (Luo et al. 2016). In Wanshan Hg mining area of China, the taxonomically annotated sequences were related to δ-Proteobacteria, Firmicutes, Chloroflexi, and Euryarchaeota (Liu et al. 2014). In temperate and tropical wetland soils, hgcA gene sequences were attributed to δ-Proteobacteria, Chloroflexi, and Methanomicrobia (Schaefer et al. 2014). In nine rice paddy soils sampled in three mining areas in China, hgcA+ microbes were dominated by Proteobacteria or Euryarchaeota in six and three sites,
Table 1. Published studies on hgcA diversity and quantification in environmental samples. Sequences and position of primers used, amplicon sizes, targets, and used methods are given.

| References                  | Primer sequences 5' > 3'                          | Target       | Amplicon size (bp) | Methods                       |
|-----------------------------|---------------------------------------------------|--------------|--------------------|-------------------------------|
| Bae et al. 2014             | hgcA_268F GGNRTYAAY RTNTGGTGYGC                   | hgcA-hgcB    | 888 to 945         | PCR and cloning               |
|                             | hgcB_1198R CADGCNCCRCAYTCVATRCA                   |              |                    |                               |
| Bowman et al. 2019          | NA                                                |              |                    | Metagenomics                  |
| Bravo et al. 2018a          | As Schaefer et al. 2014                           |              |                    | PCR and high throughput       |
|                             |                                                   |              |                    | sequencing                    |
| Bravo et al. 2018b          | As Schaefer et al. 2014                           |              |                    | PCR and high throughput       |
|                             |                                                   |              |                    | sequencing                    |
| Bravo et al. 2016           | hgcA_262F GGNRTYAAYRTNTGGTGYGC                   | hgcA         | 650                | qPCR                           |
|                             | hgcA_912R GGTGATGGGGGTACGCCGCTGWRKLT             |              |                    |                               |
| Christensen et al. 2016, 2018| ORNL-HgcAB-uni-268F AAYGTCTGGTGYGCNGCGVGG       | hgcA-hgcB    | 818 to 1020        | PCR                           |
|                             | ORNL-HgcAB-uni-1198R CABGCNCCRCAYTCVATRCA        |              |                    |                               |
|                             | ORNL-Delta-HgcA-181F GCCAATCAAGMTGASCTWC         | hgcA of SRB  | 107                | qPCR                           |
|                             | ORNL-Delta-HgcA-287R CCSCGCNCRACCCAGACRTT        |              |                    |                               |
|                             | ORNL-SRB-firm-HgcA-444F GGTGTAGGGGGTGCAGCCSGTRW | hgcA of Firmicutes | 167                | qPCR                           |
|                             | ORNL-archaea-HgcA-184F AAYTAYWCTSAAGTTYGAYGC     | hgcA of Archa | 125                | qPCR                           |
|                             | ORNL-archaea-HgcA-308R TCDGTCCCAABGTSCCYTT       |              |                    |                               |
| Dranguet et al. 2017        | As Bravo et al. 2016                              |              |                    | qPCR                           |
| Du et al. 2017              | As Schaefer et al. 2014                           |              |                    | qPCR, PCR, and cloning         |
| Gionfriddo et al. 2016      | NA                                                |              |                    | Metagenomics                   |
| Lei et al. 2019             | As Christensen et al. 2016                        |              |                    | qPCR                           |
| Liu et al. 2014             | hgcA_626F GGNRTYAAYRTNTGGTGYGC                   | hgcA         | 315                | PCR and cloning, qPCR          |
| Liu et al. 2018a            | hgcA_941R CGCATYCTTYYBACNNCC                     |              |                    |                               |
|                             | hgcA_515F GTGCCAGCMGCCGCGGTAA                    | hgcA         | 291                | qPCR                           |
|                             | hgcA_806R GGACTACHVGGGTWTCTAA                     |              |                    |                               |
| Liu et al. 2018b            | As Christensen et al. 2016                        | hgcA         |                    | qPCR, metagenomics             |
|                             |                                                   | hgcAB        |                    | PacBio sequencing              |
| Ma et al. 2017              | As Bae et al. 2014                               |              |                    | qPCR                           |
| Ndu et al. 2018             | As Christensen et al. 2016                        |              |                    | PCR and cloning, qPCR          |
| Podar et al. 2015           | NA                                                |              |                    | Metagenomics                   |
| Schaefer et al. 2014        | hgcA_261F CGGCATCAAYGTCTGGTGYGC                  | hgcAB        |                    |                               |
|                             | hgcA_912R GGTGATGGGGGTACGCCGCTGWRKLT             |              |                    |                               |
| Villar et al. 2019          | NA                                                |              |                    | Metagenomics                   |
| Vishnivetskaya et al. 2018  | As Christensen et al. 2016                        |              |                    | PCR and cloning, qPCR          |
| Xu et al. 2019              | As Schaefer et al. 2014                           |              |                    | PCR and high throughput       |
|                             |                                                   |              |                    | sequencing                    |
Table 2. Main characteristics and outcomes of published studies on *hgcA* biodiversity in environmental samples.

| Method                   | Studied environment          | Number of sequence/reads | Number of OTUs | Dominant group          | Dominant Deltaproteobacteria | Reference          |
|--------------------------|------------------------------|--------------------------|----------------|-------------------------|------------------------------|--------------------|
| PCR and cloning          | Florida Everglades           | 220                      | 168            | *Deltaproteobacteria*   | *Syntrophobacterales*        | Bae et al. 2014    |
|                          | Wetlands                     | 108                      | 40             | *Methanomicrobia*       | *Geobacter*                  | Schaefer et al. 2014|
|                          | Three gorges reservoir       | 151                      | 151            | Unidentified            | Unidentified                 | Du et al. 2017     |
|                          | Rice paddy soils             | ~1800                    | 190            | *Proteobacteria*        | *Europarchaeota*             | Liu et al. 2018a   |
|                          | Laboratory and sediment slurr|                          |                | Unidentified            | Unidentified                 | Vishnivetskaya et al.2018|
|                          | Rice paddy soils             | 5                        | ?              | Unidentified            | Unidentified                 | Ndu et al. 2018    |
|                          | Sulfate-impacted lakes       | 300                      | 174            | *Geobacteraceae*        | *Methanomicrobia*            | Jones et al. 2019  |
| PCR and high throughput sequencing | Boreal lakes                | 78,642                   | 225            | *Deltaproteobacteria*   | Unidentified                 | Bravo et al. 2018a |
|                          | Lake Geneva                  | 741,890                  | 356            | *Deltaproteobacteria*   | Unidentified (Geobacter)     | Bravo et al. 2018b |
|                          | Rice paddy soils             | 1,257,577                | 573            | *Deltaproteobacteria*   | Unidentified (Geobacter)     | Liu et al. 2018a   |
|                          | Boreal forest soils          | 5                        | ?              | *Deltaproteobacteria*   | Unidentified (Geobacter)     | Xu et al. 2019     |
| Metagenomics             | Global                       | 823,000,000              |                | Environmental compartment | dependent Nitrospinae         | Podar et al. 2015  |
|                          | Antarctic Sea-ice and brine  |                          |                | *Methanoregula* spp.    | *Geobacter*                  | Gionfriddo et al. 2016|
|                          | Rice paddy soils             | 901,610,484              | 27             | *Deltaproteobacteria*   | *Geobacter*                  | Liu et al. 2018b   |
|                          | Sulfate-impacted lakes       | 885,923                  | 27             | *Deltaproteobacteria*   | *Geobacter*                  | Jones et al. 2019  |
|                          | Eight sites                  | 381–102                  |                | *Deltaproteobacteria*   | *Deltaproteobacteria*        | Christensen et al. 2019|
|                          | Tara gene catalogs oceans    | 111,530,851              | 10             | *Nitrospinae*           | *Desulfovibrionales*         | Villar et al. 2019 |
| Metaproteomics           | EFPC and Hinds Creek, TN     | 15,270–16,852            |                | *Deltaproteobacteria*   |                             | Christensen et al. 2019|
respectively. Only nine of the 190 operational taxonomic unit (OTUs) found in these rice paddy soils were common to all sites (Liu et al. 2018a).

Based on a higher number of sequenced microbial genomes now available, Christensen et al. (2016) developed a broad range hgcAB primer pair that improved the coverage of prior developed primers by 10% (Bae et al. 2014; Schaefer et al. 2014), and several clade-specific PCR primers to improve amplification of the various members of the HgII-methylating community (Christensen et al. 2016) (Table 1). In sediments, these clade-specific primers were useful to detect HgII-methylating \( \delta \)-Proteobacteria and Archaea but failed to detect HgII-methylating Firmicutes (Christensen et al. 2017).

