Methylmercury production below the mixed layer in the North Pacific Ocean

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Mercury enters marine food webs in the form of microbially generated monomethylmercury. Microbial methylation of inorganic mercury, generating monomethylmercury, is widespread in low-oxygen coastal sediments. The degree to which microbes also methylate mercury in the open ocean has remained uncertain, however. Here, we present measurements of the stable isotopic composition of mercury in nine species of marine fish that feed at different depths in the central North Pacific Subtropical Gyre. We document a systematic decline in $\delta^{202}Hg$, $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values with the depth at which fish feed. We show that these mercury isotope trends can be explained only if monomethylmercury is produced below the surface mixed layer, including in the underlying oxygen minimum zone, that is, between 50 and more than 400 m depth. Specifically, we estimate that about 20–40% of the monomethylmercury detected below the surface mixed layer originates from the surface and enters deeper waters either attached to sinking particles, or in zooplankton and micronekton that migrate to depth. We suggest that the remaining monomethylmercury found at depth is produced below the surface mixed layer by methylating microbes that live on sinking particles. We suggest that microbial production of monomethylmercury below the surface mixed later contributes significantly to anthropogenic mercury uptake into marine food webs.

Mercury (Hg) is a globally distributed atmospheric pollutant that can form monomethyl-Hg (MMHg), which is neurotoxic and bioaccumulative in aquatic food webs. The main pathway for human exposure to MMHg is through consumption of piscivorous marine fish[1]. In the ocean Hg primarily exists as inorganic Hg(II) or Hg(0)$_{aq}$, but microbial reactions can methylate Hg(II) to MMHg and dimethyl-Hg (DMHg), and microbial and photochemical processes can degrade MMHg and DMHg back to Hg(II) or Hg(0)$_{aq}$ (ref. 2). In the North Pacific Ocean these processes result in nutrient-like vertical concentration profiles for methylated mercury, with very low concentrations in open ocean surface waters and higher concentrations in subthermocline low-O_2 deeper intermediate waters[3,4]. Similar profiles have also been documented in the Equatorial Pacific, Mediterranean Sea and Southern Ocean[5–7]. In agreement with these vertical profiles, total Hg (THg) levels in commercially important predatory pelagic fish and their prey increase with median depth of occurrence[8,9], which is indicative of both foraging depth and habitat utilization. However, the biogeochemical factors controlling the distribution and speciation of Hg in the ocean and the bioaccumulation of MMHg in marine food webs are still not well understood[10].

Microbial activity and both dark and photochemical inorganic reactions ultimately control the chemical speciation and subsequent bioavailability of Hg (ref. 2), thus an understanding of the competing processes that produce and degrade MMHg at different depths in the ocean is critical to tracing its bioaccumulation in fish and biomagnification in marine food webs. MMHg typically comprises only a small fraction of THg in North Pacific sea water (<15%; ref. 4) but nearly all of the Hg (>95%) in large marine fish[2,11] and most of the Hg (>80%) in small fish with trophic position (TP) as low as 2.5 (ref. 12). DMHg concentrations are generally small relative to MMHg (refs 3,4,13), although at some

marine locations DMHg can be a significant fraction of MMHg (refs 13,14). As DMHg is not bioaccumulated and because its Hg isotopic composition has not been measured, it is not treated in detail here. MMHg production in the oxygen minimum zone (OMZ) of the oceanic water column has been hypothesized[7,13] and recent field studies have offered new evidence supporting *in situ* methylation not only in OMZs (refs 3,6) but also in oxic surface waters[4,5,15–17]. Vertical differences in ocean biogeochemistry and the relative contributions of different Hg sources to the overall MMHg burdens in fish have wide-ranging implications for understanding how MMHg levels in marine biota will respond to future changes in anthropogenic Hg emissions[3,18].

