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Mercury Stable Isotopes Reveal the Vertical Distribution and Trophic Ecology of Deep-Pelagic Organisms over the North-East Atlantic Ocean Continental Slope

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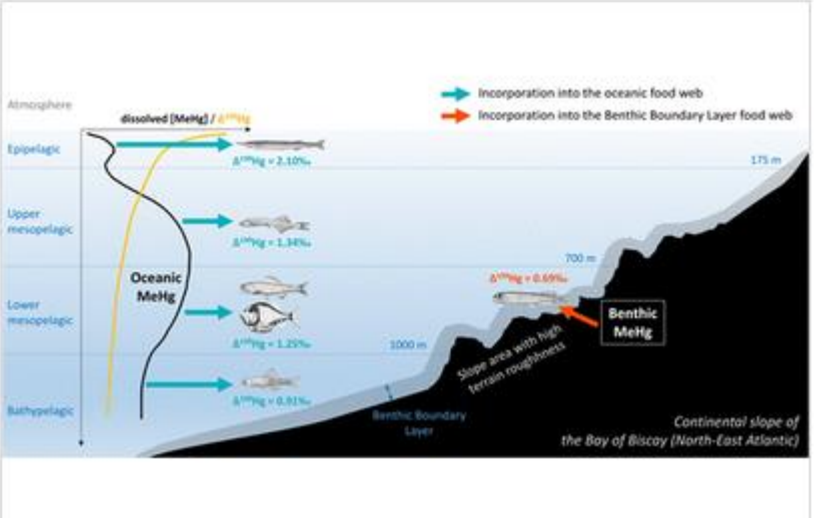
Abstract :

Deep-pelagic species are central to marine ecosystems and increasingly vulnerable to global change and human exploitation. To date, our understanding of these communities remains limited mainly due to the difficulty of observations, calling for complementary innovative tools to better characterize their ecology. We used mercury (Δ199Hg, δ202Hg, Δ201Hg, and Δ200Hg), carbon (δ13C), and nitrogen (δ15N) stable isotope compositions to segregate deep-pelagic species caught on the continental slope of the Bay of Biscay (NE Atlantic) according to their foraging depth and trophic ecology. Decreasing fish Δ199Hg values with corresponding depth estimates from the surface to down to 1,800 m confirmed that mercury isotopes are able to segregate deep species over a large vertical gradient according to their foraging depth. Results from isotopic compositions also identified different mercury sources, likely reflecting different trophic assemblages over the continental slope, in particular, the demersal influence for some species, compared to purely oceanic species. Overall, our results demonstrate how mercury stable isotopes can inform the vertical foraging habitat of little-known species and communities feeding in the deep.

Graphical abstract



Keywords : meso- and bathypelagic zones, foraging depth, benthic boundary layer, mercury sources, micronekton, Bay of Biscay

Introduction

The deep pelagic realm – the open-ocean water column greater than ~200m depth – is the largest and least-studied animal living space of the planet [1]. Its importance to services supporting life on Earth, including carbon sequestration and nutrient recycling [2], is widely acknowledged yet still poorly understood. Our limited knowledge of this environment is increasingly problematic as it is vulnerable to global threats such as climate change [3], pollution and habitat perturbation [4], and exploitation of deep-sea resources [5].

The deep pelagic ocean is dominated by mid-trophic level communities, also referred as micronekton, which include small (2-20 cm) fishes, crustaceans, molluscs, and gelatinous life forms [6]. These communities channel primary production to the top of marine food webs, as they are important consumers of zooplankton [7] and the fundamental foraging base for top predators such as marine mammals, seabirds, and economically valuable fishes like tunas [8–10]. A substantial proportion of micronekton species perform diel vertical migrations, inhabiting deeper waters during the day to avoid visual predation, then ascending through the water column, sometimes over a hundred meters, at night to feed. This vertical migration represents the largest movement of animals on Earth [11] and largely contributes to the sequestration of atmospheric CO_2 and the downward flux of nutrients and particulate organic matter via respiration, excretion and predation processes [12–14].

In oceanic pelagic ecosystems, depth is by far the primary factor determining species assemblage composition [15–17]. Each vertical stratum, i.e., epipelagic (~0-200m), mesopelagic (~200-1,000m), and bathypelagic (>1,000m) is indeed characterized by the penetration of sunlight and an associated biota [18]. Trawling and acoustic surveys also highlighted the importance of topography, with enhanced micronekton abundance and diversity found in the vicinity of seamounts, continental and island slopes, and bathyal and abyssal depths [15,17,19,20]. In these areas of abrupt changes in topography, a homogeneous layer of suspended particulate matter and enhanced zooplankton biomass [21,22], often referred as the benthic boundary layer (BBL), is generally observed within 100m of the seafloor, sometimes extending up to 1,000m if turbulent mixing occurs. The deep-pelagic species assemblage within the BBL, hereafter defined as "BBL assemblage", varies among regions and can differ from that of the surrounding water column, namely the "oceanic assemblage", and is suspected to represent a vital trophic linkage between neritic and oceanic realms at oceanic islands and on continental slopes [19]. To date, the large intrinsic micronekton diversity [23], and the bias associated to trawling data (e.g., possible contamination resulting from the absence of opening and closing nets) complicate the complete understanding of vertical distribution patterns within offshore micronekton communities. Our understanding of vertical assemblages of micronekton is also challenged by the different patterns of diel vertical migrations and their intra-species variability, including ontogenetic shifts in foraging habitat and individual behaviours, which may restrict individual depth ranges compared to the mean species-specific depth range. All these uncertainties call for complementary innovative tools to segregate deep-pelagic communities according to their foraging depth and trophic ecology.