Recent studies based on hgcAB biodiversity analyzed both hgcA (using one single pair of primers, Table 1) and 16S rRNA genes by high-throughput illumina sequencing techniques, allowing a deeper sequencing than cloning-sequencing and hence resulting in a higher number of OTUs. Results evidenced that microbial HgII-methylating community was composed of members of various clades, including SRB, FeRB, methanogens and syntrophs in temperate and boreal lake sediments (Bravo et al. 2018a) as well as in boreal forest soils (Xu et al. 2019). In boreal lakes, besides the identification of HgII-methylating methanogens and Geobacteraceae, authors further showed thanks to inhibition of sulfate reduction with molybdate that only 40% of MMHg was dependent on SRB (Bravo et al. 2018a). Another study performed in sediments impacted by a sewage treatment plant showed that HgII methylating Geobacteraceae seemed to have an important role in HgII methylation in sediments showing ferruginous conditions (Bravo et al. 2018b). Importantly, those studies suggested that the differences in the distributions of HgII methylating taxa among the different sites might derive primarily from different species of the same family having different niche requirements (Bravo et al. 2018a). In particular, the high relative abundance of phytoplankton-derived OM and the presence of specific strains of non-HgII-methylating bacteria involved in OM decomposition (e.g., Rhizobiales, Fibrobacterales, Holophagales, etc.) seems to be essential in creating a niche that promotes HgII methylation (Bravo et al. 2018a; Lei et al. 2019). Another study conducted in sulfate-impacted lakes combined cloning-sequencing of hgcA with metagenomics targeting hgcA gene and genes involved in other metabolic functions (Jones et al. 2019). This approach yielded after in silico assembly of reads with overlapping sequences in a relatively low number of contigs (27), but revealed a high occurrence of hgcA genes together with genes involved in sulfate-reduction and fermentation, but also that some abundant hgcA+ microbes were related to uncultivated microbes, such as Aminicenantes, Kir-ิตติmutielaeota, Spirochaetes, as well as completely unidentified microbes. Data showed that potential methylators from uncultivated organisms occurred more abundantly than previously anticipated in these overlooked clades and that they can dominate the methylating community in certain circumstances (Jones et al. 2019).

A recent study conducted in rice paddy soils combining metagenomics illumina sequencing and long-read PacBio sequencing, which allows overcoming the inherent risk of short-reads chimeric assembly, revealed the dominance of Geobacter spp. for bacteria and Methanoregula spp. for Archaea (Liu et al. 2018b). These authors hypothesize a syntrophic interaction between both species and in addition reported a significant correlation between Geobacter hgcA+ DNA relative abundance and MMHg concentration in soils (Liu et al. 2018b), supporting an important role of this genus for MMHg production in iron-rich paddy soils. A recent study in sediments collected in eutrophic lakes showing cyanobacteria blooms in China also reported a correlation between Archaea hgcA+ DNA relative abundance and MMHg concentration (Li et al. 2019). However, several studies that tried to correlate the level of expression of hgcA mRNA with HgII methylation rates (Goñi-Urriza et al. 2015; Bravo et al. 2016; Christensen et al. 2019) were mostly unsuccessful. For example, in pure cultures of Desulfovibrio dechloroaerovorans BerOc1, the level of expression of hgcA was not correlated with HgII methylation rates (Goñi-Urriza et al. 2015). Similarity, in sediments collected in a river impacted by effluents from a chlor-alkali plant, data suggested that physico-chemistry varied significantly among reservoirs, while functional gene activities, including hgcA, were very similar and did not correlate with MMHg concentrations (Bravo et al. 2016). In contrast, in Hg-contaminated paddy soils, the hgcAB copy number increased with both increasing THg and MMHg concentrations (Vishnivetskaya et al. 2018).

Despite the differences in primer pairs used in the studies mentioned earlier (Table 1), until now, data globally suggested that in hgcA+ \( \delta \)-Proteobacteria communities are abundant in surface sediments, but in some sites hgcA+ methanogens and other hgcA+ uncultivated groups are prevalent (Christensen et al. 2017, 2019; Vishnivetskaya et al. 2018; Bravo et al. 2018a; Jones et al. 2019). Among \( \delta \)-Proteobacteria, syntrophs and Geobacter spp. appear more prevalent in hgcA- community than previously expected. Moreover, other groups of hgcA- microbes seem to be of high importance for HgII-methylating species, certainly by providing some kind of dependence or mutualistic relationship in sediments (Bravo et al. 2018a; Liu et al. 2018b). For example, syntrophs have been shown to modulate HgII methylation of hgcA strains in controlled exposures (Yu et al. 2018). Syntrophy between methanogens or propionate utilizing syntrophs and SRB is hypothesized to enhance methylation in environments devoid of sulfate or where the type and concentration of energy sources are limiting.

Studies listed above focused in freshwaters and therefore the microorganisms processing HgII to MMHg in the ocean are still barely described. Podar et al. (2015) showed that hgcAB appeared to be abundant in marine sediments but they rarely found it in pelagic marine water column, as from 138 metagenome samples analyzed, only seven showed evidence of hgcAB. A recent analysis of 243 seawater metagenome samples from 68 different sites of the Tara Oceans revealed high abundances of hgcAB corresponding to taxonomic relatives of known HgII methylators from
Deltaproteobacteria, Firmicutes, and Chloroflexi across all oceans, with the exception of the Arctic that was not studied (Villar et al. 2019). More recently, Bowman et al. (2019), combining PCR amplification and shotgun metagenomics, searched the hgcAB gene cluster in Arctic Ocean seawater without success. Out of all the hgcA-like genes found in the queries of marine metagenomes, the Nitrospina phylum, a marine nitrite oxidizing bacteria abundant in oxygen-deficient zones, appeared to be widespread, predominant and likely a key player for MMHg production in the oxic subsurface waters of the global ocean (Villar et al. 2019), including the Arctic (Bowman et al. 2019) as well as Antarctic sea ice–brine–sea water interfaces (Gionfriddo et al. 2016). However, despite metagenomic evidence for the abundance of Nitrospina in the global ocean, the few cultured strains harboring a fused hgcAB-like gene (Methanococoides methylutens and Pyrococcus furiosus) were unable to produce MMHg in experimental conditions (Podar et al. 2015; Gilmour et al. 2018). Moreover, there is yet no experimental or observational report on the expression of hgcAB-like genes in Nitrospina bacteria. As such, an experimental evidence of the HgII methylating capacity in Nitrospina is awaited to confirm their role as important HgII methylators in the global ocean. As MMHg has been detected in the water column of every ocean basin, except for the Indian Ocean (Bowman et al. 2019), it is crucial to unveil the role of the microorganisms involved in both MMHg and degradation in seawaters.

These results and observations reveal the diversity of HgII-methylating microbial communities’ structure across ecosystems and point for the need of a thorough investigation of their functioning. Notably further work is necessary to better understand the contribution of overlooked microbial groups in HgII methylating, highlighted by the high proportion of unidentified OTUs found in recent studies concerning hgcAB biodiversity (Table 2). However, it would be useful to agree on a standardized protocol to conduct hgcAB biodiversity studies and have an hgcAB open-access library, as published studies are currently difficult to directly compare due to differences in methods, including primer pairs, alignment algorithms used and depth of sequencing.

**HgII methylators are part of a complex microbial community**

As described in the previous sections, current knowledge established that MMHg net production was linked to biotic and abiotic variables. Microorganisms behave differently from one system to another due to interactions with the physico-chemical variables but also with other organisms of their (micro)environment (Andersson et al. 2014; Bravo et al. 2018a). Studies with one strain can only describe the metabolism of this strain in a batch (Andersson et al. 2014), which is useful for a mechanistic understanding of its potential metabolism. However, it cannot be straightforwardly applied for environmental predictions because its metabolism is likely modified by the activity of other microbial groups and the ambient physico-chemistry. In this sense, one of the most insightful discoveries is the syntrophic HgII methylation recently described in both laboratory (Kerin et al. 2006; Ranchou-Peyruse et al. 2009) and field studies (Yu et al. 2018). Syntrophy is just a “proof of concept” illustrating the complexity of microbial communities carrying out HgII methylation. It is also important to consider that within a microbial community, besides HgII, some bacteria carrying out the merB gene or other genes yet to be discovered, might demethylate MMHg (Barkey et al. 2003).

Electron donors are also essential for HgII methylation. Different microbial clades are involved in the anaerobic oxidation of OM from complex organic compounds generally that goes through several steps and processes (Gilmour et al. 2013; Bae et al. 2014). For example, an initial hydrolysis of large organic substances is followed by a fermentation of intermediates into smaller organic molecules, such as lactate, propionate, butyrate, acetate, and formate, as well as CO₂ and H₂. These fermentation products might then be used as electron donors for Geobacteria, Desulfovibrionales, and Syntrophobacterales known to host HgII methylators. HgII methylators thus likely rely on other microorganisms involved in the degradation of large organic compounds. Furthermore, it was also demonstrated that the specific metabolism of one strain may provoke a new metabolism (unknown) in another strain; this is called the Quorum sensing (Lovley and Chapelle 1995). It is therefore very important to better tackle the complexity of microbial communities and describe the compondium of metabolic processes that can affect directly or indirectly HgII methylation.