Mercury isotope ratios can provide insight into the biogeochemical cycling of Hg (refs 19,20). Mercury displays mass-dependent fractionation (MDF; $\delta^{202}Hg$) during all biotic and abiotic chemical reactions that have been investigated and large magnitude mass-independent fractionation (MIF; $\Delta^{199}Hg$, $\Delta^{201}Hg$) during photochemical radical pair reactions (see Methods for definition of $\delta^{202}Hg$, $\Delta^{199}Hg$ and $\Delta^{201}Hg$). The combination of Hg MDF and MIF provides a probe into the reaction pathways and biogeochemical history of Hg and can also provide a fingerprint of sources of Hg to ecosystems[19–22]. Coupling estimates of TP with Hg concentrations can allow for an understanding of MMHg biomagnification in marine food webs[5,21].

Here we measured the Hg isotopic compositions and established TPs of pelagic fish from the North Pacific Subtropical Gyre (NPSG) near Hawaii, for which Hg concentration and median depth of occurrence were known (Supplementary Tables S1 and S2)[8]. We used amino acid compound-specific N isotope analyses (AA-CSIA) to establish organism TP because this technique provides information on time-integrated TP independent of the $\delta^{15}N$ values at the base of the food web[23]. Our aim was to use MDF ($\delta^{202}Hg$) and MIF ($\Delta^{199}Hg$, $\Delta^{201}Hg$) of Hg in a suite of fish feeding at

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different depths and across different TPs to test hypotheses for the depth of microbial Hg methylation, and both microbial and photochemical Hg demethylation, before introduction into the pelagic marine food web.

## Species sampled and their depth of occurrence

We analysed samples of muscle tissue from either three or four individuals from each of nine species of fish (six high-TP predatory species, three low-TP prey species; Supplementary Table S1 for species names and data on core depth ranges and median depths of occurrence) collected in the central NPSG near Hawaii and previously analysed for THg concentrations[8]. Electronic tagging and trawling studies provide very useful information on vertical habitat utilization. Median depth of occurrence based on core day and night depth ranges taken from the literature for the species we sampled range from 10 to 590 m. Accurately estimating depth of forage is difficult due to complexities in daily vertical migrations, periodic deep dives by some species and vertical movements of prey, among other factors. Nonetheless it is our view that the best approach to represent the range of habitats (and probable foraging depth ranges) used by these species is the median depth of occurrence.

Mercury isotope variation in Pacific ocean fish

Irrespective of the complexities in estimates of mean depths of occurrence mentioned above, we observe a systematic decrease in $\delta^{202}Hg$, $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values with median depth (Fig. 1a,b and Supplementary Tables S1 and S2). Mean THg concentrations in muscle tissue range from 16.9 ng g^{-1} (wet weight) in flying fish to 1,586 ng g^{-1} (wet weight) in swordfish. The shallowest occurring fish (flying fish) have average Hg isotope values of $\delta^{202}Hg = 1.5‰$, $\Delta^{199}Hg = 5.3‰$ and $\Delta^{201}Hg = 4.3‰$; much higher than values for the deepest occurring fish (lanternfish-distofax), which average $\delta^{202}Hg = 0.2‰$, $\Delta^{199}Hg = 1.5‰$ and $\Delta^{201}Hg = 1.2‰$.

Laboratory experiments reveal that microbial methylation produces MMHg with $\delta^{202}Hg$ values lower than that of the reactant Hg(II) (ref. 24) and microbial demethylation leaves residual MMHg with higher $\delta^{202}Hg$ values than reactant MMHg (ref. 25). Furthermore, microbial reactions do not produce changes in values of $\Delta^{199}Hg$ or $\Delta^{201}Hg$ (ref. 25). In contrast, photochemical degradation of MMHg in fresh water complexed with natural dissolved organic carbon (DOC) causes increases in $\delta^{202}Hg$, $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values in the residual MMHg with an experimentally derived $\Delta^{199}Hg/\delta^{202}Hg$ ratio of 2.43 ± 0.10 and $\Delta^{199}Hg/\Delta^{201}Hg$ ratio of 1.34 ± 0.04 (ref. 20). Photochemical reduction of Hg(II) in fresh water complexed with natural DOC yields residual Hg(II) with an experimentally derived $\Delta^{199}Hg/\delta^{202}Hg$ ratio of 1.15 ± 0.07 and $\Delta^{199}Hg/\Delta^{201}Hg$ ratio of 1.00 ± 0.01 (ref. 20). Published experiments have not been carried out in sea water or at marine MMHg/DOC ratios, but those in fresh water are adequate to guide the general interpretation of isotopic trends observed in natural systems[20–22,26–30].