Mercury (Hg) isotopes have been recently identified as promising tools to investigate species foraging depth and Hg transfer in marine food webs across oceanic and coastal environments [24–26]. Emitted to the atmosphere by natural and anthropogenic sources, Hg is deposited to or taken up by the ocean, where it is microbially converted into methylmercury (MeHg), its organic form. Methylmercury is demethylated by photochemical or microbial degradation in surface waters. A fraction of the net MeHg production can be incorporated into primary producers and biomagnified into marine food webs, mainly through dietary pathways. Many abiotic (e.g., photoreduction, volatilization) and biotic processes (e.g., (de)methylation) result in mass-dependent Hg isotope fractionation (reported as δ^{202}Hg) [27,28], making this ratio useful to trace Hg sources and transformations [29]. Conversely, mass-independent fractionation is primarily observed during photochemical reactions [27] and is not modified throughout food webs [30,31]. Odd-Hg mass-independent fractionation (commonly reported as Δ^{199}Hg) occurring in aquatic environment during the photodegradation of MeHg is therefore used to trace the foraging depth of aquatic species, with decreasing Δ^{199}Hg values from the surface to the aphotic waters following light attenuation [24]. The recent finding about even-Hg mass-independent fractionation (Δ^{200}Hg) provides additional information about atmospheric deposition pathways of both elemental (Hg^0) and inorganic (Hg^{II}) forms [32]. Coupled to carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope signatures, Hg stable isotopes, in particular Δ^{199}Hg and Δ^{200}Hg values, are increasingly used to characterize MeHg sources, vertical distribution, and trophic partitioning in marine top predators like tunas, sharks and birds [25,33,34]. Within the Hawaii marine bottomfish community, the combined use of these stable isotope ratios allowed identifying individuals that are exposed to different sources of MeHg, therefore likely feeding from separate food webs [35]. They could help filling research gaps regarding vertical distribution patterns within deep-pelagic communities, in particular the poorly understood demersal influence for some species, compared to purely oceanic species. To date, Hg isotope ratios measured in deep-pelagic species remain scare, being limited to the first 800m of the water column near Hawaii [24,35], and to a few species compared to the large intrinsic micronekton diversity, therefore calling for further investigation in other deep-sea communities.

We measured different stable isotope compositions (δ^{13}C, δ^{15}N, δ^{202}Hg, Δ^{199}Hg, Δ^{201}Hg and Δ^{200}Hg values) in a wide array of fish species (n = 24) that are characterized by different nocturnal depths down to 1,800m, and are suspected to display contrasted relationships to the seafloor in canyons of the Bay of Biscay continental slope (North-East Atlantic) [15]. We used these isotopic compositions to i) explore the biogeochemical history of Hg in deep-pelagic species from the Bay of Biscay continental slope, and ii) deduce their foraging depth and trophic ecology, in particular their belonging to the BBL assemblage *versus* purely oceanic assemblage. We also characterized the isotope compositions of six additional deep-pelagic species (five crustacean and one fish) with no information of vertical distribution patterns in the Bay of Biscay to date, to estimate their vertical foraging habitat. Finally, we explored the potential of Hg stable isotopes at revealing species-specific ontogenetic shifts of foraging habitat.

Material and methods

Study site

The Bay of Biscay is an open bay in the North-East Atlantic Ocean (Fig. 1A). It is characterized by diverse topography including a continental shelf, a very pronounced continental slope (10-12% of inclination) intersected by numerous canyons, and an abyssal plain. Horizontal circulation in the bay depends on multiple factors such as bathymetry, tides, density-driven currents, and winds [36]. The continental shelf of the Bay of Biscay receives significant amounts of freshwater and nutrients via river plumes from Loire, Gironde and Adour, which can also enhance the local productivity [37].

Sample collection

Samples were collected in October-November between 2017 and 2022, during annual "EVHOE" fishery surveys (https://doi.org/10.18142/8) (Table S1). A total of 339 deep-pelagic specimens belonging to 20 fish and 5 crustacean species were collected on the French continental slope. All deep-pelagic specimens were caught at night, when they are supposed to feed. To explore the vertical distribution patterns over a broad vertical gradient, we completed this dataset with more surface dwelling fish species, corresponding to the oceanic *Scomberesox saurus*, and the four pelagic neritic species *Engraulis encrasicolus*, *Sardina pilchardus*, *Sprattus sprattus*, and *Trachurus Trachurus*, which may interact occasionally with the bottom in shallower waters from the shelf. Each specimen was measured onboard to the lowest mm (i.e., standard length for fishes, and cephalothorax length for crustaceans) and stored frozen at -20°C prior analyses. At the laboratory, white dorsal (fish) or abdominal (crustacean) muscle was sampled on each individual for $\delta^{13}C$ and $\delta^{15}N$ analyses, before grinding whole bodies (digestive tracts emptied and rinsed with ultra-pure water) for total and isotopic Hg analyses. When insufficient material was available for chemical analyses, tissues of small individuals of the same size and collected at similar depth were pooled (Table S1). Additional information on sample collection are available in the Supporting Information.

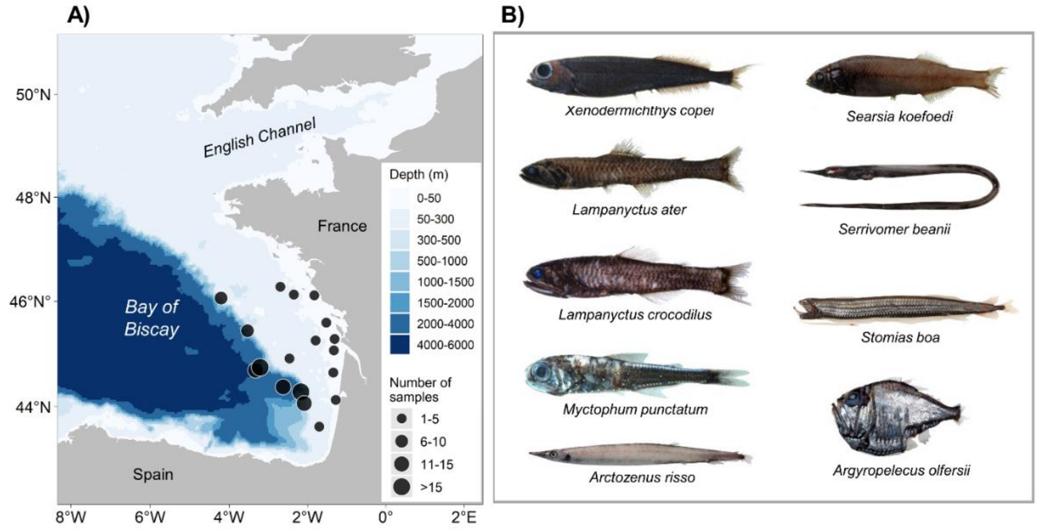


Figure 1. Fish and crustacean sample collection in the Bay of Biscay (North-East Atlantic). A) Map of sample provenance, with the size of the circles being proportional to the number of samples collected, and the colour gradient showing the bathymetry in meters (m) within the Bay of Biscay. **B)** Pictures of some studied meso- and bathypelagic fish species, ©Samuel P. Iglésias/MNHN. Note that the size of the pictures are not proportional to mean observed species sizes.