**Physico-chemistry plays a pivotal role in HgII methylation**

Besides the presence and diversity of HgII methylating microbes, HgII methylation depends on the amount of HgII bio-available for methylation (Schaefer et al. 2011; Jonsson et al. 2014), which is determined by chemical speciation of HgII, solubility of the Hg-S particles (Hsu-Kim et al. 2013; Liem-Nguyen et al. 2017) as well as the availability of electron donors and acceptors for HgII methylating microorganisms (Desrochers et al. 2015). HgII methylation is a bio-physico-chemical conundrum because both the amount of HgII available for methylation and the activity of microorganisms involved in the process are determined by multiple physico-chemical variables such as sulfur (Skylberg et al. 2003; Drott et al. 2007), iron (Bravo et al. 2015) and OM concentration and speciation (Schartup et al. 2013; Bravo et al. 2017) as well as Eh, pH, nutrient availability, and temperature (Ullrich et al. 2001; Paramjane and Hall 2017) (Fig. 1).

**Variables affecting HgII chemical speciation**

Salinity, sulfur, iron, and OM affect HgII chemical speciation. Sea salt ions may also affect HgII speciation and/or methylation in estuarine and marine environments. In marine waters, HgII forms compounds with chlorine (HgCl₄²⁻ and HgCl₂⁻) to a greater extent than oxides, that are in turn formed in freshwaters (Mason and Fitzgerald 1993). A lower HgII methylating activity in marine and estuarine sediments than in freshwater sediments...
has been attributed to the formation of charged chloride but also sulfide complexes, that undergo a slower methylation processes than other HgII forms (Gårdfeldt et al. 2003; Jonsson et al. 2012; Gworek et al. 2016).

In the context of the biological and chemical interplays controlling HgII methylation, sulfur plays a central role by directly affecting HgII speciation and solubility (Jonsson et al. 2012; Graham et al. 2013; Hsu-Kim et al. 2013; Liem-Nguyen et al. 2017) and consequently its bioavailability (Schaefer and Morel 2009; Chiasson-Gould et al. 2014; Schartup et al. 2015; Mazrui et al. 2016). Reactions between HgII and sulfide control the formation of the solid phase metacinnabar, β-HgS(s) but also aqueous complexes such as Hg(SH)2, HgS2H, and HgS2 2− or polysulfides HgSnSH−(aq) (n = 4–6) (Liem-Nguyen et al. 2017). Elevated sulfide concentrations, eventually limits HgII bioavailability for methylation (Drott et al. 2007; Hsu-Kim et al. 2013; Bigham et al. 2017).

Conversely low sulfide concentrations might enhance HgII methylation processes (Benoit et al. 1999; Graham et al. 2012). Natural OM often contain thiols, which sulfhydryl group has a high capacity to complex HgII and MMHg (Skyllberg et al. 2006; Skyllberg 2008). Iron plays also a key role on HgII methylation as its reduced form Fe2+ can scavenge sulfide, form stable iron–sulfur compounds (FeS, Fe3S4, or FeS2) and then might let HgII bioavailable for methylation (i.e., ferruginous conditions; Bravo et al. 2015). Each of these HgII complexes, i.e., inorganic sulfides, polysulfides, and OM in aqueous, solid, and adsorbed phases, show different reactivity in the environment. For example, HgII methylation rate constants in estuarine sediments spanned over two orders of magnitude depending on the chemical form: metacinnabar (β-HgS (s)) < cinnabar (α-HgS(s)) < HgII reacted with mackinawite (⇌FeS–HgII) < HgII bonded to natural OM (NOM–HgII) < (Hg(NO3)2(aq)) that is a typical aqueous tracer (Jonsson et al. 2012).

The effect of natural OM on the sediment and pore water-partitioning coefficient for HgII (Hammerschmidt et al. 2008; Liem-Nguyen et al. 2016) needs still to be determined. Under low OM and porewater sulfide concentrations, HgII partitioning coefficient becomes a major factor for methylation (Mitchell and Gilmour 2008; Hollweg et al. 2009). Some studies have shown that increases in natural OM concentrations might also raise partitioning coefficients in sediment and pore water and have shown to decrease HgII concentration in the pore water, and thus its bioavailability for uptake by methylating microorganisms (Liem-Nguyen et al. 2016). In contrast, other studies concluded that OM content did not explain variations in HgII partitioning, most likely only a limited fraction of OM was relevant for Hg complexation or because it is also possible that the composition, rather than amount, of OM controls HgII partitioning (Schartup et al. 2013).

High concentrations of OM decreased HgII bioavailability in laboratory experiments (Chiasson-Gould et al. 2014) and in marine sediments (Hammerschmidt et al. 2008). Moreover, OM might also be important in determining HgII bioavailability by stabilizing HgS particles at nanoscale that can be methylated by anaerobic bacteria (Graham et al. 2013; Hsu-Kim et al. 2013). To progress further in the understanding of HgII methylation in future research, it is of the utmost importance to measure and model HgII chemical speciation, which is ultimately determining the HgII availability for methylating bacteria (Fig. 1, violet box).
**Variables affecting microbial activity**

Sulfur, iron, OM, redox, pH, nutrients, and temperature affect microbial activity. Both sulfur and iron have oxidized forms (SO$_4^{2−}$ and Fe$^{3+}$, respectively) that can serve as electron acceptor for some Hg$^{II}$ methylating bacteria (e.g., SRB and FeRB). Natural OM, besides its strong capacity to bind Hg$^{II}$ and influence Hg$^{II}$ speciation, controls microbial activity and Hg$^{II}$ methylation (Graham et al. 2013; Hsu-Kim et al. 2013). The molecular composition of OM shows a central role in controlling Hg$^{II}$ methylation (Bravo et al. 2017), notably phytoplankton derived OM and fresh humic matter were associated with both high bacterial activity and Hg$^{II}$ methylation rates in sediments (Graham et al. 2013; Schartup et al. 2013; Mazrui et al. 2016; Bravo et al. 2017; Christensen et al. 2017; Herrero Ortega et al. 2018). Also, an increase in nutrients, associated to an enhanced algae biomass production, increased MMHg formation in sediments (Bravo et al. 2017; Herrero Ortega et al. 2018). Changes in redox conditions might also affect iron, sulfur and Hg$^{II}$ speciation (Liem-Long 2013). The activity of the microorganisms (Grégoire and Poulin 2018) and consequently Hg$^{II}$ methylation (Fig. 1, orange boxes and arrows). Furthermore, Hg$^{II}$ methylation strongly depends on the activity of the whole microbial community (Weber et al. 2006; DeAngelis et al. 2010). Therefore, all the physico-chemical variables causing increased microbial activity in sediments, such as temperature (Gudasz et al. 2010), might indirectly lead to enhanced Hg$^{II}$ methylation (Bravo et al. 2017; Dijkstra et al. 2011).

From all the studies mentioned earlier, we can conclude that both microbial activity and Hg$^{II}$ chemical speciation control the formation of MMHg in the environment. Therefore, Hg cycling and in particular, the methylation of Hg$^{II}$ is an intricate process regulated by both physico-chemical and biological constrains that needs holistic approaches to be fully understood.

**Knowledge gaps and uncertainties**

A high number of unidentified putative Hg$^{II}$ methylators

The complexity of microbial communities and its implications for Hg cycling is one of the main current challenges. First biodiversity studies used 16S rRNA gene to investigate the diversity of Hg$^{II}$ methylating microbial communities. But biodiversity of this gene cannot provide reliable and robust identification of Hg$^{II}$ methylator diversity and abundance, because hgcAB+ strains are too rare (<1%) and not well identified in 16S rRNA databases (<300 species) (Miller and Bassler 2001; Christensen et al. 2019).

Recent studies targeting hgcAB are interesting, but these molecular approaches entail several limits. First, as for all microbial biodiversity studies, the DNA extraction protocols need to be well planned to ensure clean subsampling avoiding contamination and may need to be optimized for the efficient recovery of DNA and the elimination of potential inhibitors for PCR and/or sequencing technologies. Indeed, DNA extraction and PCR amplification are known to be prone to artifacts due to any combination of high primer mismatch, low abundance, and/or low DNA extraction efficiency that can significantly affect results (Bravo et al. 2018a; Epp et al. 2019). Although claimed as “universal,” primers inherently show preferences and limitations in covering all species equally among different environmental samples. More specifically, currently available primer pairs for the gene hgcA are predicted to cover 84% (Schaef er et al. 2014) and 94% (Christensen et al. 2016) of the whole biodiversity by PCR-based approaches. However, within amplified sequences of hgcA a significant proportion cannot be identified above the clade level because of (1) the lack of identified organisms in the databases, and (2) the low conservation of hgcA gene that is not ideal for biodiversity studies. Consequently, a significant proportion of OTUs are attributed to unidentified species. Indeed, identification of hgcA sequences from short reads is made by aligning metagenomics data with a set of known gene sequences isolated from cultivated strains. This approach is prone to inaccuracies, especially if the data are evaluated down to the genus level. For example, in recent studies 62% (Bravo et al. 2018b) and 57% (Bravo et al. 2018a) of the identified OTUs could be taxonomically assigned at the order level. More in detail, different algorithms are available to identify OTUs e.g., simple sequence alignment-based algorithms (e.g., Basic Local Alignment Search Tool [BLAST]) or profile hidden Markov model (HMM)-based searches, which are expected to be more sensitive and accurate in identifying homologs. Nonetheless, the HMM model is satisfying for hgcA but not for hgcB that cannot be confidently differentiated from other ferredoxin-encoding genes due to homology (Christensen et al. 2019). Besides, both primers and algorithms are developed on sequences from cultivated strains and consequently identify those species with a higher efficiency (Podar et al. 2015; Bravo et al. 2018b; Liu et al. 2018b). Moreover, only few hundred sequenced genomes of methylating strains are currently available in databases. Although this number is increasing regularly, databases still do not include reliably microorganisms that cannot be grown in the laboratory. Besides we do not have information on the rates and methylation capabilities of these uncultivated populations and thus we cannot affirm that the genes identified code for MMHg production in these organisms. Further, we cannot evaluate how Hg methylation rates in these organisms compare to other methylators in culture. In sum, available sequences are far from being sufficient to confidently identify all OTUs homologs of hgcA and might not allow identifying unknown microbes. This is a current inherent technical limit that has to be considered when interpreting data.