Although photochemical degradation of MMHg is an important process in the upper ocean[16,21], degradation by microbes may also be important[25]. Our measured trends of Hg isotope values in fish can be used to test whether microbial or photochemical degradation of MMHg is the dominant process affecting MMHg before it enters the food web. The $\Delta^{199}Hg/\delta^{202}Hg$ ratio in the fish we analysed is 2.64 ± 0.24 (Fig. 2a), close to the experimentally derived ratio of 2.43 ± 0.10 for photochemical degradation[20] and the data fall on a linear trend. If the dominant demethylation process was microbial we would expect a slope not statistically different from 0 and if both processes were important we would expect a slope intermediate between 0 and 2.43, with considerable scatter if the relative importance of these processes changed with water depth. We estimate based on experimentally derived fractionation
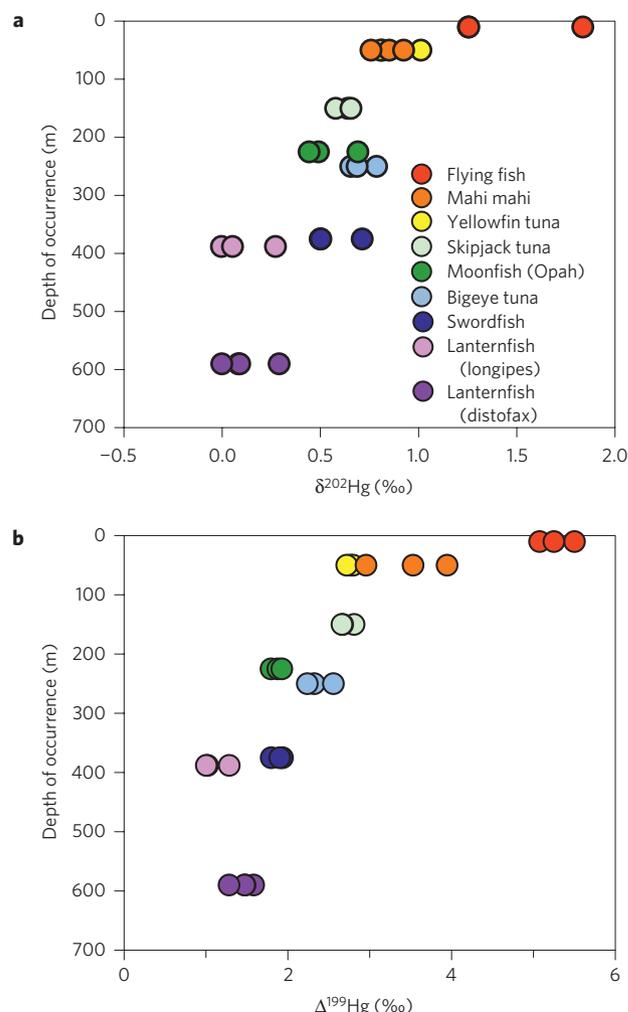
Figure 1 | Mercury isotope diagrams illustrating MDF and MIF variation in fish with depth. a,b, $\delta^{202}Hg$ (**a**) and $\Delta^{199}Hg$ (**b**) values (in permil) for fish from the NPSG near Hawaii plotted as a function of median depth of occurrence (in metres) for six species of predatory pelagic fish, as well as surface-dwelling flying fish and two species of lanternfish that undergo diel vertical migration.

factors that the deviation of isotopic compositions from the linear trend on Fig. 2a can accommodate a maximum of ~15% microbial demethylation[25,31], with most owing to photochemical degradation. As the linear array of data on the plot of $\delta^{202}Hg$ versus $\Delta^{199}Hg$ is caused primarily by photochemical degradation, the regressed line should pass through the isotopic composition of MMHg that occurred before marine photochemical degradation. On Fig. 2a we also plot the range of measured Hg isotopic compositions in precipitation on the west coasts of California[32] and Florida[33] (which reveal only a small amount of atmospheric photochemical MIF). If the $\delta^{202}Hg$ values of Hg(II) in precipitation were decreased by ~0.5‰ during microbial methylation (as observed in laboratory experiments[24]) they would be compatible with the predicted values for marine MMHg before any photochemical degradation (Fig. 2a).