Chemical analyses

Carbon and nitrogen stable isotopes: For all species, except the four neritic ones, $\delta^{13}C$ and $\delta^{15}N$ were measured on 0.50 ± 0.05 mg aliquots of freeze-dried and powdered muscle tissues, packed into tin cups and analysed with an elemental analyser (Flash EA, 2000, Thermo Scientific) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific) at LIENSs laboratory (La Rochelle, France). Based on repeated measurements of the internal standards USGS-61 and USGS-62, the analytical precision was < 0.15‰ for $\delta^{15}N$ and < 0.10‰ for $\delta^{13}C$ (Supporting information).

Total mercury concentrations: Total Hg concentrations were measured in all species on 10-30 mg aliquots of freeze-dried and powdered samples by thermal decomposition, gold amalgamation, and atomic adsorption (Altec AMA-254, Altec Ltd) at LIENSs (La Rochelle, France, $n = 60$) and GET (Toulouse, France, $n = 54$). Mercury concentrations were measured on whole organism samples, except for *Aphanopus carbo* samples ($n = 3$), which was by far the largest species considered, for which analyses were performed on muscle tissues. Blanks and certified reference materials (TORT-3, lobster hepatopancreas, total Hg = 292 ± 22 $ng.g^{-1}$ dry weight, dw) were routinely analysed every 5-10 samples to check total Hg measurement accuracy and traceability. The precision of total Hg measurements was below 10% for $n = 3$ replicates of each sample. Total Hg content is considered to reflect MeHg concentrations as most of the total Hg (> 70%) has been reported to consist of MeHg in whole myctophids and euphausiid (i.e., similar crustaceans as ours) [38].

Mercury stable isotopes: Aliquots of 50-200 mg of freeze-dried and powdered samples were left overnight at room temperature in 5 mL of concentrated (16N) commercial nitric acid (HNO_3). Next morning, 1 mL of commercial hydrogen peroxide (H_2O_2) was added and samples were digested on a hotplate for 6h at 110°C. 1.67 mL of commercial hydrochloric acid (HCl) was then added to reach 100 vol% inverse aqua regia. Upon cooling to room temperature, 100 µL of 0.2 M BrCl were added to the cold digestion solutions to ensure a full oxidation of MeHg to inorganic Hg. Two types of certified reference materials (TORT-3; and BCR-464, tuna fish muscle, total Hg = 5240 ± 100 $ng.g^{-1}$ dw) and blanks were prepared in the same way.

To avoid matrix effect, we used a standard stannous chloride ($SnCl_2$) reduction method and a custom-made purge-and-trap system [32], based on the USEPA method 1361 [39]. An aliquot or the entire digest solution was diluted in ~150 mL Milli-Q water in a 500 mL pre-cleaned Pyrex bottle to reach a final inorganic Hg concentration in trap solutions between 0.5 and 2.0 $ng.g^{-1}$. 10 mL of 10 %vol $SnCl_2$ were added to the bottle and purged with Hg-free argon for 2h at 75 $mL.min^{-1}$ into a 7 mL, 40 %vol inverse aqua regia trap. Final trap solutions were diluted with Milli-Q water to 20 %vol inverse aqua regia and kept refrigerated at 2-4°C until Hg isotope analysis.

The total Hg isotopic composition was measured at GET using a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS, Thermo Finningan Neptune Plus) with continuous-flow cold vapor generation using Sn(II) reduction. Mercury isotopic values are expressed in the δ unit notation and reported in ‰ deviation from the bracketing standard NIST-3133 (Supplementary Information). To verify efficient sample digestion, and purge and trap recovery, total Hg in the diluted digest mixtures was monitored by MC-ICP-MS using ^{202}Hg signals with the following mean recoveries: 83 ± 12 % (n = 109). This is within the 80-120% uncertainty range of the combined analysis methods, and no relationship between Hg isotope signatures and recovery were observed. Isotopic measurement reproducibility was assessed by analysing ETH-Fluka (n = 20), UM-Almadén (n = 19), and the certified reference materials TORT-3 and BCR-464 (n = 12). Measured isotope signatures and analytical reproducibility of standards agreed with previously published values (Table S2).

Nocturnal depth estimation

To assess the ability of $\Delta^{199}Hg$ values to proxy the foraging depth of micronekton species, we used species-specific estimates of the mean nocturnal depth of deep-pelagic fishes on the Bay of Biscay canyons, inferred with trawling data [15]. The foraging depth of *S. saurus* was set at 30 m (www.fishbase.org), while for the four neritic species, we used the approximate trawling depth. In total, estimates of the mean nocturnal depth were available for 24 (only fishes) of the 30 studied deep-pelagic species, and were missing for one fish and the five crustacean species (Table S1). We divided the pelagic domain into four zones following the definition of Loutrage et al (2023) which accounts for environmental specificities of our study area: epipelagic (25-175m), upper mesopelagic (175-700m), lower mesopelagic (700-1,000m), and bathypelagic (> 1,000m).

Statistical analyses

Fish species were first grouped visually according to their $\Delta^{199}Hg$ values and estimated nocturnal depths. We used a permutational analysis of variances (PERMANOVA) to test for significant differences in the isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\Delta^{199}Hg$, $\delta^{202}Hg$, $\Delta^{201}Hg$ and $\Delta^{200}Hg$ values) between the two pre-defined BBL and oceanic assemblages. Differences in individual isotope signatures between assemblages were tested with an analysis of variance (ANOVA) or a non-parametric Kruskal-Wallis test, depending on the distribution of isotope values (tested with a Shapiro test). We performed a Principal Component Analysis (PCA) to visualise in two dimensions whether our two pre-defined assemblages differ according to their isotopic ratios, and which of these ratios best explain the segregation of species into two assemblages. Finally, to further explore the biogeochemical history of Hg in fishes, we fitted linear models, including the variable "assemblage", between i) Δ^{199}Hg and Δ^{201}Hg values to explore photochemical reactions, and ii) Δ^{199}Hg and δ^{202}Hg values to explore the MeHg sources prior incorporation in fishes.