However, studies conducted on DNA do not reflect the activity of the protein or the enzyme preforming the process, but only the possibility that a strain could sometime methylate Hg$^{II}$. Although the use of RNA should theoretically provide more detailed information on the activity of the organisms involved in Hg$^{II}$, up to now the abundance of hgcAB transcripts could not be correlated to Hg$^{II}$ methylation or MMHg concentration (Goni-Urriza et al. 2015; Bravo et al. 2016; Vishnivetskaya et al. 2018; Christensen et al. 2019), except in one study (Ledeker and De Long 2013). The precise factors regulating hgcA gene and protein
expression are not identified yet but it seems that hgCA gene could be constitutively expressed or regulated by carbon, metabolism, but Hg does not appear to be a key regulator (Goñi-Urriza et al. 2015; Christensen et al. 2019). Moreover, the correlation between mRNA and protein abundance is not necessarily linear as posttranslational regulations can take place. In addition to the factors regulating hgCA gene expression, it is important to consider that net MMHg production also depends on MMHg demethylation activity. Therefore, the absence of correlation between hgCA gene and MMHg concentration is not surprising and highlights the complexity of predicting MMHg concentration dynamics in the environment.

Currently, the scientific community lacks a straightforward method to directly measure the activity of hgCA at the protein level. New metaproteomic approaches are currently developed and could in the near future allow a direct analysis of hgCA protein abundance in the environment (Meier et al. 2019), but this approach has been tested only once to study hgCA biodiversity yet (Christensen et al. 2019). Metagenomics, metatranscriptomics, and metaproteomics offer a deeper sequencing and a wealth of information than earlier but they are cost and time-consuming to conduct analysis of hundreds of samples. This highlights that we still lack of a simple and accurate method to identify HgII-methylating microbial activity in environmental samples.

Another issue is that there is currently no medium or protocol to reliably culture a whole microbial community including FeRB, SRB, firmicutes, Clostridia, and so forth. Even studies with intact sediments in controlled experimental conditions can only approximate interactions of microbial community in situ and need to be confirmed in the field. For this reason, it is difficult to study and explain interactions of microbial communities through controlled experiments using a single strain. For example, microbial strains showing maximum methylation rates in the laboratory may not be as active in complex consortia under real field conditions. Metagenomics, metatranscriptomics, and metaproteomics could help to progress in the elucidation of the biological metabolism behind HgII methylation in the field. Instead of identifying different taxa, these approaches combine genes coding for all metabolic pathways of the different taxa to describe the potential function of the whole community (metagenomics) or its activity in one compartment (sediment, water, soil, etc.) at the moment of the sampling (metatranscriptomics and metaproteomics). Nonetheless, available metagenomics approaches have seldom identified hgCA sequences (i.e., 63 out of 203 metagenome projects revealed hgCA occurrence; Podar et al. 2015; and 77 out 243, Villar et al. 2019) supporting that new study should be conducted in environments relevant for HgII methylation. Furthermore, metatranscriptomics and metaproteomics are still rarely applied, most likely because they are expensive and time-consuming. However, although we believe that these approaches are promising and their use will increase in coming years, they will need to be coupled with a detailed analysis of environmental conditions (e.g., physico-chemistry analysis) to be fully informative.

Are we measuring realistic HgII methylation rates?

Last but not least, besides limitations to directly measure biological process responsible for HgII methylation, there are several pitfalls on the methods used to determine HgII methylation at environmentally relevant HgII concentrations. Several approaches have been used to estimate MMHg formation: using labeled HgII forms with radio-isotopes (Goñi-Urriza et al. 2015; Bravo et al. 2016), stable isotopes (Ramlal et al. 1986), by measuring the percentage of total Hg and MMHg (% MMHg) (Hintelmann et al. 2000) or the change in MMHg concentration over time (Drott et al. 2008). The amendment of standards enriched in HgII stable isotope tracers to environmental samples is now widely used in different laboratories because they allow determining HgII methylation rates and MMHg demethylation rates simultaneously. However, it is known that the geochemical form of the HgII isotope used as tracer determines its reactivity (i.e., methylation rate) in the environment. For example, HgII methylation rate constants in estuarine sediments spanned over two orders of magnitude from metacinnabar to a typical aqueous tracer such as Hg(NO3)2(aq) (Jonsson et al. 2012). Therefore, HgII methylation rate constants measured using a highly available tracer might result in an overestimation of the in situ HgII methylation rate, when the tracer is a poor representative of the indigenous HgII chemical forms. The %MMHg and the change of MMHg concentration overtime represent the net MMHg, accounting for MMHg degradation processes and while it might be useful to predict MMHg concentrations in the environment, its use is limited to provide mechanistic understanding of HgII methylation processes.

From this literature review, we observe that after 50 yr of efforts to study HgII methylation through the world, knowledge has greatly increased, but there are still many aspects within the bio-physico-chemistry of MMHg formation that need to be unveiled in the natural environment because: (1) there is a lack of techniques and methods for the measurements of the different HgII chemical species available for methylation; (2) the physico-chemical factors affecting HgII chemistry are still only partly understood; (3) the biological mechanisms involved in the whole bio-physico-chemical process of MMHg net production remains to be understood and described more accurately.

How to study methylmercury formation in future research?

Besides current limits inherent to used analysis described earlier, main questions lacking a clear answer concerning the behavior of Hg in environmental systems include: What are all the HgII chemical species available for methylation? How diverse are hgCA species? How does microbial community consortium impact the amount and speciation of HgII available for methylation? And what is the impact of the hgCA−...
microbial species on the activity of Hg\textsuperscript{II} methylators? Do techniques exist to tackle these questions?

Laboratory studies are essential to identify mechanisms driving Hg\textsuperscript{II} methylation but need to include more realistic scenarios and/or be validated under field conditions. Currently, in situ studies to elucidate the biodiversity of Hg\textsuperscript{II} methylators and determine the drivers of their activity need to be conducted more widely and in more diverse environments. For in situ studies, Hg\textsuperscript{II} speciation and Hg\textsuperscript{II} rates need to be determined, and accompanied by the study of the factors controlling the activity of Hg\textsuperscript{II} methylating microorganisms. Determination of the methylating activity of natural microbial assemblages in relation to sediment characteristics, specific environmental conditions and the level of Hg contamination needs to be undertaken to validate laboratory-based measurements and to improve our understanding of Hg cycling in contaminated environments (Fig. 2). In this context, interactions between microbial communities and the physico-chemistry are key to predict Hg\textsuperscript{II} methylation as several studies pointed out that physico-chemistry rather than the microbial community structure were determining Hg\textsuperscript{II} methylation rate constants in lake sediments (Bravo et al. 2016, 2017, 2018\textsuperscript{a}; Liu et al. 2018\textsuperscript{b}). This is likely true but it should not be forgotten that the local physico-chemistry is also the result of the microbial activity (Bravo et al. 2017, 2018\textsuperscript{b}).

We can use existing and future new molecular biology and chemistry techniques to study Hg\textsuperscript{II} methylation, but studies have now to put more effort on combining metagenomics, metatranscriptomics, and metaproteomics approaches with experiments on bacterial isolates and also complex matrices (i.e., sediments, marine waters, etc.) to explain more in detail environmental mechanisms (Fig. 2). Last, we should keep in

---

**Fig. 2.** Schematic representation of the proposed conceptual iterative strategy for studying Hg\textsuperscript{II} methylation.
mind that MMHg in aquatic systems is the net result of different processes: (1) formation (Hg$^\text{II}$ methylation), (2) degradation (MMHg demethylation), and (3) the inputs and outputs of the system (Fig. 3). Therefore, when studying Hg$^\text{II}$ methylation, MMHg demethylation, and the inputs and outputs of the system, we target only a limited part of the whole Hg cycling determining MMHg concentrations in the environment. Future studies need to be invested in studying concomitantly the drivers of MMHg methylation and MMHg demethylation mechanisms in situ as well as the transport of MMHg within the aquatic network, which is indeed highly controlled by hydrological processes (Fig. 3).