The $\Delta^{199}Hg/\Delta^{201}Hg$ ratio can be diagnostic of reactions that cause Hg MIF. $\Delta^{201}Hg$ is highly correlated with $\Delta^{199}Hg$ for all marine fish that have been analysed including those from the Gulf of Mexico[21], San Francisco Bay estuary[30] and the NPSG (this study), with a slope of 1.20 ± 0.01 (Fig. 2b). These field results suggest that photochemical degradation mechanisms are similar
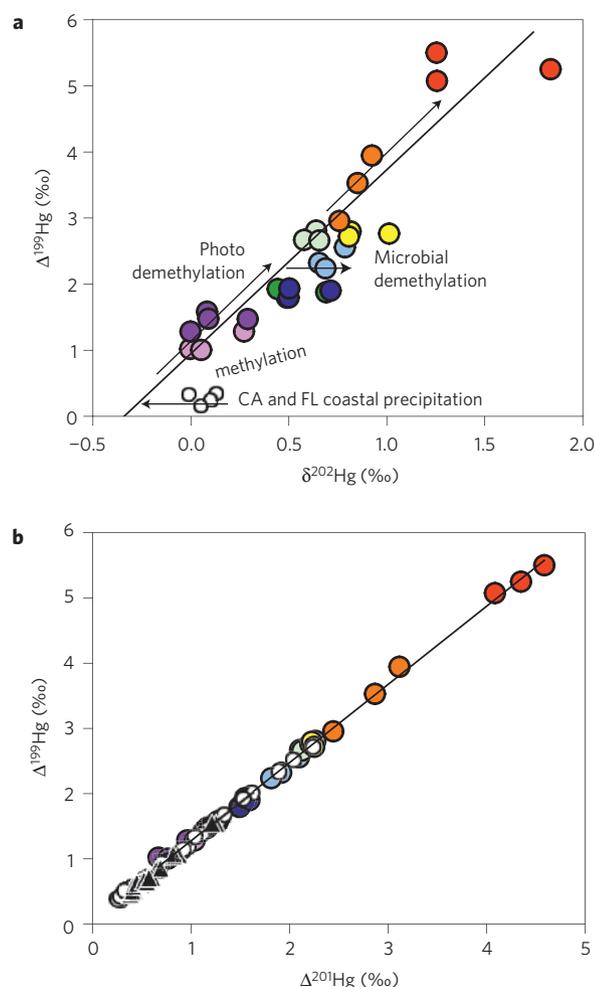
Figure 2 | Mercury isotope diagrams illustrating correlations between MIF and MDF and between MIF of Δ^{199}Hg and MIF of Δ^{201}Hg. a,b, δ^{202}Hg versus Δ^{199}Hg values (**a**) and Δ^{201}Hg versus Δ^{199}Hg values (**b**) for the marine fish shown in Fig. 1 using the same symbols as in Fig. 1. Also shown in **a** are values for δ^{202}Hg and Δ^{199}Hg in coastal precipitation (white circles) from California[32] and Florida[33], and the estimated shift in values due to microbial methylation[24] before photochemical degradation, and due to ~15% microbial demethylation[25] following photochemical degradation (black arrows). Also plotted in **b** are marine fish from the Gulf of Mexico[21] (white circles) and the San Francisco estuary[30] (triangles).