To investigate possible ontogenetic shifts of micronekton foraging habitat, we fitted linear regressions between Δ^{199}Hg values (as a proxy for foraging depth) and fish size.

For the two assemblages separately, we fitted a logarithm model to predict foraging depth as a function of Δ^{199}Hg values [25,40]. We used these results to predict the foraging layer (i.e., epipelagic, mesopelagic, or bathypelagic) of the six deep-pelagic species for which no estimated nocturnal depth was available yet in the study area, and for which little is known more globally regarding their vertical distribution and trophic ecology.

All statistical analyses were performed with the statistical open source R software 4.2.2 [41].

Results and discussion

Variation of Δ^{199}Hg values with depth

In the Bay of Biscay, Δ^{199}Hg values measured in all fish and crustacean samples varied between 0.53‰ and 2.10‰ (Fig. 2 and S1, Table S3). These positive Δ^{199}Hg values in fishes from meso- and bathypelagic waters (> 600m depth) are similar to the observations reported at same depths in Hawaiian waters [35,42], and reflect the trophic transfer of MeHg that has previously undergone significant photodegradation in the environment prior to incorporation into marine food webs [27,43], as discussed in the following section.

In general, except for some species, fish Δ^{199}Hg values declined predictably with estimated nocturnal depth between 30m and 1,800m, as observed in other marine systems in the Pacific Ocean [24,33,35,42] (Fig. 2). Nevertheless, some species (*n* = 9, see the squares on Fig. 2) did not fall on the expected trend of Δ^{199}Hg values with depth, with Δ^{199}Hg ratios generally lower than expected. Among them, the four neritic species (i.e., *E. encrasicolus*, S. *pilchardus*, *S. sprattus*, and *T. Trachurus*) had lower Δ^{199}Hg values (0.89 ± 0.16‰, all species combined) than *S. saurus* (2.10‰), an oceanic epipelagic species known to forage within the same depth range (0-100m) (Fig. 2). Such an isotopic shift in Δ^{199}Hg values has already been observed in the coastal species *Caranx ignobilis* from Hawaii, and was thought to reflect a different biogeochemical cycling of Hg in coastal areas compared to the open ocean, prior incorporation into marine food webs [35]. In shallower waters of the French continental shelf of the Bay of Biscay, the four neritic fishes can forage at the vicinity of the seafloor, likely in the benthic boundary layer (BBL), which may explain their distinct Δ^{199}Hg values compared to a purely oceanic species like *S. saurus*. Lower Δ^{199}Hg values in nine species (i.e., four neritic and five deep-pelagic fishes) would therefore suggest that these species rely, at least partly, on another food web, and thus on a contrasted MeHg pool with a source of production different from the main pelagic oceanic MeHg production likely occurring in the oxygen minimum zone of the water column. To further explore these possible differences in foraging strategies, we pre-defined two fish assemblages based on Figure 2: an oceanic assemblage corresponding to species for which Δ^{199}Hg values decreased predictably with estimated foraging depth (*n* = 15, symbolised by dots), and a BBL assemblage corresponding to species with unexpected lower Δ^{199}Hg values ($n = 9$, symbolised by squares). We then explored their Hg, carbon and nitrogen stable isotope compositions to further characterize these two assemblages (see below).

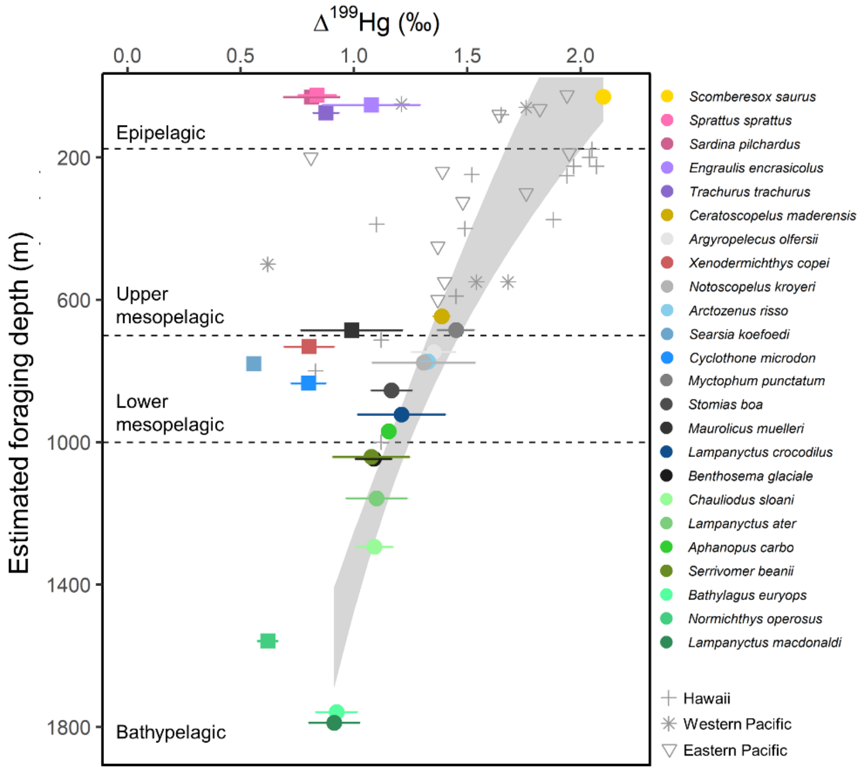


Figure 2. Variation with depth of Δ^{199}Hg values in fishes. Relationship between Δ^{199}Hg values (‰) and estimated foraging depth (nocturnal depth) of 19 deep-pelagic and five epipelagic fish species caught in the Bay of Biscay (NE Atlantic) (filled colored symbols) in comparison to published data (open grey symbols) from Hawaii [24,35,42], and the western and eastern Pacific [33]. Colors of the filled symbols refer to fish species, while shapes distinguish oceanic species (dots) from species under a Benthic Boundary Layer (BBL) influence (squares). The grey band represents the 95% confidence interval of the logarithm model fitted between depth estimates and Δ^{199}Hg values in oceanic fishes only (p-value < 0.05, $R^2 = 0.89$; depth $= 2022 * \log(\Delta^{199}\text{Hg}) - 1364$). No significant logarithm model was found for fishes under the BBL influence (p-value $= 0.40$).