**References**

Achá, D., H. Hintelmann, and J. Yee. 2011. Importance of sulfate reducing bacteria in mercury methylation and demethylation in periphyton from Bolivian Amazon region. Chemosphere 82: 911–916. doi:10.1016/j.chemosphere.2010.10.050

Achá, D., H. Hintelmann, and C. A. Pabón. 2012. Sulfate-reducing bacteria and mercury methylation in the water column of the Lake 658 of the experimental lake area. Geomicrobiol. J. 29: 667–674. doi:10.1080/01490451.2011.606289

Åkerblom, S., A. Bignert, M. Meili, L. Sonesten, and M. Sundbom. 2014. Half a century of changing mercury levels in Swedish freshwater fish. Ambio 43: 91–103. doi:10.1007/s13280-014-0564-1

Amos, H. M., D. J. Jacob, D. G. Streets, and E. M. Sunderland. 2013. Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. Global Biogeochem. Cycles 27: 410–421. doi:10.1002/gbc.20040

Andersson, M. G. I., M. Berga, E. S. Lindström, and S. Langenheder. 2014. The spatial structure of bacterial communities is influenced by historical environmental conditions. Ecology 95: 1134–1140. doi:10.1890/13-1300.1

Bae, H. S., F. E. Dierberg, and A. Ogram. 2014. Syntrophs dominate sequences associated with the mercury methylation-related gene hgcA in the water conservation areas of the Florida Everglades. Appl. Environ. Microbiol. 80: 6517–6526. doi:10.1128/AEM.01666-14

Barckay, T., S. M. Miller, and A. O. Summers. 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27: 355–384. doi:10.1016/S0168-6445(03)00046-9

Benoit, J. M., C. C. Gilmour, R. P. Mason, and A. Heyes. 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Environ. Sci. Technol. 33: 951–957. doi:10.1021/es9808200

Bigham, G. N., K. J. Murray, Y. Masue-Slowey, and E. A. Henry. 2017. Biogeochemical controls on methylmercury in soils and sediments: Implications for site management. Integr. Environ. Assess. Manag. 13: 249–263. doi:10.1002/ieam.1822

Bjerklund, G., M. Dadar, J. Mutter, and J. Aaseth. 2017. The toxicology of mercury: current research and emerging trends. Environ. Res. 159: 545–554. doi:10.1016/j.envres.2017.08.051

Bloom, N. S. 1992. On the chemical form of m00ercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. 49: 1010–1017. doi:10.1139/f92-113

Bouchet, S., and others. 2013. MMHg production and export from intertidal sediments to the water column of a tidal lagoon (Arcachon Bay, France). Biogeochemistry 114: 341–358. doi:10.1007/s10533-012-9815-z

Bouchet, S., and others. 2018. Linking microbial activities and low-molecular-weight thiols to Hg methylation in biofilms and periphyton from high-altitude tropical lakes in the Bolivian Altiplano. Environ. Sci. Technol. 52: 9758–9767. doi:10.1021/acs.est.8b01885

Bowman, K. L., R. E. Collins, A. M. Agather, C. H. Lamborg, C. R. Hammerschmidt, D. Kaul, C. L. Dupont, G. A. Christensen, and D. A. Elias. 2019. Distribution of mercury-cycling genes in the Arctic and equatorial Pacific oceans and their relationship to mercury speciation. Limnol. Oceanogr.: 1–11. doi:10.1002/lio.11310

Bravo, A. G., C. Cosio, D. Amouroux, J. Zopfi, P.-A. Chevalley, J. E. Spangenberg, V.-G. Ungureanu, and J. Dominik. 2014. Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant. Water Res. 49: 391–405. doi:10.1016/j.watres.2013.10.024

Bravo, A. G., S. Bouchet, S. Guédron, D. Amouroux, J. Dominik, and J. Zopfi. 2015. High methylmercury production under ferruginous conditions in sediments impacted by sewage treatment plant discharges. Water Res. 80: 245–255. doi:10.1016/j.watres.2015.04.039

Bravo, A. G., J. L. Loizeau, P. Dranguet, S. Makri, E. Björn, V. G. Ungureanu, V. I. Slaveykova, and C. Cosio. 2016. Persistent 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

Bravo, A. G., S. Bouchet, J. Tolui, E. Björn, A. Mateos-Rivera, and S. Bertilsson. 2017. Molecular composition of organic matter controls methylmercury formation in boreal lakes. Nat. Commun. 8: 14255. doi:10.1038/ncomms14255

Bravo, A. G., and others. 2018a. Methanogens and iron-reducing bacteria: the overlooked members of mercury-methylating microbial communities in boreal lakes. Appl. Environ. Microbiol. 84: 0–16. doi:10.1128/AEM.01774-18

Bravo, A. G., J. Zopfi, M. Buck, J. Xu, S. Bertilsson, J. K. Schaefer, J. Poté, and C. Cosio. 2018b. Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases. ISME J. 12: 802–812. doi:10.1038/s41396-017-0007-7

Celo, V., D. R. S. Lean, and S. L. Scott. 2006. Abiotic methylation of mercury in the aquatic environment. Sci. Total
Environ. 368: 126–137. doi:10.1016/j.scitotenv.2005.09.043

Chételat, J., B. Braune, J. Stow, and S. Tomlinson. 2015. Special issue on mercury in Canada’s north: summary and recommendations for future research. Sci. Total Environ. 509–510: 260–262. doi:10.1016/j.scitotenv.2014.06.063

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

Christensen, G. A., and others. 2016. Development and validation of broad-range qualitative and clade-specific quantitative molecular probes for assessing mercury methylation in the environment. Appl. Environ. Microbiol. 82: 6068–6078. doi:10.1128/AEM.01271-16

Christensen, G. A., and others. 2017. Carbon amendments alter microbial community structure and net mercury methylation potential in sediments. Appl. Environ. Microbiol. 84: 1–14. doi:10.1128/AEM.01049-17

Christensen, G. A., A. C. Somenahally, J. G. Moberly, C. M. Miller, A. J. King, C. C. Gilmour, S. D. Brown, M. Podor, C. C. Brandt, S. C. Brooks, A. V. Palumbo, J. D. Wall, and D. A. Elias. (2018) Carbon amendments alter microbial community structure and net mercury methylation potential in sediments. Applied and Environmental Microbiology 84.

Christensen, G. A., and others. 2019. Determining the reliability of measuring mercury cycling gene abundance with correlations with mercury and methylmercury concentrations. Environ. Sci. Technol. 53: 8649–8663. doi:10.1021/acs.est.8b06389

Clarkson, T. W., and L. Magos. 2006. The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36: 609–662. doi:10.1080/1040840060845619

Cleekner, L. B., C. C. Gilmour, J. P. Hurley, and D. P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. Limnol. Oceanogr. 44: 1815–1825. doi:10.4319/lo.1999.44.7.1815

Compeau, G., and R. Bartha. 1984. Methylation and demethylation of mercury under controlled redox, pH and salinity conditions. Appl. Environ. Microbiol. 48: 1203–1207.

Compeau, G. C., and R. Bartha. 1985. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. 50: 498–502.

Cossa, D., B. Averty, and N. Pirrone. 2009. The origin of methylmercury in open Mediterranean waters. Limnol. Oceanogr. 54: 837–844. doi:10.4319/lo.2009.54.3.0837

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microrganisms communities acclimate to recurring changes in soil redox potential status. Environ. Microbiol. 12: 3137–3149. doi:10.1111/j.1462-2920.2010.02286.x

Depew, D., and others 2013. An overview of mercury concentrations in freshwater fish species: a national fish mercury dataset for Canada. Can. J. Fish Aquat. Sci. 451: 436–451. doi:10.1139/cjfas-2012-033

Desrochers, K. A. N., K. M. A. Paulson, C. J. Ptacek, D. W. Blowes, and W. D. Gould. 2015. Effect of electron donor to sulfate ratio on mercury methylation in floodplain sediments under saturated flow conditions. Geomicrobiol. J. 32: 924–933. doi:10.1080/01490451.2015.1035818

Devereux, R., M. R. Winfrey, J. Winfrey, and D. A. Stahl. 1996. Depth profile of sulfate-reducing bacterial ribosomal RNA and mercury methylation in an estuarine sediment. FEMS Microbiol. Ecol. 20: 23–31. doi:10.1111/j.1574-6941.1996.tb00301.x

Dijkstra, P., S. C. Thomas, P. L. Heinrich, G. W. Koch, E. Schwartz, and B. A. Hungate. 2011. Effect of temperature on metabolic activity of intact microbial communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. Soil Biol. Biochem. 43: 2023–2031. doi:10.1016/j.soilbio.2011.05.018