at these diverse marine sites, which include coastal and open ocean environments. In comparison, the Δ^{199}Hg/Δ^{201}Hg ratio obtained in MMHg photochemical degradation experiments (with 1 mg l^{-1} freshwater DOC) was 1.36 ± 0.04 (ref. 20). Analyses of fish from lakes yield a wider range of Δ^{199}Hg/Δ^{201}Hg (0.99–1.33; refs 20,26–29), but most are ~1.3. Investigations have shown that variation in the Δ^{199}Hg/Δ^{201}Hg ratio during photochemical reduction of Hg(II) can depend on the organic ligand to which Hg(II) is bonded[34]. It seems likely that a similar phenomenon occurs with MMHg and that this explains the slightly higher experimentally derived Δ^{199}Hg/Δ^{201}Hg ratio compared with what is observed in marine fish.

The high Δ^{199}Hg (and Δ^{201}Hg and δ^{202}Hg) values in surface-foraging fish and the decreases in all three isotope values with increasing fish depth of occurrence clearly supports the hypothesis that MMHg at shallower depths undergoes a greater extent of photochemical degradation before introduction into the marine food web compared with MMHg at greater depths (Figs 1–2).

Mapping observed Δ^{199}Hg values to experimental data[20] and to a proposed extrapolation of these data to the MMHg/DOC ratio of North Pacific surface water[22] suggests that these fish preserve the isotopic composition of residual MMHg that has undergone between 46 and 80% photochemical degradation at a mean depth of 10 m and between 9 and 21% photochemical degradation at a depth of 590 m (Methods). Estimates of percentage photochemical degradation could be even higher for lower TP fish, which may have up to 20% inorganic Hg (ref. 12), but this does not change our interpretation of the data appreciably.

We investigated the effects of biomagnification on Hg concentration and isotopic compositions using our estimated TPs for the fish (Supplementary Table S3). There is a significant positive correlation between TP and THg ($R^2 = 0.78$), but there is not a significant correlation between TP and Δ^{199}Hg values ($R^2 = 0.079$) or δ^{202}Hg values ($R^2 = 0.003$; data given in Supplementary Tables S2 and S3). This is consistent with the conclusion of a previous study[35] that trophic transfer does not result in appreciable isotopic fractionation of Hg and demonstrates that, although feeding ecology can be an important factor controlling marine fish Hg levels, trophic transfer does not significantly affect Hg isotope values.

Implications for Hg biogeochemical cycling in the ocean

Our measurement of the depth dependence of the Hg isotopic composition of fish from the NPSG has implications for models of MMHg production and degradation in the open ocean. The trends in δ^{202}Hg and Δ^{199}Hg values (Fig. 1) cannot be explained by production of MMHg exclusively in the photic zone, deposition of MMHg from the atmosphere, or lateral advection of MMHg from coastal regions. In these cases we would not expect to see sharp decreases with depth in Δ^{199}Hg and Δ^{201}Hg values in fish, because there is no known mechanism in aquatic systems to erase the MIF signal imparted by photochemical degradation in the photic zone, except through dilution by newly formed MMHg at depth. Instead, the Hg isotope data require that methylation occurs below the surface mixed layer (SML), in agreement with a previous study of MMHg/P ratios in open Mediterranean waters[6].

Photochemical demethylation leaves a residual pool of unreacted MMHg with increased δ^{202}Hg, Δ^{199}Hg and Δ^{201}Hg values, which is available for bioaccumulation. Δ^{199}Hg and Δ^{201}Hg values can be lowered only by addition of MMHg that has undergone very little or no photochemical degradation before bioaccumulation. Therefore, if all MMHg was formed in the SML it would be expected to show high positive values of Δ^{199}Hg and Δ^{201}Hg owing to exposure to sunlight in the photic zone, and this signature would be retained in Hg exported to greater depths. Decline of Δ^{199}Hg values with depth must, therefore, be a consequence of MMHg production below the SML, probably on sinking particles of organic matter[3,7,17], which does not undergo photochemical degradation and mixes with residual photodegraded MMHg exported to depth from the SML either attached to sinking particles or by zooplankton and micronekton that undergo diel vertical migration. Fish from 590 m depth preserve a highly attenuated, but still clearly identifiable, MIF signature attributable to photochemical demethylation. A simplified depiction of our model for Hg deposition, methylation, photodemethylation and incorporation into the marine food web is shown in Fig. 3.