Biogeochemical history of mercury in fishes

Fish isotope composition (δ^{13}C, δ^{15}N, Δ^{199}Hg, δ^{202}Hg, Δ^{201}Hg, and Δ^{200}Hg values) varied significantly between the two pre-defined assemblages (PERMANOVA, p-value < 0.05), which was also confirmed by the Principal Component Analysis (Fig. 3A), and supports our classification of two distinct assemblages. The first principal component (PC1) explained 50% of the variance and was primarily and positively associated to Δ^{199}Hg and Δ^{201}Hg values (Fig. 3A and S2), therefore mainly reflecting MeHg photodegradation and fish foraging depth. This axis is also, to a lesser extent, negatively associated to δ^{15}N values and shows generally higher δ^{15}N values in micronekton fishes foraging in deeper waters, as already found in other regions of the global ocean [44–47], and probably due to the preferential bacterial degradation of ^{15}N-depleted organic matter at the base of marine food webs at depth [48]. The second principal component (PC2) explained 25% of the variance and was correlated primarily and positively to δ^{13}C and δ^{202}Hg values, and was, to a lesser extent, negatively related to Δ^{200}Hg values

(Fig. 3A and S2). This finding likely reflects differences of Hg biogeochemical cycling (i.e., sources and/or transformation reactions) prior incorporation into marine food webs. Compared to the oceanic species, BBL fishes displayed generally negative PC1 values, which may reflect deeper foraging depths in general for this assemblage. More probably, this result may be biased by the absence of surface BBL species (i.e., the four neritic species) in the PCA because of missing $\delta^{13}C$ and $\delta^{15}N$ values (see Material and Methods, Table S3). Fishes of the BBL assemblage were also characterized by lower PC2 values compared to the oceanic species, which suggests differences in Hg sources and/or biogeochemical processes.

Although fish $\Delta^{200}Hg$ values were the third explanatory variable of the PC2, there was no significant difference of $\Delta^{200}Hg$ values between the two assemblages (ANOVA, *p*-value = 0.73), suggesting that the inorganic Hg precursor to fish MeHg of both assemblages is likely the same, originating from a common atmospheric Hg source. Here, the mean fish $\Delta^{200}Hg$ value of 0.05‰ agrees with the $\Delta^{200}Hg$ values reported globally in seawater, marine sediments, and marine biota, and lies in between the deposition signature of atmospheric elemental Hg^0 (~ -0.05‰) and divalent Hg^{II} (~ 0.14‰) (Fig. S3), which illustrates the equal contribution of Hg^{II} wet and dry deposition, and gross Hg^0 gas exchange [32].. This result suggests that the inorganic Hg source at the base of MeHg formation is likely similar for the two fish assemblages, but that differences in carbon sources and/or mechanisms involved in the formation of MeHg prior to fish incorporation are at play.

Also similar between the two assemblages are the correlations between $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values, commonly used to investigate photochemical reactions in the ocean. Here, the linear slope of 1.17 ± 0.06 (Fig. 3B) for all fishes is consistent with slopes (~1.20) reported in other marine fishes exposed to photodegraded MeHg [24,25,33,35,42], evidencing similar ocean MeHg photodegradation processes prior incorporation into the two trophic assemblages.

The similar $\Delta^{199}Hg/\delta^{202}Hg$ slope of ~0.4 between the two assemblages (Fig. 3C) are consistent with those observed in other coastal marine ecosystems (~0.4), but lower than values (~2.0) observed in oligotrophic regions of the central Pacific Ocean [49]. Yet, the offset of the $\Delta^{199}Hg/\delta^{202}Hg$ ratios between the two assemblages may indicate different MeHg sources prior incorporation into both assemblages. It is generally assumed that MeHg in pelagic fish originates mainly from MeHg produced in the water column by remineralization of sinking particulate organic matter in the oxygen minimum zone [50], leading to fish $\Delta^{199}Hg$ values generally higher than 1‰ [24,25,35]. Methylmercury production also occurs in shelf sediments [51], where the absence of light leads to MeHg characterized by lower $\Delta^{199}Hg$ values compared to MeHg produced within the water column, typically lying between 0‰ and 1‰ [26,52]. Here, significantly lower $\Delta^{199}Hg$ values in BBL fishes (Fig. 2; Kruskal-Wallis, *p*-value < 0.05) likely reflect the lower $\Delta^{199}Hg$ signature of MeHg produced in sediments, suggesting that BBL fishes rely, at least in part, on a benthic food web as a source of food, as previously suggested for bottom fishes in coastal ecosystems of Hawaii [35].

This benthic dietary contribution might be nuanced by the absence of significant differences of $\delta^{13}C$ values between BBL and oceanic fishes (Fig. S4; Kruskal-Wallis, *p*-value = 0.10), which does not mirror the expected higher $\delta^{13}C$ values in benthic sources (~ -17‰) compared to pelagic sources (~ -22‰) in strictly marine systems [53]. Yet, contrary to a strictly oceanic system, the Bay of Biscay shelf is influenced by the Loire and the Gironde River plumes [37], and may receive significant amounts of terrestrial inputs that are generally characterized by lower $\delta^{13}C$ values [54], which may in turn impact the benthic carbon signature on the continental slope. Taken together, our results suggest that a nonnegligible part of the deep-pelagic fishes caught in canyons of the Bay of Biscay are not purely oceanic, and that some species may feed on benthic food web resources during the night.