Dranguet, P., S. Le Faucheur, and V. I. Slavoykova. 2017. Mercury bioavailability, transformations, and effects on freshwater biofilms. Environ. Toxicol. Chem. 36: 3194–3205. doi:10.1002/etco.3934

Blum, J. D., B. N. Popp, J. C. Drazen, M. W. Johnson, and C. Anela Choy. 2013. Methylmercury production below the mixed layer in the North Pacific Ocean. Nat. Geosci. 6: 879–884. doi:10.1038/ngeo1918

Drott, A., L. Lambertsson, E. Björn, and U. Skyllberg. 2007. Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. Environ. Sci. Technol. 41: 2270–2276. doi:10.1021/es061724z

Drott, A., L. Lambertsson, E. Björn, and U. Skyllberg. 2008. Do potential methylation rates reflect accumulated methyl mercury in contaminated sediments? Environ. Sci. Technol. 42: 153–158. doi:10.1021/es0715851

Du, H., M. Ma, T. Sun, X. Dai, C. Yang, F. Luo, D. Wang, and Y. Igarashi. 2017. Mercury-methylating genes dsrB and hgcA in soils/sediments of the three gorges reservoir. Environ. Sci. Pollut. Res. 24: 5001–5011. doi:10.1007/s11356-016-8213-9

Du, H., M. Ma, Y. Igarashi, and D. Wang. 2019. Biotic and abiotic degradation of methylmercury in aquatic ecosystems: A review. Bull. Environ. Contam. Toxicol. 102: 605–611. doi:10.1007/s00128-018-2530-2

Eagles-Smith, C. A., and others. 2016. Spatial and temporal patterns of mercury concentrations in freshwater fish across the Western United States and Canada. Sci. Total Environ. 568: 1171–1184. doi:10.1016/j.scitotenv.2016.03.229

Eckley, C. S., and H. Hintelmann. 2006. Determination of mercury methylation potentials in the water column of lakes across Canada. Sci. Total Environ. 368: 111–125. doi:10.1016/j.scitotenv.2005.09.042

Eckley, C. S., C. J. Watras, H. Hintelmann, K. Morrison, A. D. Kent, and O. Regnell. 2005. Mercury methylation in the hypolimnetic waters of lakes with and without connection.
to wetlands in northern Wisconsin. Can. J. Fish. Aquat. Sci. 411: 400–411. doi:10.1139/F04-205

Eklöf, K., J. Fölster, L. Sonesen, and K. Bishop. 2012. Spatial and temporal variation of THg concentrations in run-off water from 19 boreal catchments, 2000-2010. Environ. Pollut. 164: 102–109. doi:10.1016/j.envpol.2012.01.024

Eklöf, K., K. Bishop, S. Bertilsson, E. Björn, M. Buck, U. Skyllberg, O. A. Osman, R. M. Kronberg, and A. G. Bravo. 2018. Formation of mercury methylation hotspots as a consequence of forestry operations. Sci. Total Environ. 613–614: 1069–1078. doi:10.1016/j.scitotenv.2017.09.151

Epp, L. S., H. H. Zimmermann, and K. R. Stoof-Leichsenring. 2019. Sampling and extraction of ancient DNA from sediments, p. 31–44. In Ancient DNA: Methods and protocols, methods in molecular biology. Springer. doi:10.1007/978-1-4939-9176-1_5

Fernández-Gómez, C., A. Drott, E. Björn, S. Díez, J. M. Bayona, S. Tesfai, A. Lindfors, and U. Skyllberg. 2013. Towards universal wavelength-specific photodegradation rate constants for methyl mercury in humic waters, exemplified by a boreal lake-wetland gradient. Environ. Sci. Technol. 47: 6279–6287. doi:10.1021/es404373s

Fleck, J. A., and others. 2015. Mercury and methylmercury in aquatic sediment across western North America. Sci. Total Environ. 568: 727–738. doi:10.1016/j.scitotenv.2016.03.044

Fleming, E. J., E. E. Mack, P. G. Green, and D. C. Nelson. 2006. Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. Appl. Environ. Microbiol. 72: 457–464. doi:10.1128/AEM.72.1.457

Gårdfeldt, K., and others. 2003. Evasion of mercury from coastal and open waters of the Atlantic Ocean and the Mediterranean Sea. Atmos. Environ. 37: 73–84. doi:10.1016/S1352-2310(03)00238-3

Gascón Díez, E., J. L. Loizeau, C. Cosio, S. Bouchet, T. Adatte, D. Amouroux, and A. G. Bravo. 2016. Role of settling particles on mercury methylation in the oxic water column of freshwater systems. Environ. Sci. Technol. 50: 11672–11679. doi:10.1021/acs.est.6b03260

Gilmour, C. C., E. A. Henry, and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26: 2281–2287. doi:10.1021/es00035a029

Gilmour, C. C., and others. 2013. Mercury methylation by novel microorganisms from new environments. Environ. Sci. Technol. 47: 11810–11820. doi:10.1021/es403075t

Gilmour, C. C., A. L. Bullock, A. Mcburney, and M. Podar. 2018. Robust mercury methylation across diverse methanogenic archaea. mBio 9: 1–13. doi:10.1128/mBio.02403-17

Gionfriddo, C. M., and others. 2016. Microbial mercury methylation in Antarctic Sea ice. Nat. Microbiol. 1: 16127. doi:10.1038/nmicrobiol.2016.127

Goñi-Urriza, M., Y. Corsellis, L. Lanceleur, E. Tessier, J. Gury, M. Monperrus, and R. Guyoneaud. 2015. Relationships between bacterial energetic metabolism, mercury methylation potential, and hgCA/hgcB gene expression in Desulfovibrio dechloroacetivorans BerOc1. Environ. Sci. Pollut. Res. 22: 13764–13771. doi:10.1007/s11356-015-4273-5

Graham, A. M., G. R. Aiken, and C. C. Gilmour. 2012. Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions. Environ. Sci. Technol. 46: 2715–2723. doi:10.1021/es303658f

Graham, A. M., G. R. Aiken, and C. C. Gilmour. 2013. Effect of dissolved organic matter source and character on microbial hg methylation in hg-S-DOM solutions. Environ. Sci. Technol. 47: 5746–5754. doi:10.1021/es400414a

Grégoire, D. S., and A. J. Poulain. 2018. Shining light on recent advances in microbial mercury cycling. Facets 3: 858–879. doi:10.1139/facets-2018-0015

Gudasz, C., D. Bastviken, K. Steger, K. Premke, S. Sobek, and L. J. Tranvik. 2010. Temperature-controlled organic carbon mineralization in lake sediments. Nature 466: 478–481. doi:10.1038/nature09383

Guimaraes, J. R. D., J. B. N. Mauro, M. Meili, M. Sundbon, A. L. Haglund, S. A. Coelho-Souza, and L. D. Hylander. 2006. Simultaneous radioassays of bacterial production and mercury methylation in the periphyton of a tropical and a temperate wetland. J. Environ. Manage. 81: 95–100. doi:10.1016/j.envrman.2005.09.023

Gworek, B., O. Bemowska-Kalabun, M. Kijeriska, and J. Wrozek-Jakubowska. 2016. Mercury in marine and oceanic waters—a review. Water Air Soil Pollut. 227: 371. doi:10.1007/s11270-016-3060-3

Hamelin, S., M. Amyot, T. Barkay, Y. Wang, and D. Planas. 2011. Methanogens: principal methylators of mercury in lake periphyton. Environ. Sci. Technol. 45: 7693–7700. doi:10.1021/es2010072

Hammerschmidt, C. R., W. F. Fitzgerald, P. H. Balcom, and P. T. Visscher. 2008. Organic matter and sulfide inhibit methylmercury production in sediments of New York/New Jersey Harbor. Mar. Chem. 109: 165–182. doi:10.1016/j.marchem.2008.01.007

Herrero Ortega, S., and others. 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

Hines, M. E., E. N. Poitras, S. Covelli, J. Faganeli, A. Emili, S. Žižek, and M. Horvat. 2012. Mercury methylation and demethylation in Hg-contaminated lagoon sediments (Marano and Grado lagoon, Italy). Estuar. Coast. Shelf Sci. 113: 85–95. doi:10.1016/j.ecss.2011.12.021

Hintelmann, H., K. Keppel-Jones, and D. Evans. 2000. Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury
availability. Environ. Toxicol. Chem. 19: 2204–2211. doi: 10.1002/etc.5620190909

Hollweg, T. A., C. C. Gilmour, and R. P. Mason. 2009. Methylmercury production in sediments of Chesapeake Bay and the mid-Atlantic continental margin. Mar. Chem. 114: 86–101. doi:10.1016/j.marchem.2009.04.004

Hsu-Kim, H., K. H. Kucharzyk, T. Zhang, and M. A. Deshusses. 2013. Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. Environ. Sci. Technol. 47: 2441–2456. doi: 10.1021/es303470g

