Check for updates

LETTER

Vertical stratification of environmental DNA in the open ocean captures ecological patterns and behavior of deep-sea fishes

Oriol Canals [iD],[1]* Iñaki Mendibil,[1] María Santos,[1] Xabier Irigoien,[1,2] Naiara Rodríguez-Ezpeleta [iD][1]*
[1]AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Sukarrieta, Bizkaia, 48395, Spain; [2]Ikerbasque, Basque Foundation for Science, Bilbao, Bizkaia, Spain

Scientific Significance Statement

The deep sea provides global vital functions such as sequestration of carbon from the atmosphere. The increased anthropogenic pressures and interest in expanding deep-sea fisheries make this pristine ecosystem particularly vulnerable, whose conservation largely depends on rapid knowledge acquisition. In view of the limitations of traditional methods to explore the biodiversity of this vast ecosystem, the analysis of traces of macroorganismal DNA released into the water column arises as a cost-effective, noninvasive alternative. Yet, the success of this approach requires understanding of the stratification of DNA traces in the ocean. This study provides evidence that fish DNA traces can be used to establish depth-specific fish diversity and abundance throughout the water column, opening a promising avenue for gathering knowledge about the deep-sea ecosystem.

Establishing the foundations for a sustainable use of deep-sea resources relies on increasing knowledge on this inaccessible ecosystem, which is challenging with traditional methods. The analysis of environmental DNA (eDNA) emerges as an alternative, but it has been scarcely applied to deep-sea fish. Here, we have analyzed the fish eDNA contained in oceanic vertical profile samples (up to 2000 m depth) collected throughout the continental slope of the Bay of Biscay. We detected 52 different fish species, of which 25 were classified as deep-sea fish. We found an increase of deep-sea fish richness and abundance with depth, and that eDNA reflects day–night community patterns and species-specific vertical distributions that are consistent with the known diel migratory behavior of many mesopelagic fishes. These findings highlight the potential of eDNA to improve knowledge on the fish species inhabiting the dark ocean before this still pristine ecosystem is further exploited.

*Correspondence: ocanalsd@gmail.com, nrodriguez@azti.es

Additional Supporting Information may be found in the online version of this article.

The deep sea, that is, the dark oceanic zone below 200 m depth, is the largest, least explored, and most pristine ecosystem on Earth (Sutton 2013; Duarte et al. 2020; Martin et al. 2020). Recent estimates suggest that the upper deep-sea layer (from 200 to 1000 m depth), referred to as the mesopelagic zone, may contain 90% of the global fish biomass (Irigoien et al. 2014), which, in the current context of increasing global human population and need to end overfishing, uncovers an alternative source of food for direct human consumption or as feed supply for aquaculture (Food and Agriculture Organization [FAO] 2018). Yet, uncontrolled exploitation of this resource could result in irremediable damage (Mengerink et al. 2014; Martin et al. 2020) since mesopelagic fish play an important role in carbon sequestration (Davison et al. 2013; Thurber et al. 2014; Klevjer et al. 2016), and in trophic connectivity, by linking upper and lower ocean layers (St. John et al. 2016). This is due to the diel vertical migration (DVM) of many mesopelagic fishes moving into upper layers for feeding at nighttime and into deeper layers to avoid predation at daytime (Sutton 2013), which has been referred to as the "largest daily animal migration on Earth" (St. John et al. 2016).

Understanding the role of mesopelagic organisms in global biogeochemical cycles and evaluating the ecosystem services they provide is urgent before uninformed extraction of these resources starts (Martin et al. 2020). Fundamental knowledge gaps need to be filled for this purpose, such as which animals inhabit the mesopelagic zone, how are they distributed in space and time, and which factors control their distribution. This lack of information is mostly due to the inaccessibility of deep-sea ecosystems, challenging traditional sampling methods (Martin et al. 2020). Although net tows, video plankton recorders, bioacoustics, and autonomous or remotely operated underwater vehicles are being applied, they are either extremely time-consuming, expensive, selective, highly specialized, hardly available for most research groups, and/or reliant on often scarce taxonomic expertise (Hansen et al. 2018). Thus, new technologies are required to acquire the needed insights on deep-sea ecosystems at a global scale to set the basics for studies aiming at evaluating the role of mesopelagic organisms as diet of commercially important and emblematic species, at understanding their role in carbon sequestration, and at exploring their potential as food and bioactive compound sources.

