

Author Response

Dear Reviewer,
Thank you very much for reviewing the manuscript and for your invaluable input. Below, we have posted your comments and suggestions in grey boxes, with our responses highlighted in blue boxes and proposed manuscript changes shown in red boxes. We hope you find our proposed revisions appropriate and agree with the suggested changes to the manuscript.

Reviewer Comment

The manuscript addresses a significant and persistent anomaly in marine ecotoxicology: the observation that marine sponges, particularly those with high microbial abundance, accumulate high concentrations of inorganic Hg while maintaining unexpectedly low levels of MeHg. The authors employ a coupled 1D hydrodynamic-biogeochemical model to test the hypothesis that trophic dilution via DOM consumption, rather than microbial mediated MeHg demethylation, drives these patterns. Furthermore, the study posits a potentially massive ecosystem service, suggesting that sponge grounds could reduce MeHg bioaccumulation in benthic fish by up to 45%. The overall manuscript is well organized, and a minor revision is needed. My specific comments are as below:

Reviewer Comment

Title: MeHg demethylation

Author Response

We suggest to update the title as below:

Suggested edit

DOM consumption and demethylation of MeHg as potential drivers of low MeHg in Mediterranean Sea sponges and benthic fish: a modelling perspective

Reviewer Comment

Abstract: The second sentence is too long. The complex role of sponges and their significance should be more clearly emphasized.

Reviewer Comment

Line 6: demethylation of MeHg

Reviewer Comment

Line 11-12: This conclusion is overly speculative and requires additional supporting evidence.

Author Response

We would update the abstract as posted below to take above 3 points into account. We suggest to rewrite the first 3 sentences to explain why we think sponges might play an important role in MeHg bioaccumulation. Additionally, we suggest we remove the statement about sulfated polysaccharides from the abstract. It is an interesting point for

the discussion indeed but lacks the supporting evidence to be placed in the abstract.

Suggested edit

Methylmercury (MeHg) is a bioaccumulative neurotoxin that poses a risk to human health through seafood consumption. Sponges have unique mercury (Hg) profiles. Measurements show an unusually high inorganic Hg (iHg) content in Low Microbial Abundance (LMA) sponges and an even higher iHg content in High Microbial Abundance (HMA) sponges, while MeHg concentrations remain low, particularly in HMA sponges. Combined with the recently improved understanding of the important ecological role of sponges as a food source for other biota, suggests their low MeHg content may influence MeHg transfer within benthic food webs. In this study, we used a 1D water-column model to investigate the bioaccumulation of MeHg in sponges. It has been hypothesized that the low MeHg content in HMA sponges may result from active MeHg demethylation. Our model results indicate that the consumption of dissolved organic matter (DOM) can already explain the low observed MeHg content in LMA sponges, and higher DOM consumption in HMA sponges can account for the even lower MeHg levels in HMA species. Alternatively, if MeHg demethylation occurs, a low rate of 1% per day could explain the differences between LMA and HMA sponges. Although DOM consumption increases iHg bioaccumulation in both sponge types, it does not explain the extremely high iHg concentrations observed. Finally, our model suggests that HMA sponges could potentially reduce MeHg concentrations in benthic fish by up to 45% when they dominate at the base of the food web. These findings highlight the potentially important role of sponges in Hg cycling and indicate that sponge-dominated systems could help reduce MeHg accumulation in benthic food webs.

Reviewer Comment

Lines 17-24: Links between sponges Hg and fish Hg are needed here.

Author Response

I would suggest to add the following statement

Suggested edit

MeHg bioaccumulation in marine food webs is primarily driven by basal concentrations and trophic position, as shown by consistent trophic magnification slopes across ecosystems (Lavoie et al., 2013). Since sponges are actively consumed by higher trophic levels and can dominate benthic biomass (Mortimer et al., 2021), unique MeHg accumulation patterns in sponges compared to other megabenthos could influence seafood contamination levels.

Reviewer Comment

Line 36: ?

Author Response

Apology that was a reference mistake which we overlooked. Corrected it to (Hao et al., 2022).

Reviewer Comment

Lines 33-43: This section is overly general and would benefit from greater conciseness.

Author Response

We would suggest to shorten and rewrite that section as below.