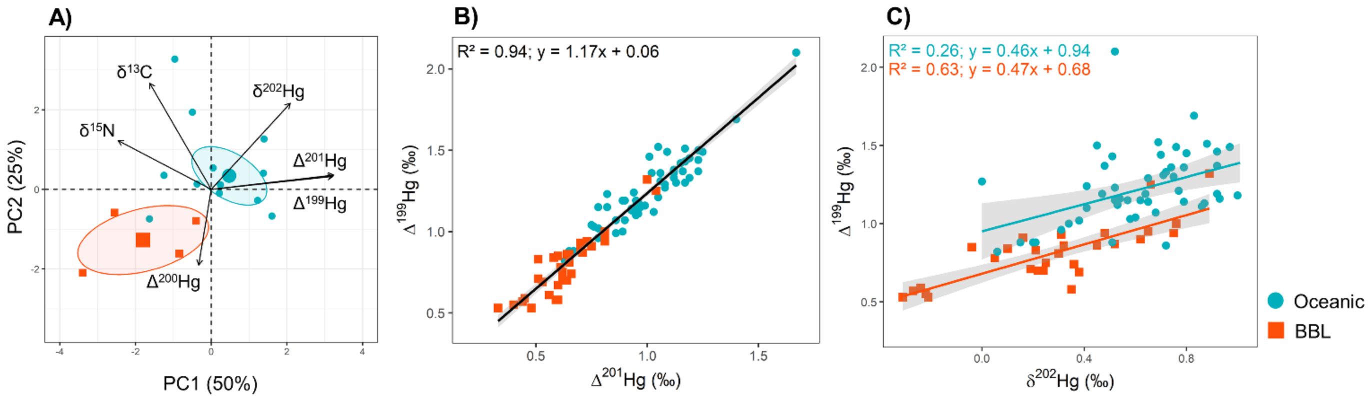


Figure 3. Variability of isotope compositions in meso- and bathypelagic fishes. The blue dots and the orange squares distinguish fishes belonging to the oceanic and the benthic boundary layer (BBL) assemblages, respectively. **A)** Principal Component Analysis and associated 95% confidence ellipses discriminating oceanic and BBL fishes, based on their carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and mercury ($\Delta^{199}Hg$, $\delta^{202}Hg$, $\Delta^{201}Hg$, $\Delta^{200}Hg$) stable isotope compositions. The small symbols represent the species while the two larger symbols are the barycentres of the 95% confidence ellipses. **B)** Relationship between $\Delta^{199}Hg$ and $\Delta^{201}Hg$ values (‰) measured in oceanic and BBL fishes. The variable "assemblage" and the interaction were not significant in the linear model, that is why a unique slope (represented by the black line and grey shadow) was fitted to all data. **C)** Relationship between $\Delta^{199}Hg$ and $\delta^{202}Hg$ values (‰) measured in oceanic and BBL fishes. The variable "assemblage" was significant in the linear model so different linear regressions were fitted for the two fish assemblages (as represented by the two colored solid lines).

Implications for foraging depth segregation

Among the 19 deep-pelagic fishes characterized with both isotopic values and mean nocturnal depth estimates, 14 appeared to be purely oceanic (symbolised by dots on Fig. 2), i.e., remaining within the water column to feed. Among them, some are resident of a given vertical layer, while others perform daily vertical migrations ascending to the surface at night (Table S1). All of them are suspected to feed primarily at night, although this assumption remains to be evidenced. Because MeHg accumulates in marine organisms mainly through the dietary pathway, $\Delta^{199}Hg$ values in fishes are assumed to reflect the foraging depth in particular. Here, fish $\Delta^{199}Hg$ values increased predictably with nocturnal depth estimates, which seems to confirm the general assessment that oceanic deep-pelagic micronektonic species mainly feed at night. If fish performing daily vertical migrations were indeed also feeding during the day in deeper waters, we would not have found a correlation between their $\Delta^{199}Hg$ values and nocturnal depth estimates, as observed here.

In addition to the oceanic assemblage, our results suggest the presence of a distinct bottom-associated community of deep-sea fishes on the continental slope of the Bay of Biscay, as previously suggested with trawling data in this area [15]. This BBL assemblage includes *Maurolicus muelleri*, *Xenodermichthys copei*, *Searsia koefoedi*, *Normichthys operosus,* and *Cyclothone microdon* (symbolised by squares on Fig. 2). Complementary stomach content data in these species remain scarce to evaluate the benthic contribution to their diet. Yet, some fish families like Platytroctidae (including *S. koefoedi* and *N. operosus*) and Alepocephalidae (including *X. copei*) are suspected to be gelatinovorous [7], which could explain an affinity to the BBL where gelatinous blooms occur. In addition, catch data in different oceanic regions, including ours, seem to validate the particular link of these five species to the seafloor. *X. copei* and *N. operosus* have been previously reported in both pelagic and demersal trawls in the NE Atlantic [55]. *M. muelleri* has been found to occur primarily in association with continental slope and shelf regions in the west African Benguela Current [56], and with seamounts in Hawaii [19,20,57]. More locally, it is also regularly caught in benthic trawls carried by fisheries surveys on the Bay of Biscay and Celtic Sea continental shelves, as well as in the North Sea [58]. To date, there are no reports of *C. microdon* close the seafloor, yet this species has been shown to display a broad range of vertical distribution in the Mid-Atlantic Ridge (0-3000m) [17,59], which could hide a close relationship to the seafloor for some individuals. Overall, the drivers of the BBL assemblages at the vicinity of seamounts and continental and island slopes remain poorly known and likely result from multiple processes including concentration of food resources, importance of the seafloor for reproduction, and active horizontal migration [18]. For instance, the presence of *X. copei* near the bottom possibly reflects a demersal spawning behavior, as commonly observed in some oceanic species, given that its spawning period has been reported in October-November [60] when the survey took place.

When discussing the two assemblages species composition, it is worthwhile mentioning that some species (e.g., *Serrivomer beanii* and *Bathylagus euryops*), classified here as purely oceanic according to their isotope compositions, were previously found close to the bottom at night in trawling data in the Bay of Biscay [15] and on the Mid Atlantic Ridge [17]. This suggests that BBL assemblages generally reflect that of the surrounding water column, although some fish families have been reported to occur exclusively in the BBL, being absent or less abundant in oceanic waters [19,20]. Such vertical variability may also exist within a same species. Some species can be indeed found over a large depth range, but not all individuals necessarily use this entire depth range. Overall, this illustrates the interconnectivity of the different pelagic ocean layers, and highlights the need to measure Hg isotopes in more individuals per species to better characterize the diversity of vertical distribution patterns within deep-pelagic communities.