In this scenario, environmental DNA (eDNA)-based approaches arise as cost-effective alternatives to explore the deep sea. Water column eDNA includes DNA traces released by macroorganisms (including fish) into the environment in form of tissue, cells, scales, mucus, or feces (Gilbey et al. 2021). This material can be analyzed through metabarcoding, which allows community characterization by simultaneously sequencing a short region of the genome of multiple species, which is then compared to a reference database (Hansen et al. 2018). eDNA collected from water samples can provide biodiversity information of virtually any marine habitat (Thomsen et al. 2012) including deep-sea environments (Thomsen et al. 2016; Laroche et al. 2020; McClenaghan et al. 2020). However, inferring the macroorganisms present in marine ecosystems by analyzing their DNA released into the environment requires an understanding of how DNA traces behave in the water column, and many factors related to extra-organismal DNA production rate, degradation and transport have been reported to influence its detection and concentration in the ocean (Hansen et al. 2018).

Although eDNA vertical structuring has been reported from inshore sediment samples (DiBattista et al. 2019) and in lakes (Littlefair et al. 2021), to date and to the best of our knowledge, no study has assessed whether eDNA is vertically structured or mixed up along the water column in the open ocean, which is crucial to evaluate the potential of eDNA to assess diversity and behavior of deep-sea fish communities. Here, we have applied eDNA metabarcoding to eight vertical profiles (up to 2000 m depth) collected during day and night-time along the continental slope of the Bay of Biscay. We hypothesized that fish eDNA is vertically stratified along the oceanic water column and can therefore provide information on fish vertical structuring. Evidence of eDNA-based approaches being capable of inferring deep-sea fish ecology, including diversity and vertical distribution, will greatly broaden the horizon of exploring new approaches for further understanding the remote deep sea, which is key to ensure a sustainable exploitation of its resources.

Material and methods

Sampling

Vertical profile samples from eight stations along the continental slope of the Bay of Biscay (Fig. 1; Supporting Information Table S1) were collected in Spring 2018 on board the Ramón Margalef research vessel at 5, 50, 200, 500, and 1000 m depth and at 50 m above the seafloor (thereafter >1000 m) using a rosette sampler and, from five of the stations, also at 4.4 m depth using the continuous clean circuit intake of the ship. On board, 2–5 L of 50-μm pore-sized mesh prefiltered water samples were immediately filtered through Sterivex 0.45-μm pore-sized enclosed filters (Millipore) using a peristaltic pump and kept at $-20°$C until further processing. All the material used in the filtration process was cleansed with bleach (10%) and Milli-Q water between filtrations to prevent cross contaminations. In total, 52 samples from 8 vertical profiles (13 from the surface and 8 from each 50, 200, 500, 1000, and > 1000 m depth) were collected (Rodríguez-Ezpeleta 2021).