Suggested edit

The primary pathway of MeHg bioaccumulation in aquatic ecosystems is direct uptake from the water column via respiration, diffusion, or ingestion. When this uptake results in organism concentrations exceeding those in surrounding water, the process is termed bioconcentration. This occurs because MeHg binds strongly to organic matter, particularly thiol (-SH) groups in proteins (Hao et al., 2022), increasing internal concentrations by 10^5 – 10^6 -fold relative to water (Lee & Fisher, 2016). Bioconcentration is surface-area dependent and thus especially efficient in small organisms, making it critical at the food web base, such as phytoplankton (Mason et al., 1995). Efficient trophic transfer of bioconcentrated MeHg results in increasing concentrations with trophic position, a process known as biomagnification. This typically increases MeHg bioaccumulation 3–10-fold per trophic level (Lavoie et al., 2013; Mason et al., 1996). This drives high MeHg levels in top predators, including human-consumed seafood.

Reviewer Comment

Lines 45-48: In the case of inorganic mercury (iHg), iHg-DOM binding determines the bioavailability of iHg for methylation.

Author Response

We suggest to add the following statement to line 48 to include that.

Suggested edit

Dissolved organic matter (DOM) binding to MeHg varies by source: marine DOM enhances phytoplankton MeHg uptake by facilitating active transport through membrane channels (Garcia-Arevalo et al., 2024; Schartup et al., 2015), whereas terrestrial DOM inhibits uptake of MeHg by biota due to stronger thiol binding (Seelen et al., 2023). DOM also binds iHg, influencing its speciation and bioavailability for microbial methylation (Graham et al., 2012).

Reviewer Comment

Section2: In this section, the role of sponges in Hg cycling and MeHg accumulation in biota should be clearly highlighted. This is important and helps readers understand the significance of this study.

Author Response

We suggest to add the following addition to the current section 1.2 (starting at line 96). This helps highlight why we think the unique bioaccumulation patterns of sponges are significant and should be studied.

Suggested edit

Most ecosystems rely on phytoplankton as the base of the food web, meaning that MeHg bioaccumulation can often be estimated from trophic level and phytoplankton MeHg concentrations. Sponge-dominated systems differ in that sponges can efficiently utilize DOM as a food source and exhibit extremely low MeHg concentrations compared to other megabenthos (Orani et al., 2020). This is important because the main predictor of MeHg concentrations in high trophic levels is the MeHg concentration at the base of the food web and the trophic level itself (Lavoie et al., 2013; Wu et al., 2019). Because sponges can form the majority of the benthic biomass in sponge reefs and are commonly predated upon, the MeHg content of sponges is likely directly linked to the MeHg content of higher trophic level animals, although this relationship remains understudied. We therefore hypothesize that the low MeHg concentrations in sponges lead to reduced MeHg bioaccumulation in high trophic level animals inhabiting sponge grounds compared to animals of similar trophic position in phytoplankton-based ecosystems.

Reviewer Comment

Section 1.3: Why DOM consumption can result in low MeHg concentrations?

Author Response

We agree that this is underexplained. We suggest to update the sentence at line 86 to clarify that.

Suggested edit

An alternative explanation for the low MeHg concentrations observed in HMA sponges is that these low levels result from the consumption of dissolved organic matter (DOM). This mechanism was proposed in (Amptmeijer et al., 2025), which presents a modelling study examining how sponge feeding strategies influence iHg and MeHg dynamics. In that study, the partitioning of iHg and MeHg to DOM follows the implementation of the the MERCY v2.0 model (Bieser et al., 2023), based on the emperical research of (Tesán Onrubia et al., 2020).

While MeHg is efficiently bioaccumulated by biota such as phytoplankton, this is not the case for iHg, resulting in higher MeHg than iHg concentrations in phytoplankton relative to seawater (Garcia-Arevalo et al., 2024). For DOM, however, this relationship is not present. Rather, the efficient partitioning of iHg to DOM, combined with the higher dissolved concentration of iHg compared to MeHg, produces DOM with high iHg and low MeHg levels. When suspension-feeding sponges consume DOM in the model of Amptmeijer et al., 2025, they therefore accumulate elevated iHg and very low MeHg as a direct consequence of the DOM composition.

It is important to note that the feeding strategy in that model is considered in isolation, and biological processes that may further influence bioaccumulation such as *in vivo* demethylation of MeHg, the long lifespan of sponges, and their low metabolic rates are not included.