Vertical foraging habitats estimation

Based on our assemblages definition, the relationships between fish Δ^{199}Hg and estimated nocturnal depths, and the vertical stratification in the Bay of Biscay [15], we used Δ^{199}Hg values measured in six additional deep-pelagic species (i.e., five crustacean and one fish), with no information of nocturnal depth, to estimate their main vertical foraging habitat (Fig. 4). Among them, the fish *Maulisia microlepis*, and the crustacean *Ephyrina figueirai* displayed low Δ^{199}Hg values (0.80 and 0.63‰, respectively, Table S3), similar to the nine BBL species, which could suggest a BBL influence as well for these two species. *M. microlepis* has been shown to display a wide depth range (750 – 2,300 m) over the mid-Atlantic Ridge [17], but no particular record within the BBL have been reported to date. Yet, this species belongs to the Platytroctidae family, the same family as the two BBL fishes *N. operosus* and *S. koefoedi*, which could support our hypothesis of a BBL behavior in the study area. To date, the vertical distribution patterns of the genus *Ephyrina* (crustaceans) are poorly documented. Although *E. figueirai* is mainly observed in the deepest layers and more in bathypelagic than mesopelagic environments in the Bay of Biscay (J. Spitz, personal communication), further research are required to characterize its foraging habitat and its possible contribution to the BBL assemblage in the Bay of Biscay.

For the four other crustacean species, namely *Meganyctiphanes norvegica*, *Acanthephyra pelagica*, *Systellaspis debilis*, and *Pasiphaea multidentata*, Δ^{199}Hg values fell within the expected relationship between Δ^{199}Hg values and nocturnal depths for oceanic fishes (i.e., their mean Δ^{199}Hg values crossed the blue band corresponding to the logarithm model fitted on oceanic species) (Fig. 4), suggesting a purely oceanic foraging habitat for these four crustaceans. More specifically, Δ^{199}Hg values in *M. norvegica* suggest that this crustacean forages mainly within the mesopelagic waters, which generally aligns with similar biomass and density records in the 0-400 m and the 400-800 m depth in the Irminger Sea (North Atlantic) [61]. *P. multidentata* is also supposed to forage within the mesopelagic layer given its Δ^{199}Hg signature, which is consistent with previously reported depth range (128-871 m) in the northwestern Mediterranean Sea [62]. Conversely, our results suggest that *A. pelagica* and *S. debilis* primarily forage within bathypelagic waters. *A. pelagica* has already been reported in deep waters in the eastern North Atlantic (800-2000m at 40°N) [63] and the north Atlantic Subtropical convergence zone (200-2,000 m but mainly at 700-1,800 m) [64], which seems to validate our foraging habitat estimation. Rare depth records of *S. debilis* in the Gulf of Mexico suggest that this species can be found on a broad depth range (0-1,500 m), but with a preferential epipelagic habitat during the night [65], which contrasts with our bathypelagic foraging habitat and calls for further research.

Overall, these results illustrate the enhanced power of resolution of Δ^{199}Hg values at estimating vertical foraging habitat of deep-pelagic species. Yet, they also highlight the importance of biological and ecological knowledge to properly interpret this signature, especially on continental slopes or seamounts, where a fourth vertical assemblage, namely the BBL, adds to the classical epipelagic, mesopelagic and bathypelagic layers found in the open ocean.

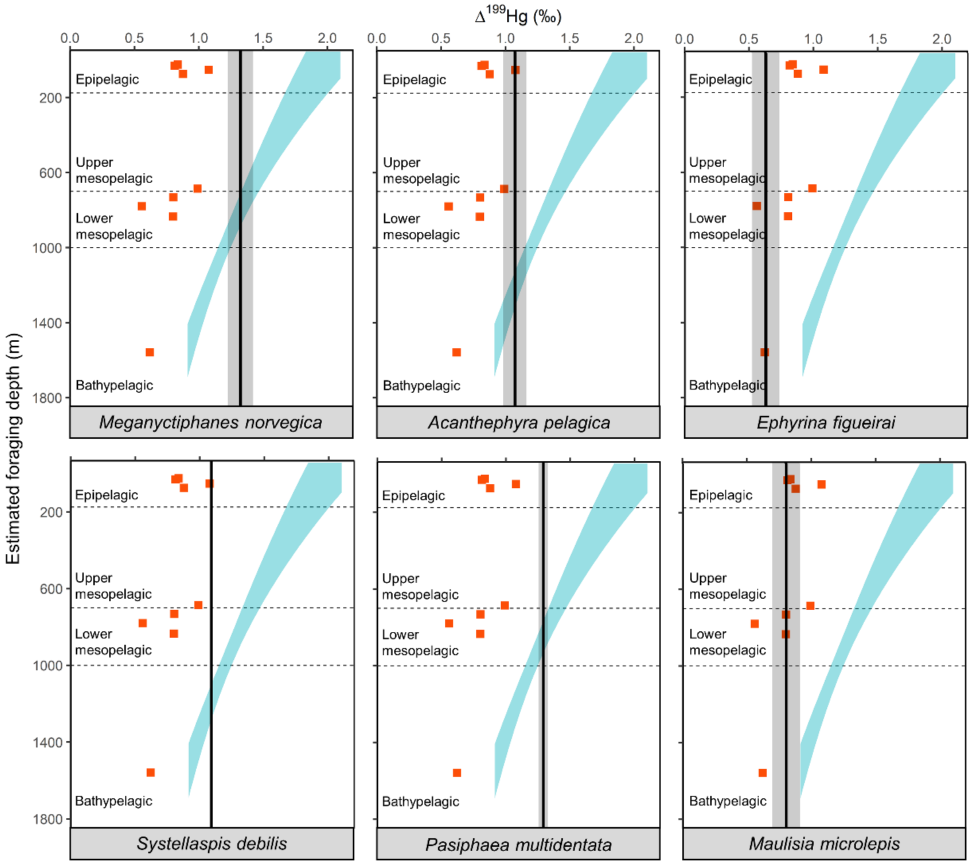


Figure 4. Vertical foraging habitat estimation of deep-pelagic species using their Δ^{199}Hg values. The black solid lines and shadow give the mean ± SD of Δ^{199}Hg values (‰), respectively, of each crustacean and fish species. The blue band represents the 95% confidence interval of the logarithm model fitted between depth estimates and Δ^{199}Hg values in oceanic fishes only (p-value < 0.05, $R^2 = 0.89$). The red squares represent the variation with depth of Δ^{199}Hg values measured in fish species under the Benthic Boundary Layer (BBL) (see Fig. 2).