Δ^{199}Hg values of MMHg in the deep ocean (~590 m) food web can be explained using a simple mixing calculation as a combination of 20–40% MMHg from the SML foodweb and 60–80% MMHg formed below the SML in the pycnocline (Fig. 3). Published depth profiles of MMHg from the central Pacific[3,4] show increases with depth from ~20 to 50 fM near the surface to ~40 to 400 fM at 600 m depth. Our results indicate that between 46 and 80% of
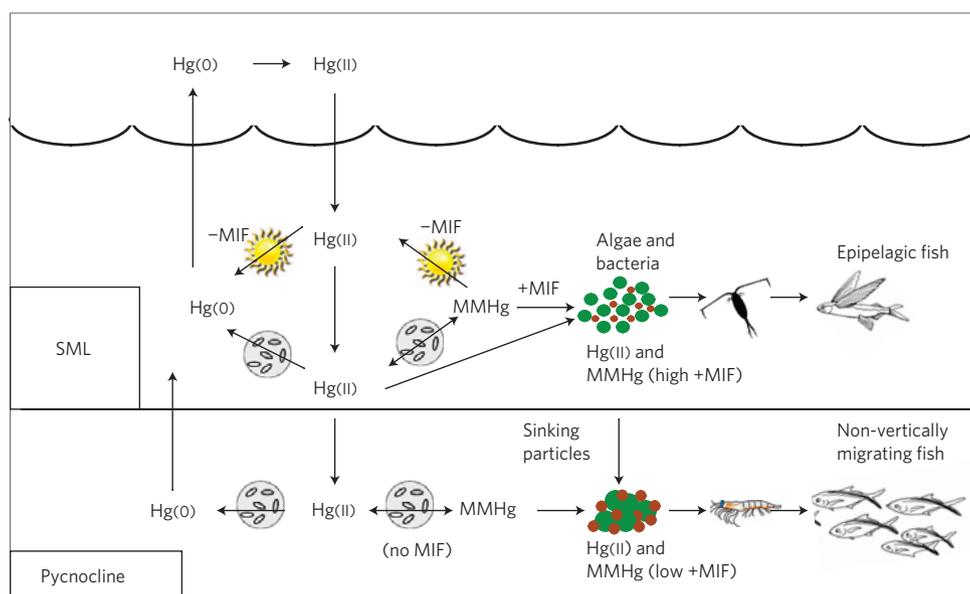
Figure 3 | Schematic representation of a simplified model for Hg cycling between atmosphere, SML, pycnocline and marine food web. Hg(II) is deposited from precipitation to the ocean surface, where some is photochemically reduced to Hg(0), some is microbially reduced, some is microbially methylated to MMHg and some is transported to greater depths attached to particles. Photochemical degradation of MMHg in the SML leaves a residual MMHg pool with high positive values of MIF that is propagated through the shallow-water food web to epipelagic fish. Sinking particles carry MMHg with MIF and Hg(II) without significant MIF to the pycnocline where additional microbial methylation occurs, producing additional MMHg without MIF, which dilutes the MIF in MMHg exported from the SML, resulting in MMHg with lower positive values of MIF. Thus MMHg with lower MIF is incorporated into the mesopelagic food web and therefore to deeper foraging fish.

MMHg produced in the photic zone is photodegraded there and that 60–80% of MMHg bioaccumulated at 590 m is produced below the SML. Thus the observed MMHg vertical concentration gradient could primarily be attributed to increased photochemical degradation near the surface. There is supporting evidence from MMHg mass balance calculations for production of significant amounts of MMHg in the mixed layer. It has been suggested[4,10] that the vertical flux of MMHg in the North Pacific is small compared with rates of bioaccumulation and photochemical degradation; in agreement with the Hg isotopic results, horizontal transport of MMHg by surface currents is considered to be minor[10]. MMHg concentration profiles between the SML and ∼600 m were used to model the vertical flux of MMHg to surface water and estimated rates of bioaccumulation and of photochemical degradation of MMHg in surface waters greatly exceeded this vertical flux[4]. This conclusion is consistent with our Hg isotopic data that suggest MMHg production in the mixed layer and down to a depth of 590 m within the OMZ (>400 m).