DNA extraction and metabarcoding

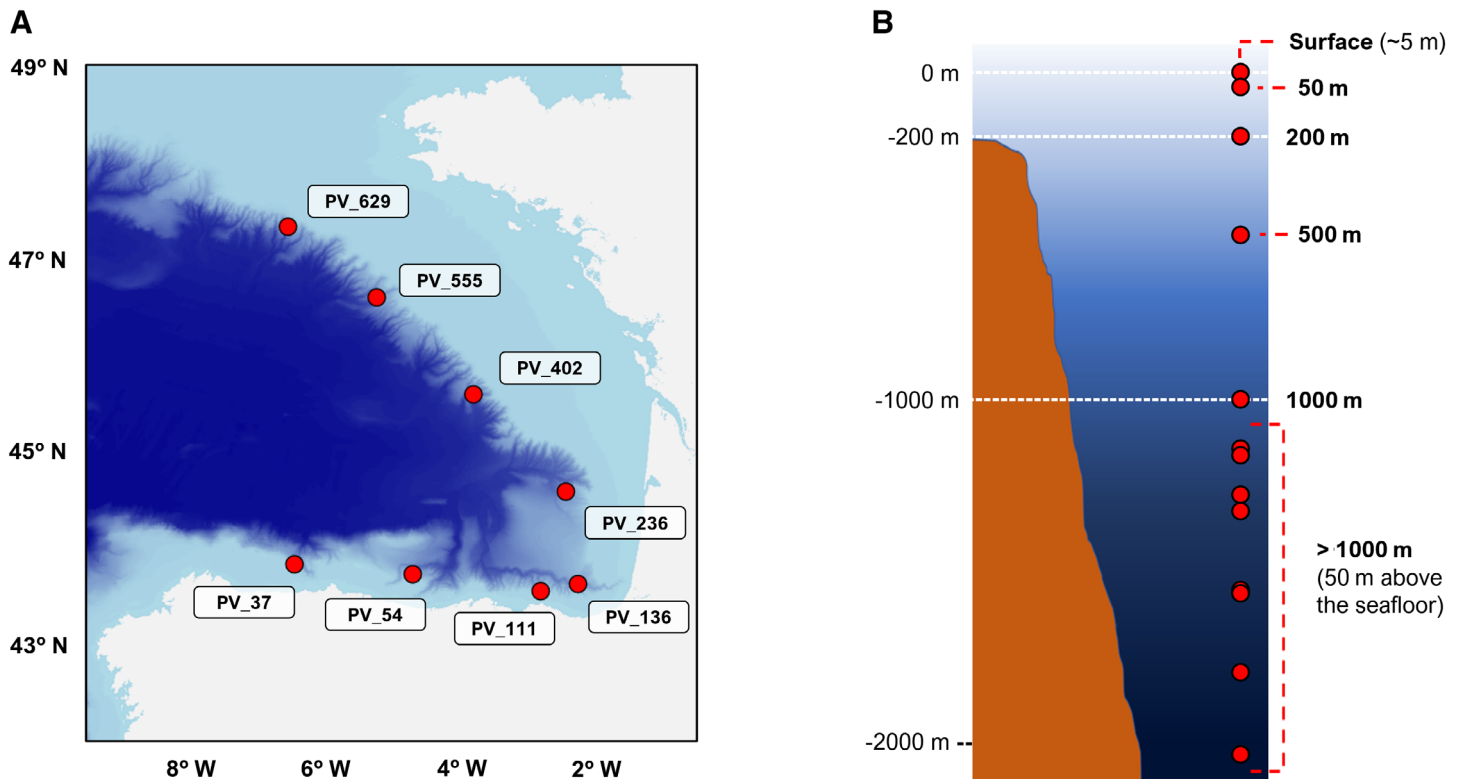Laboratory and bioinformatic analysis procedures for eDNA metabarcoding were performed as described in Fraija-

A

B

Fig. 1. (**A**) Map of the Bay of Biscay displaying the eight stations from where samples used in this study were collected. (**B**) Depths at which samples were collected in each of the eight vertical profiles.

Fernández et al. (2020) and are detailed in Supporting Information. Briefly, a short region of the *12S* ribosomal RNA gene (thereinafter *tele01*; Valentini et al. 2016) was amplified from the extracted eDNA and sequenced. Polymerase chain reaction (PCR) mixtures were prepared under the hood in the pre-PCR laboratory using dedicated micropipettes and disposable plastic ware that were previously decontaminated under the UV light, and all postamplification steps were carried out in the post-PCR laboratory. Resulting sequences were quality filtered and taxonomically assigned using an in-house curated database from Fraija-Fernández et al. (2020) restricted to the fish species (Myxini, Petromyzonti, Holocephali, Elasmobranchii, and Actinopterygii) expected in the Northeast Atlantic and Mediterranean areas. Because our aim was to compare depths and not locations, and in order to avoid biases due to different sequencing intensity across samples, raw reads from samples from the same depth were pooled into one integrated sample. Only reads assigned to the species and genus levels were considered for subsequent analyses, and those reads assigned to species of the same genus were grouped. Those fish species whose individuals are known to inhabit below 200 m depth (including demersal species exhibiting a wide range of depth tolerance such as *Lophius piscatorius*) were classified as deep sea based on FishBase (http://www.fishbase.org/).

Results

Fish diversity in the continental slope of the Bay of Biscay

Overall, eDNA metabarcoding analyses identified 52 fish genus/species (47 Actinopterygii and 5 Elasmobranchii) (Supporting Information Table S2) representing 96.8% of the reads, suggesting that the reference data set covered most of the fish diversity in the area. The European anchovy (*Engraulis encrasicolus*) was the most abundant species (Supporting Information Table S3), as in previous studies conducted in the same area (Fraija-Fernández et al. 2020). Almost half of species (25), accounting for 14% of the reads, were classified as deep-sea fish (Fig. 2); among them, *L. piscatorius*, *Maurolicus muelleri*, *Xenodermichthys copei*, *Benthosema glaciale*, *Merluccius merluccius*, and *Micromesistius poutassou* representing 95.9% of the deep-sea fish reads, being widely spread along the water column, and detected at all depths (except *X. copei*, which was absent at 50 m depth). Interestingly, also the most abundant epipelagic fish species were detected at all depths along the water column (Fig. 3).