Hylander, L. 2000. Relationship of mercury with aluminum, iron and manganesoxy-hydroxides in sediments from the Alto Pantanal, Brazil. Sci. Total Environ. 260: 97–107. doi: 10.1016/S0048-9697(00)00544-1

Jackson, T. a. 1997. Long-range atmospheric transport of mercury to ecosystems, and the importance of anthropogenic emissions—a critical review and evaluation of the published evidence. Environ. Rev. 5: 207. doi:10.1139/er-5-3-4-207

Jensen, S., and A. Jernelöv. 1969. Biological methylation of mercury in aquatic organisms. Nature 223: 753–754. doi: 10.1038/223753a0

Jones, D. S., G. M. Walker, N. W. Johnson, C. P. J. Mitchell, J. K. Coleman Wasik, and J. V. Bailey. 2019. Molecular evidence for novel mercury methylating microorganisms in sulfate-impacted lakes. ISME J. 13: 1659–1675. doi: 10.1038/s41396-019-0376-1

Jonsson, S., U. Skyllberg, M. B. Nilsson, P. Westlund, A. Shchukarev, E. Lundberg, and E. Björn. 2012. Mercury methylation rates for geochemically relevant HgII species in sediments. Environ. Sci. Technol. 46: 11653–11659. doi: 10.1021/es3015327

Jonsson, S., U. Skyllberg, M. B. Nilsson, E. Lundberg, A. Andersson, and E. Bjo. 2014. Differentiated availability of geochemical mercury pools controls methylmercury levels in estuarine sediment and biota. Nat. Commun. 5: 4624–4634. doi: 10.1038/ncomms5624

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

Kim, K.-H., E. Kabir, and S. Ara Jahan. 2016. A review on the distribution of Hg in the environment and its human health impacts. J. Hazard. Mater. 310: 278–279. doi: 10.1016/j.jhazmat.2016.02.043

King, J. K., J. E. Kostka, M. E. Frischer, and F. M. Saunders. 2000. Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. Appl. Environ. Microbiol. 66: 2430–2437. doi:10.1128/AEM.66.6.2430-2437.2000

King, J. K., J. E. Kostka, M. E. Frischer, F. M. Saunders, and R. A. Jahnke. 2001. A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria. Environ. Sci. Technol. 35: 2491–2496. doi:10.1021/es001813q

Klapstein, S. J., and N. J. O. Driscoll. 2018. Methylmercury biogeochemistry in freshwater ecosystems: a review focusing on DOM and photodemethylation. Bull. Environ. Contam. Toxicol. 100: 14–25. doi: 10.1007/s00128-017-2236-x

Kocman, D., S. J. Wilson, H. M. Amos, K. H. Telmer, F. Steenhusen, E. M. Sunderland, R. P. Mason, P. Outridge, and M. Horvat. 2017. Toward an assessment of the global inventory of present-day mercury releases to freshwater environments. Int. J. Environ. Res. Public Health 14: 138. doi:10.3390/ijerph14020138

Korthals, E. T., and M. R. Winfrey. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl. Environ. Microbiol. 53: 2397–2404.

Lamborg, C. H., and others. 2014. A global ocean inventory of anthropogenic mercury based on water column measurements. Nature 512: 65–68. doi:10.1038/nature13563

Lavoie, R. A., A. Bouffard, R. Maranger, and M. Amyot. 2018. Mercury transport and human exposure from global marine fisheries. Sci. Rep. 8: 1–9. doi:10.1038/s41598-018-24938-3

Lederer, B. M., and S. K. De Long. 2013. The effect of multiple primer-template mismatches on quantitative PCR accuracy and development of a multi-primer set assay for accurate quantification of pcaA gene sequence variants. J. Microbiol. Methods 94: 224–231. doi:10.1016/j.mimet.2013.06.013

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

Lehnherr, I., V. L. S. Louis, and J. L. Kirk. 2012. Methylmercury cycling in high arctic wetland ponds: controls on sedimentary production. Environ. Sci. Technol. 46: 10523–10531. doi: 10.1021/es300577e

Lei, P., L. M. Nunes, Y. R. Liu, H. Zhong, and K. Pan. 2019. Mechanisms of algal biomass input enhanced microbial Hg methylation in lake sediments. Environ. Int. 126: 279–288. doi:10.1016/j.envint.2019.02.043

Liem-Nguyen, V., S. Jonsson, U. Skyllberg, M. B. Nilsson, A. Andersson, E. Lundberg, and E. Björn. 2016. Effects of nutrient loading and mercury chemical speciation on the formation and degradation of methylmercury in estuarine sediment. Environ. Sci. Technol. 50: 6983–6990. doi: 10.1021/acs.est.6b01567

Liem-Nguyen, V., U. Skyllberg, and E. Björn. 2017. Thermodynamic modeling of the solubility and chemical speciation of mercury and methylmercury driven by organic thiols and micromolar sulfide concentrations in boreal wetland soils. Environ. Sci. Technol. 51: 3678–3686. doi:10.1021/acs.est.6b04622
Bravo and Cosio
Biotic formation of methylmercury