It is unknown how the balance of increased fossil fuel use and improving emissions control technologies will affect future anthropogenic Hg emissions. But it has been suggested that Hg concentrations in surface and shallow subsurface waters of the Pacific Ocean will change significantly over the next few decades[3,10]. For example, it has been predicted[3] that the concentration of THg mercury in North Pacific intermediate waters (200–1,000 m depth) will double by the year 2050, relative to 1995, assuming contemporary atmospheric Hg deposition rates. Increases in Hg concentrations in the SML of the North Pacific are more difficult to predict because they are sensitive to regionally variable meteorological and chemical factors that control the oxidation of Hg(0) and the transport and deposition of Hg(II) (ref. 3). The extent to which atmospheric deposition of anthropogenic Hg may impact marine fisheries will depend in part on the depth(s) at which inorganic Hg is converted to organic Hg and at which MMHg enters the food web and subsequently bioaccumulates. Moreover,

in the Pacific (and other oceans), OMZs are expanding[36,37]. Understanding Hg biogeochemical cycling in these environments is crucial for the prediction of potential consequences for marine ecosystems. We suggest that, because of photochemical degradation in the mixed layer, the greatest input of MMHg to food webs occurs below the mixed layer and results in the observed increases in Hg concentration of high-trophic-level fish from the mixed layer down to the OMZ.

Methods

Hg concentrations. Total Hg levels in homogenized freeze-dried samples were measured by thermal decomposition, gold amalgamation and atomic absorption spectroscopy (Milestone, DMA-80) as described in US Environmental Protection Agency Method 7473. One in every ten samples was analysed in replicate and the lower limit of quantification was ∼0.2 ng g^{-1}.

Hg isotope ratios. Hg isotopic analyses were carried out on freeze-dried homogenized samples using methods previously described in detail[19–21,29,30,35]. Briefly, samples were placed in a combustion furnace that was ramped to a maximum temperature of 750 °C, Hg(0) vapour was transported in a stream of air and O_2 through a second furnace held at 1,000 °C and the gas stream was then bubbled into a solution of 1% $KMnO_4$ where Hg(0) was oxidized and retained as Hg(II). Following combustion, 1% $KMnO_4$ solutions were neutralized with NH_2OH and $SnCl_2$ and Hg(0) was sparged with Ar gas into another 1% $KMnO_4$ solution to obtain a complete separation of Hg from other combustion products. These $KMnO_4$ solutions were neutralized with NH_2OH and Hg concentrations were determined using a Nippon Instruments MA-2000 amalgamation–atomic absorption spectrometer to determine Hg separation yields. Replicate combustions of samples and standard reference materials were made along with procedural blanks. Hg isotopic compositions were determined on a Nu Instruments multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS). $KMnO_4$ sample solutions were diluted to between 1 and 5 ng g^{-1} and a National Institute of Standards and Technology (NIST) 3133 standard was matched to samples by matrix and Hg concentration. $KMnO_4$ solutions were partially neutralized with NH_2OH and then further reduced by $SnCl_2$ online. Reduced Hg(0) was separated from solution using an online phase separator and introduced to the MC-ICP-MS in a stream of Ar gas. Instrumental mass bias was corrected using an internal Tl standard (NIST 997) added as an aerosol to the gas stream and sample-standard bracketing was carried out with NIST 3133. On-peak zero corrections were applied to all analyses.

Hg isotope nomenclature. Hg isotopic ratios are reported in permil, referenced to the NIST 3133 standard, where:

$$\delta^{xxx}Hg = \{[(^{xxx}Hg/^{198}Hg)_{sample}/(^{xxx}Hg/^{198}Hg)_{NIST3133}] - 1\} \times 1{,}000$$

and *xxx* equals 199, 201 or 202. MDF is reported as $\delta^{202}Hg$.