Deep-sea fish vertical structuring

An overall approach to fish vertical structuring assessed by eDNA metabarcoding showed an increase in deep-sea fish relative richness (proportion of deep-sea species related to total
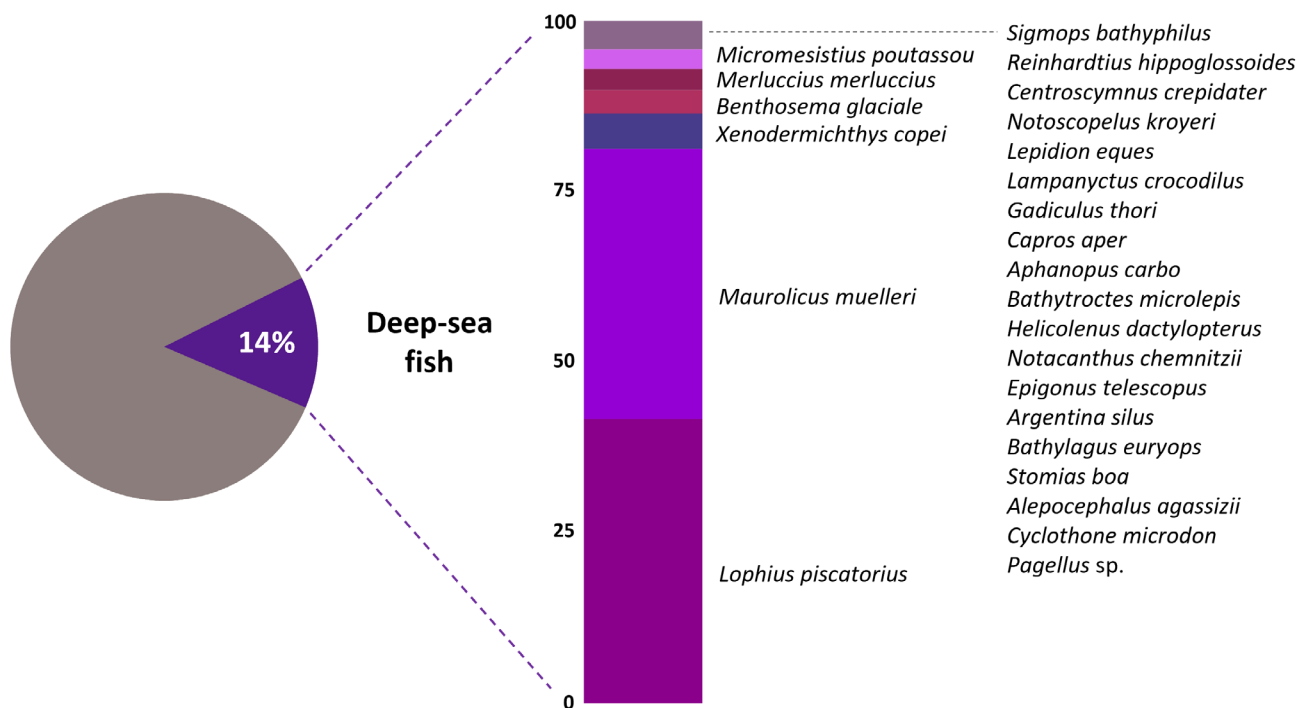
Fig. 2. Proportion of reads belonging to deep-sea fish and within, relative abundance of each deep-sea fish species detected, ordered from the most (bottom) to the least (top) abundant.

fish species) with depth (Supporting Information Fig. S1). Deep-sea fish represented approximately 35% of the richness in the epipelagic zone and over 50% in the mesopelagic and bathypelagic layers. In terms of abundance (percentage of deep-sea fish reads related to total fish reads per layer), deep-sea species accounted for less than 10% of relative abundance at the surface, and progressively increased to the mesopelagic zone, where it represented between 27% and 32% of fish abundance. The relative abundance of deep-sea species slightly dropped at > 1000 m depth, accounting for about 17% of reads.