Ontogenetic shifts of foraging depth

When discussing the foraging habitat of deep-pelagic species, it is worthwhile mentioning that some of them display ontogenetic shifts in their diet and/or habitat use [18]. To further investigate this possible intra-specific variability, we explored, when possible (i.e., more than five specimens per species), the relationship between Δ^{199}Hg values (used as a proxy for foraging depth) and individual body size (Fig. 5). Over the seven studied species, we found a significant linear relationship between foraging depth and fish size for *Lampanyctus crocodilus* only (p-value < 0.05 for this species only), with larger individuals associated to deeper foraging depths. Ontogenetic shift of both vertical habitat use and diet for this species has been already documented in the Bay of Biscay [66] and the western Mediterranean Sea [67,68], with diet shifting from epipelagic crustaceans to benthopelagic fishes at the senescent stage. This ontogenetic shift of trophic ecology likely results from a trade-off between the energetic costs and benefits of migration for feeding, associated to physiological modifications with age. The absence of significant relationships between foraging depth and fish size for *Argyropelecus olfersii, Myctophum punctatum, Notoscopelus kroyeri, S. koefoedi*, *Stomias boa*, and *X. copei* (linear regression, p-value > 0.05) aligns with the absence of ontogenetic shifts for both depth (estimated with trawling data) and δ^{15}N values for these six species in the Bay of Biscay [66]. As our dataset is relatively limited (n < 10 individuals per species), our investigation of ontogenetic shifts in foraging ecology through Δ^{199}Hg values could have been biased by limited size ranges and the number of individuals. Beyond general patterns, individual specificity in foraging and migration behaviour may exist within different species. Deep pelagic species are recorded at a wide range of depths; yet, it is currently unknown whether the entire depth range, or only a restricted part of it, is exploited at the individual level. Overall, this first exploration suggests that Hg stable isotopes could be a powerful tool at the species level as well to investigate ontogenetic shifts of foraging habitat, therefore calling for larger species-specific datasets of Δ^{199}Hg values covering broad individual fish sizes to better characterize species-specific trophic ecology and investigate individual specialization.

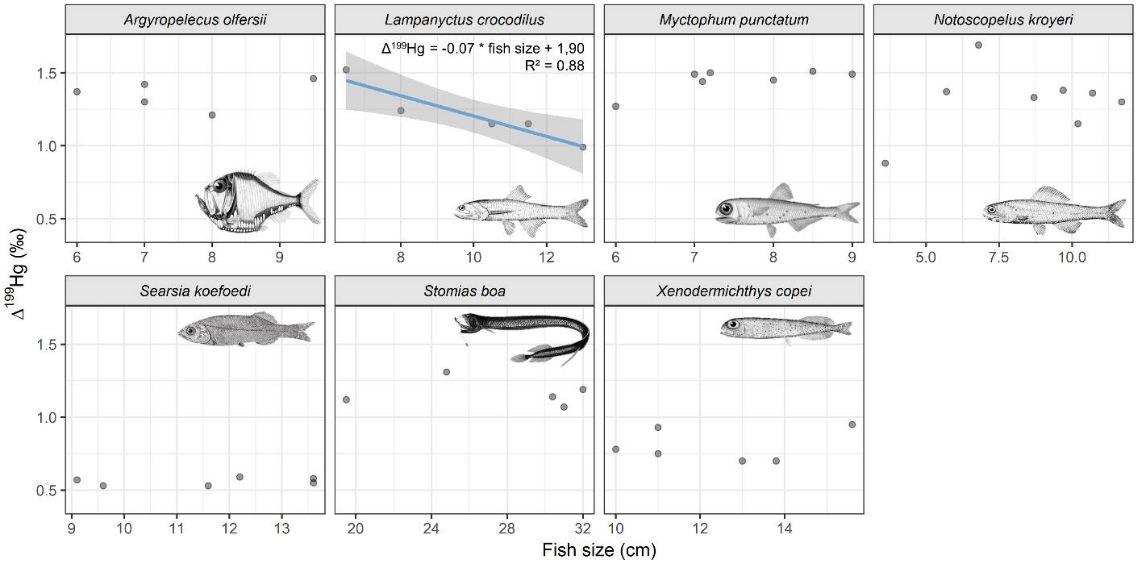


Figure 5. Exploration of ontogenetic shifts in foraging depth of meso- to bathypelagic fishes. Relationship between Δ^{199}Hg values (‰), used as a proxy of fish foraging depth, and fish size (cm). The blue line and the grey shadow represent the significant linear regression between the two variables found for *L. crocodilus* only ($p < 0.05$), and the associated standard deviation, respectively.

Perspectives for deep-pelagic ecological research

Decreasing fish Δ^{199}Hg values with corresponding depth estimates from the surface to down to 1,800 m depth confirms that Hg isotopes are able to segregate meso- and bathypelagic species over a wide vertical gradient according to their foraging depth. In addition, the complementary use of Hg, carbon, and nitrogen stable isotopes allowed identifying different trophic assemblages, in particular the demersal behavior of some oceanic species over the continental slope of the Bay of Biscay. Taken together, our results shed light on the potential of Hg stable isotopes to refine species-specific information on their vertical foraging habitat within deep-pelagic communities, which may help overcoming the possible bias associated to traditional trawling data. Notably, relationships between Δ^{199}Hg values and depth could be particularly useful in predicting the foraging depth of rare and hard-to-capture deep-diving species including fish, cephalopods, and even marine mammals.

Although some deep-pelagic species are characteristic of certain depth zones, active flux of micronekton between the various depth strata are known to occur between the surface waters to the deepest depths [18]. In complex regions like the Bay of Biscay canyons, where a BBL adds to the epipelagic, mesopelagic, and bathypelagic layers found in the open ocean, our results highlight the importance of biological and ecological knowledge to correctly interpret micronekton isotope compositions. At the species level, individuals can display changes of foraging habitat during their lifetime, adopting for instance a demersal behavior over continental slopes for spawning, or shifting to a resident pattern in bathypelagic waters at the senescent stage. This interconnectivity of the pelagic ocean vertical layers calls for big datasets of Hg, carbon, and nitrogen stable isotopes in many individuals, covering a wide range of species with contrasting ecologies to help characterizing the fluxes of deep-sea species in an ecosystem. Such characterization would be useful to help predicting the trophic linkages between micronekton and top predators, and to better understand carbon fluxes in the global ocean.

Supporting Information: Additional information about sample collection, chemical analyses, and results of stable isotope compositions and mercury concentrations.

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