MIF represents the difference between the measured $\delta^{xxx}Hg$ values and the values predicted based on MDF and the $\delta^{202}Hg$ value. MIF is reported as $\Delta^{199}Hg$ and $\Delta^{201}Hg$ in permil. For $\delta < 10\permil$ a valid approximation is[19]:

$$\Delta^{199}Hg = \delta^{199}Hg - (\delta^{202}Hg \times 0.252)$$
$$\Delta^{201}Hg = \delta^{201}Hg - (\delta^{202}Hg \times 0.752)$$

Analytical uncertainty was estimated based on replication of the UM-Almadén standard solution and full procedural analyses of fish tissue standards ERM-CE464 and DORM-3 and is given in Supplementary Table S4.

Estimate of per cent photochemical demethylation. The proportion of MMHg that was photochemically demethylated before entry into the base of the food web was estimated following the method of ref. 20 unmodified, and as modified by ref. 22, to obtain the range of values reported in the text. The first method uses the observed $\Delta^{199}Hg$ value and the fractionation factor determined experimentally[20] using $1\,mg\,l^{-1}$ DOC and much higher MMHg/DOC than present in surface ocean water. The second method extrapolates the fractionation factor to the approximate MMHg/DOC of surface ocean water using two published experiments and the assumption that there is no fractionation of the $\Delta^{199}Hg$ value at DOC $= 0$ (ref. 22). Estimates of per cent photochemical degradation using the first method are about twice as high as estimates using the second method, but the proportional shift in photochemical degradation with depth is the same with both methods. Neither method considers the effect of salinity on fractionation of $\Delta^{199}Hg$ and further experiments will be needed to refine estimates of the per cent photochemical degradation of MMHg based on $\Delta^{199}Hg$.

Trophic position. Tissue samples for AA-CSIA were prepared as described in ref. 38 by acid hydrolysis followed by derivatization to produce trifluoroacetic amino acid esters and analysed using either a Delta V plus or MAT 253 mass spectrometer. Relative trophic position was estimated using differences between the $\delta^{15}N$ values of glutamic acid ($\delta^{15}N_{glu}$) and phenylalanine ($\delta^{15}N_{phe}$) and equation 7 of ref. 39 assuming the difference between $\delta^{15}N_{glu}$ and $\delta^{15}N_{phe}$ in marine algae (β) is $3.4 \pm 0.9\permil$ and the ^{15}N enrichment in glutamic acid relative to phenylalanine during trophic transfer (Δ) is $7.6 \pm 1.1\permil$. Uncertainty in trophic position was calculated by propagation of errors[38] and is treated quantitatively using the analytical solution of differentiation of equation 7 of ref. 39:

$$\sigma_{TP}^2 = \left(\frac{\partial TP}{\partial \delta^{15}N_{glu}}\right)^2 \sigma_{\delta^{15}N_{glu}}^2 + \left(\frac{\partial TP}{\partial \delta^{15}N_{phe}}\right)^2 \sigma_{\delta^{15}N_{phe}}^2 + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma_\beta^2 + \left(\frac{\partial TP}{\partial \Delta}\right)^2 \sigma_\Delta^2$$

Analytical uncertainty in $\delta^{15}N_{glu}$ and $\delta^{15}N_{phe}$ values in samples is based on replicate analyses at least in triplicate (Supplementary Table S3). Routine bulk tissue C and N isotopic compositions were determined using an online C–N analyser coupled with an isotope ratio mass spectrometer.

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J.D.B. supervised Hg isotope measurements and co-wrote the manuscript, B.N.P. supervised determinations of trophic position and co-wrote the manuscript, J.C.D. supervised sample collection and contributed to data interpretation, C.A.C. carried out Hg concentration and N isotope measurements and contributed to data interpretation, and M.W.J. carried out Hg isotope measurements and contributed to data interpretation.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.D.B.

Competing financial interests

The authors declare no competing financial interests.