Author Response

Additionally we suggest to add this to section to the discussion

Suggested edit

The difference in bioaccumulation of iHg and MeHg highlights mercury as a unique pollutant. Certain biological pathways, such as the direct absorption of MeHg via membrane channels in phytoplankton, enable highly efficient MeHg bioaccumulation. As a result, MeHg concentrations in biota can exceed iHg concentrations, even in primary producers, despite dissolved iHg being more abundant than MeHg. In DOM, which primarily consists of dead organic particles, these preferential uptake pathways are absent, and DOM binds iHg and MeHg only through scavenging. DOM composition strongly influences its binding capacity: thiol-rich fractions of terrestrial DOM efficiently bind MeHg (Seelen et al., 2023), whereas marine DOM fractions show a higher affinity for iHg (Tesán Onrubia et al., 2020), which underpins the partitioning behavior used in the MERCY V2.0 model Bieser et al., 2023.

The lack of efficient MeHg uptake mechanisms, combined with low dissolved MeHg concentrations, results in low MeHg bound to DOM in our model, while iHg is higher due to the efficient scavenging of iHg by DOM and the higher dissolved concentration of iHg than MeHg. Consequently, when HMA sponges feed on DOM, they accumulate very low MeHg concentrations, while, on the other hand, the efficient partitioning of iHg to DOM results in elevated iHg levels, even though iHg is not typically transferred via diet very efficiently.

Reviewer Comment

Lines 567-570: Yes, The validation of the model relies on a limited sample sizes ($n=4$ for HMA and $n=6$ for LMA sponges). The use of Kolmogorov-Smirnov (KS) tests on such sparse data provides a misleading sense of statistical robustness, as the test lacks the power to reject the null hypothesis, thereby creating a "false positive" agreement between model and observation. The author should strengthen the discussion by explicitly addressing how the limited sample size may influence the validity and generalizability of the findings, rather than simply noting the limitation.

Author Response

Thank you for this excellent suggestions. We suggest to rewrite the section at line 321 to the text as below to better explains how the limited sample size affects the results.

Suggested edit

There is a large difference in sample sizes, with 3,652 model data points compared to only 4 (HMA) and 6 (LMA) observations. This imbalance reduces the power of the KS and Wilcoxon tests, making them especially susceptible to false-negative outcomes. This means that high p-values cannot confirm model fit, whereas low p-values (<0.05) are more reliable indicators of a mismatch between the model and observations.

This limitation is particularly relevant for the Kolmogorov–Smirnov test, as it evaluates the distribution. With only 4 and 6 data points, the observational distribution cannot be robustly defined, but significantly low (<0.05) p-values still strongly indicate a mismatch between the modeled distribution and the limited observations.

Therefore, these metrics are mainly used to compare which setup performs better relative to other setups and to flag poor-performing configurations. However, they cannot be used to fully determine whether the model simulations are in agreement with observations due to the limited number of observations.

Author Response

And we suggest to expand the statement at line 567 to further mention how more data can help improve the model.

Suggested edit

Beyond the limitations of the rDOM implementation, several major limitations affect the model. The most important is the low data availability. Our conclusions rely on a single study of MeHg and iHg bioaccumulation in Mediterranean sponges, with small sample sizes ($n = 4$ for HMA and $n = 6$ for LMA sponges). While the model–data comparison allows identification of clear mismatches between the model and observations, the limited number of observations prevents reliable validation of simulated Hg bioaccumulation patterns. Therefore, the agreement between model simulations and observations should be interpreted with care.

In addition, key processes remain poorly mechanistically understood. The mechanisms and rates of (r)DOM consumption by sponges, the role of sponges in ecosystem-level Hg bioaccumulation, and the functional differences between LMA and HMA sponges are not fully understood. These knowledge gaps limit process-based parameterization and increase structural uncertainty in the model.

Because of this, targeted empirical studies on Hg bioaccumulation in sponges, including controlled experiments focused on the bioaccumulation pathways that were flagged as potentially significant in this model, such as the role of the uptake of DOM by sponges on Hg bioaccumulation and the consumption and trophic transfer of Hg of sponges by predators, could substantially improve model development by enabling more robust validation.

References

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