gradient. Appl. Environ. Microbiol. 80: 2874–2879. doi:10.1128/AEM.04225-13
Liu, X., A. Ma, G. Zhuang, and X. Zhuang. 2018a. Diversity of microbial communities potentially involved in mercury methylation in rice paddies surrounding typical mercury mining areas in China. Microbiology 7: e00577. doi:10.1002/mbo3.577
Liu, Y.-R., A. Johs, L. Bi, X. Lu, H.-W. Hu, D. Sun, J.-Z. He, and B. Gu. 2018b. Unraveling microbial communities associated with methylmercury production in Paddy soils. Environ. Sci. Technol. 52: 13110–13118. doi:10.1021/acs.est.8b03052
Louis, V. L. S. T., J. W. M. Rudd, C. a. Kelly, K. G. Beaty, R. J. Flett, and N. T. Roulet. 1996. Production and loss of methylmercury and loss of total mercury from boreal forest catchments containing different types of wetlands. Environ. Sci. Technol. 30: 2719–2729. doi:10.1021/es950856h
Lovley, D. R., and F. H. Chapelle. 1995. Deep subsurface microbial processes. Rev. Geophys. 33: 365–381. doi:10.1029/95rg01305
Luo, F., C. Yang, T. Sun, Y. Igarashi, X. Dai, D. Wang, M. Ma, and H. Du. 2016. Mercury-methylating genes dsrb and hgcA in soils/sediments of the three gorges reservoir. Environ. Sci. Pollut. Res. 24: 5001–5011. doi:10.1007/s11356-016-8213-9
Ma, M., H. Du, D. Wang, S. Kang, and T. Sun. 2017. Biotically mediated mercury methylation in the soils and sediments of Nam Co Lake, Tibetan plateau. Environ. Pollut. 227: 243–251. doi:10.1016/j.envpol.2017.04.037
MacMillan, G. A., C. Girard, J. Chételat, I. Laurion, and M. Amyot. 2015. High methylmercury in arctic and subarctic ponds is related to nutrient levels in the warming eastern Canadian arctic. Environ. Sci. Technol. 49: 7743–7753. doi:10.1021/acs.est.5b00763
Malcolm, E. G., J. K. Schaefer, E. B. Ekstrom, C. B. Tuit, A. Jayakumar, H. Park, B. B. Ward, and F. M. M. Morel. 2010. Mercury methylation in oxygen deficient zones of the oceans: no evidence for the predominance of anaerobes. Mar. Chem. 122: 11–19. doi:10.1016/j.marchem.2010.08.004
Mason, R. P., and W. F. Fitzgerald. 1993. The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean. Deep. Res. Part I. 40: 1897–1924. doi:10.1016/0967-0637(93)90037-4
Mason, R. P., A. L. Choi, W. F. Fitzgerald, C. R. Hammerschmidt, C. H. Lamborg, A. L. Sørensen, and E. M. Sunderland. 2012. Mercury biogeochemical cycling in the ocean and policy implications. Environ. Res. 119: 101–117. doi:10.1016/j.envres.2012.03.013
Mauro, J., J. Guimares, H. Hintelmann, C. Watras, E. Haack, and S. Coelho-Souza. 2002. Mercury methylation in macrophytes, periphyton, and water - comparative studies with stable and radio-mercury additions. Anal. Bioanal. Chem. 374: 983–989. doi:10.1007/s00216-002-1534-1
Mazrui, N. M., S. Jonsson, S. Thota, J. Zhao, and R. P. Mason. 2016. Enhanced availability of mercury bound to dissolved organic matter for methylation in marine sediments. Geochim. Cosmochim. Acta 194: 153–162. doi:10.1016/j.gca.2016.08.019
Meier, D. V., and others. 2019. Microbial metal-sulfide oxidation in inactive hydrothermal vent chimneys suggested by metagenomic and metaproteomic analyses. Environ. Microbiol. 21: 682–701. doi:10.1111/1462-2920.14514
Miller, M. B., and B. L. Bassler. 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. 55: 165–199. doi:10.1146/annurev.micro.55.1.165
Mitchell, C. P. J., and C. C. Gilmour. 2008. Methylmercury production in a Chesapeake Bay salt marsh. J. Geophys. Res. Bioge. 113: G00C04. doi:10.1029/2008jg000765
Monperrus, M., E. Tessier, D. Amouroux, and A. Leynaud. 2007. Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea. 107: 49–63. doi:10.1016/j.jmarsc.2007.01.018
Munson, K. M., C. H. Lamborg, R. M. Boitau, and M. A. Saito. 2018. Dynamic mercury methylation and demethylation in oligotrophic marine water. Biogeoosciences 15: 6451–6460. doi:10.5194/bg-15-6451-2018
Ndu, U., G. A. Christensen, N. A. Rivera, C. M. Gionfriddo, M. A. Deshusses, D. A. Elias, and H. Hsu-Kim. 2018. Quantification of mercury bioavailability for methylation using diffusive gradient in thin-film samplers. Environ. Sci. Technol. 52: 8521–8529. doi:10.1021/acs.est.8b00647
Pak, K., and R. Bartha. 1998. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. Appl. Environ. Microbiol. 64: 1–6.
Paranjape, A. R., and B. D. Hall. 2017. Recent advances in the study of mercury methylation in aquatic systems, p. 85–119. In Recent advances in the study of mercury methylation in aquatic systems. doi:10.1139/facets-2016-0027
Parks, J. M., and others 2013. The genetic basis for bacterial mercury methylation. Science 339: 1332–1335. doi:10.1126/science.1230667
Pirrone, N., and others. 2009. Global mercury emissions to the atmosphere from natural and anthropogenic sources. In Mercury fate and transport in the global atmosphere: Emissions, measurements and models. Springer doi:10.1007/s11259-009-9255-y
Poddar, M., and others. 2015. Global prevalence and distribution of genes and microorganisms involved in mercury methylation. Sci. Adv. 1: e1500675–e1500675. doi:10.1126/sciadv.1500675
Qian, C., A. Johs, H. Chen, B. F. Mann, X. Lu, P. E. Abraham, R. L. Hetisch, and B. Gu. 2016. Global proteome response to deletion of genes related to mercury methylation and
dissimilatory metal reduction reveals changes in respiratory metabolism in Geobacter sulfurreducens PCA. J. Proteome Res. 15: 3540–3549. doi:10.1021/acs.jproteome.6b00263
Ramlal, P. S., J. W. M. Rudd, and R. E. Hecky. 1986. Methods for measuring specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. Appl. Environ. Microbiol. 51: 110–114.
Ranchou-Peyruse, M., M. Monperrus, R. Bridou, R. Duran, D. Amouroux, J. C. Salvado, and R. Guynineaud. 2009. Overview of mercury methylation capacities among anaerobic bacteria including representatives of the sulphate-reducers: implications for environmental studies. Geomicrobiol. J. 26: 1–8. doi:10.1080/01490450802599227
Regnell, O., and C. J. Watras. 2019. Microbial mercury methylation in aquatic environments: A critical review of published field and laboratory studies. Environ. Sci. Technol. 53: 4–19. doi:10.1021/acs.est.8b02709
Schaefer, J. K., and F. M. M. Morel. 2009. High methylation rates of mercury bound to cysteine by Geobacter sulfurreducens. Nat. Geosci. 2: 123–126. doi:10.1038/ngeo412
Schaefer, J. K., S. S. Rocks, W. Zheng, L. Liang, B. Gu, and F. M. M. Morel. 2011. Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. Proc. Natl. Acad. Sci. U. S. A. 108: 8714–8719. doi:10.1073/pnas.1105781108
Schaefer, J. K., R.-M. Kronberg, F. M. M. Morel, and U. Skyllberg. 2014. Detection of a key Hg methylation gene, hgcA, in wetland soils. Environ. Microbiol. Rep. 6: 441–447. doi:10.1111/1758-2229.12136
Schartup, A. T., R. P. Mason, P. H. Balcom, T. A. Hollweg, and C. Y. Chen. 2013. Methylmercury production in estuarine sediments: role of organic matter. Environ. Sci. Technol. 47: 695–700. doi:10.1021/es302566w
Schartup, A. T., U. Ndu, P. H. Balcom, R. P. Mason, and E. M. Sunderland. 2015. Contrasting effects of marine and terrestrially derived dissolved organic matter on mercury speciation and bioavailability in seawater. Environ. Sci. Technol. 49: 5965–5972. doi:10.1021/es506274x
Skyllberg, U. 2008. Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: illumination of controversies and implications for MeHg net production. J. Geophys. Res. 113: 1–14. doi:10.1029/2008JG000745
Skyllberg, U., J. Qian, W. Frech, K. Xia, and W. F. Bleam. 2003. Distribution of mercury, methyl mercury and organic sulphur species in soil, soil solution and stream of a boreal forest catchment. Biogeochemistry 64: 53–76. doi:10.1023/A:1024904502633
Skyllberg, U., P. R. Bloom, J. Qian, C.-M. Lin, and W. F. Bleam. 2006. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. Environ. Sci. Technol. 40: 4174–4180. doi:10.1021/es0600577
Smith, S. D., and others. 2015. Site-directed mutagenesis of hgcA and hgcB reveals amino acid residues important for mercury methylation. Appl. Environ. Microbiol. 81: 3205–3217. doi:10.1128/AEM.00217-15
Sunderland, E. M., D. P. Krabbenhoft, J. W. Moreau, S. A. Strode, and W. M. Landing. 2009. Mercury sources, distribution, and bioavailability in the North Pacific Ocean: insights from data and models. Global Biogeochem. Cycles 23. doi:10.1029/2008GB003425
Sundseth, K., J. Pacyna, A. Banel, E. Pacyna, and A. Rautio. 2015. Climate change impacts on environmental and human exposure to mercury in the Arctic. Int. J. Environ. Res. Public Health 12: 3579–3599. doi:10.3390/ijerph120413579
Tjerngren, I., M. Meili, E. Björn, and U. Skyllberg. 2012. Eight boreal wetlands as sources and sinks for methyl mercury in relation to soil acidity, C/N ratio, and small-scale flooding. Environ. Sci. Technol. 46: 8052–8060. doi:10.1021/es300845x
Ullrich, S. M., T. W. Tanton, S. A. Abdashitova, and A. Svetlana. 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit. Rev. Environ. Sci. Technol. 31: 241–293. doi:10.1080/20016491089226
UNEP. 2013. Global mercury assessment 2013: Sources, emissions, releases, and environmental transport. UNEP 42. doi: DTI/1636/GE
Villar, E., L. Cabrol, and L.-E. Heimbürger-Boavida. 2019. Widespread microbial mercury methylation genes in the global ocean. bioRxiv. doi:10.1101/648329
Vishnivetskaya, T. A., and others. 2018. Microbial community structure with trends in methylation gene diversity and abundance in mercury-contaminated rice paddy soils in Guizhou, China. Environ. Sci. Process. Impacts 20: 673–685. doi:10.1039/C7EM00558J
vonk, J. W., and A. K. Sijpsteijn. 1973. Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. In Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. Antonie Van Leeuwenhoek. doi:10.1007/BF02578894
Weber, K. A., L. A. Achenbach, and J. D. Coates. 2006. Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction. Nat. Rev. Microbiol. 4: 752–764. doi:10.1038/nrmicro1490
Windham-Myers, L., and others. 2014. Mercury cycling in agricultural and managed wetlands: A synthesis of methylmercury production, hydrologic export, and bioaccumulation from an integrated field study. Sci. Total Environ. 484: 221–231. doi:10.1016/j.scitotenv.2014.01.033
Xu, J., and others. 2019. Mercury methylating microbial communities of boreal forest soils. Sci. Rep. 9: 518. doi:10.1038/s41598-018-37383-z
Yu, R. Q., J. R. Flanders, E. E. MacK, R. Turner, M. B. Mirza, and T. Barkay. 2012. Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in
freshwater river sediments. Environ. Sci. Technol. 46: 2684–2691. doi:10.1021/es2033718
Yu, R.-Q., J. R. Reinfelder, M. E. Hines, and T. Barkay. 2013. Mercury methylation by the methanogen Methanospirillum hungatei. Appl. Environ. Microbiol. 79: 6325–6330. doi:10.1128/AEM.01556-13
Yu, R. Q., J. R. Reinfelder, M. E. Hines, and T. Barkay. 2018. Syntrophic pathways for microbial mercury methylation. ISME J. 12: 1826–1835. doi:10.1038/s41396-018-0106-0

Acknowledgments
Authors thank Prof. Jeffra Schaeffer (Rutger University, USA) for critical reading of the draft.

AGB acknowledges the European Commission, for the MER-CURE (Using global marine metagenomics to understand MERCury microbial associated processes: finding a CURE for mercury contaminated environments), an individual Fellowship of the Marie Skłodowska-Curie Actions.

Conflict of Interest
None declared.

Submitted 09 April 2019
Revised 17 September 2019
Accepted 02 October 2019
Associate editor: Vanessa Hatje