A more detailed analysis revealed that the vertical distribution patterns of deep-sea fish richness and abundance inferred from eDNA differed during day and night (Fig. 4), which agrees with the diel vertical migratory behavior of several deep-sea species. In daylight-collected samples, deep-sea fish abundance peaked between 500 and 1000 m depth (representing 33% and 39% of fish reads, respectively), but their DNA signal remarkably moved upward the water column at night, reaching over 75% of total fish reads in the epipelagic zone (50 and 200 m depth). Accordingly, daylight-collected samples showed an increase in deep-sea fish relative richness from 500 m depth, while the increase in deep-sea species richness moved up to 200 m depth in samples taken at night.

Fish species-specific DVM behavior

Noticeable, day–night variations along the water column emerged not only at the community level but also at the species level (Supporting Information Fig. S2), indicating that eDNA metabarcoding data could be providing valuable insights on deep-sea fish species ecology and behavior. For instance, our eDNA-based data revealed evident diel vertical migratory behavior for *M. muelleri* and *B. glaciale*, and lack of vertical migratory patterns but a distribution restricted to the low mesopelagic and/or bathypelagic layers during both day and night for *Cyclothone microdon* (Fig. 5).

Discussion

Fish eDNA stratification in the ocean responds to fish vertical distribution

Despite the complexity of the vertical structuring of pelagic communities (Sutton 2013) and the call for studies focusing on the deep-sea ecosystem (Mengerink et al. 2014; St. John et al. 2016; Martin et al. 2020), to date just a few studies have used eDNA sampling to explore the deep sea (Thomsen et al. 2016; Easson et al. 2020; Laroche et al. 2020; McClenaghan et al. 2020; Govindarajan et al. 2021), and none has provided insights on the deep, vertical stratification of eDNA in the ocean. Here, we observed a consistent detection of DNA of the most abundant epipelagic fish species along the water column (e.g., European anchovy is detected down to > 1000 m depth), whereas detection of DNA from deep-sea fish was restricted to the upper depth at which they were assumed to occur and below. This implies that in the open ocean fish eDNA can be detected at the depth where it was
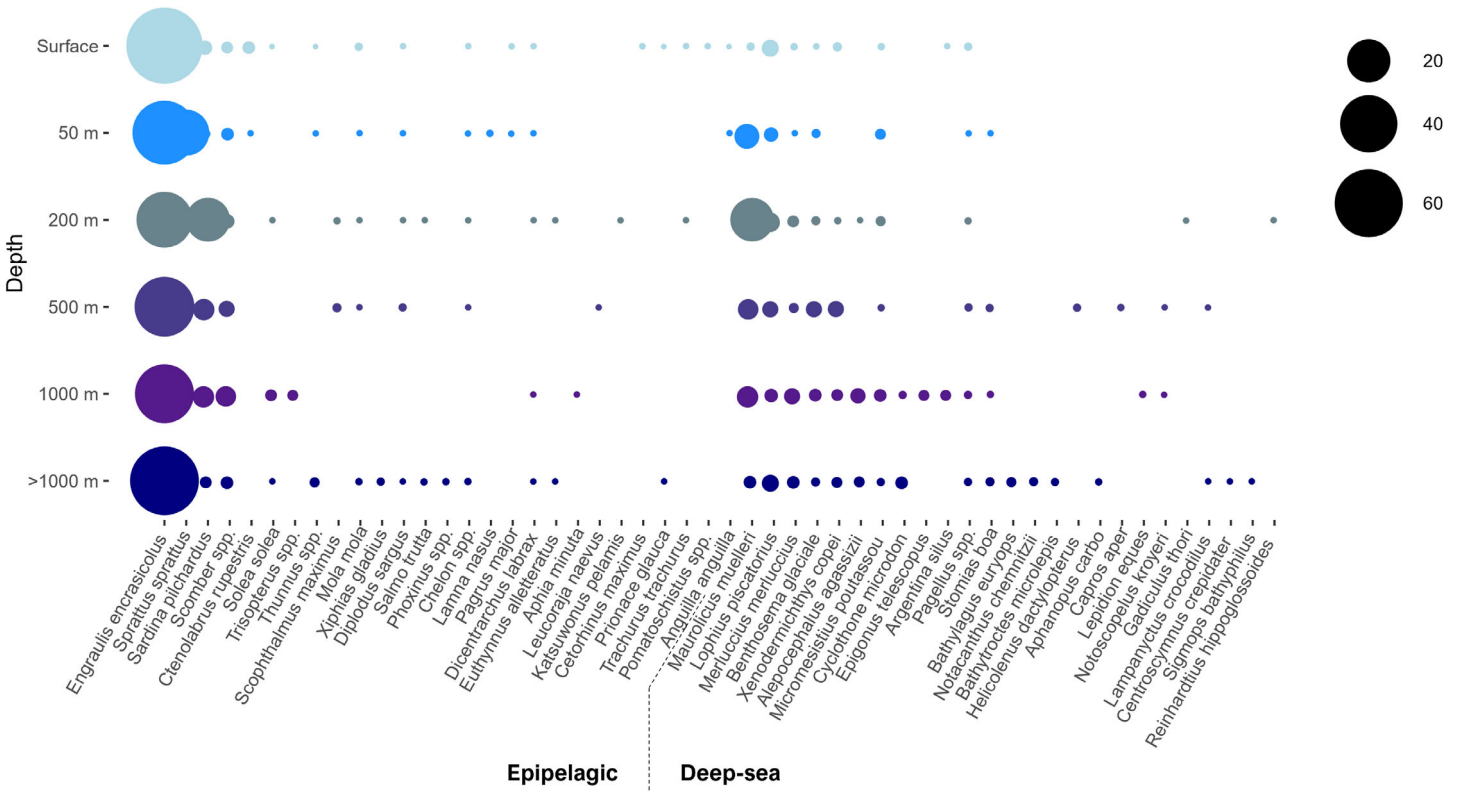
Fig. 3. Vertical distribution of all fish species detected, where the size of the bubbles indicates the relative abundance in percentage of each species at a given depth.
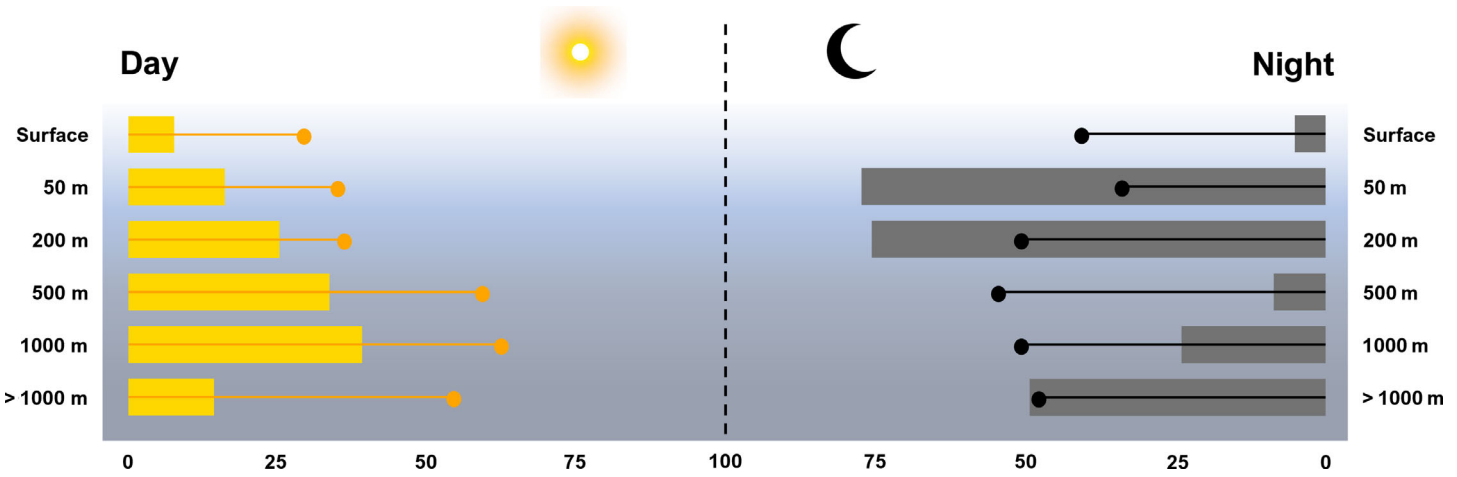

Fig. 4. Relative abundance (bars, inferred from number of reads) and relative richness (dots) of deep-sea fish at each depth during day (left) and night (right).

released and deeper, but rarely shallower. Although these findings hamper ecological conclusions regarding the oceanic vertical distribution of epipelagic species based on eDNA vertical profiles, they highlight the potential of water column eDNA metabarcoding for the study of deep-sea organism ecology,

such as diversity, spatial distribution, and their upper depth limit in the vertical gradient.

Detection and concentration of eDNA in the ocean are known to be influenced by transportation and degradation processes (Hansen et al. 2018). Here, our findings point to a
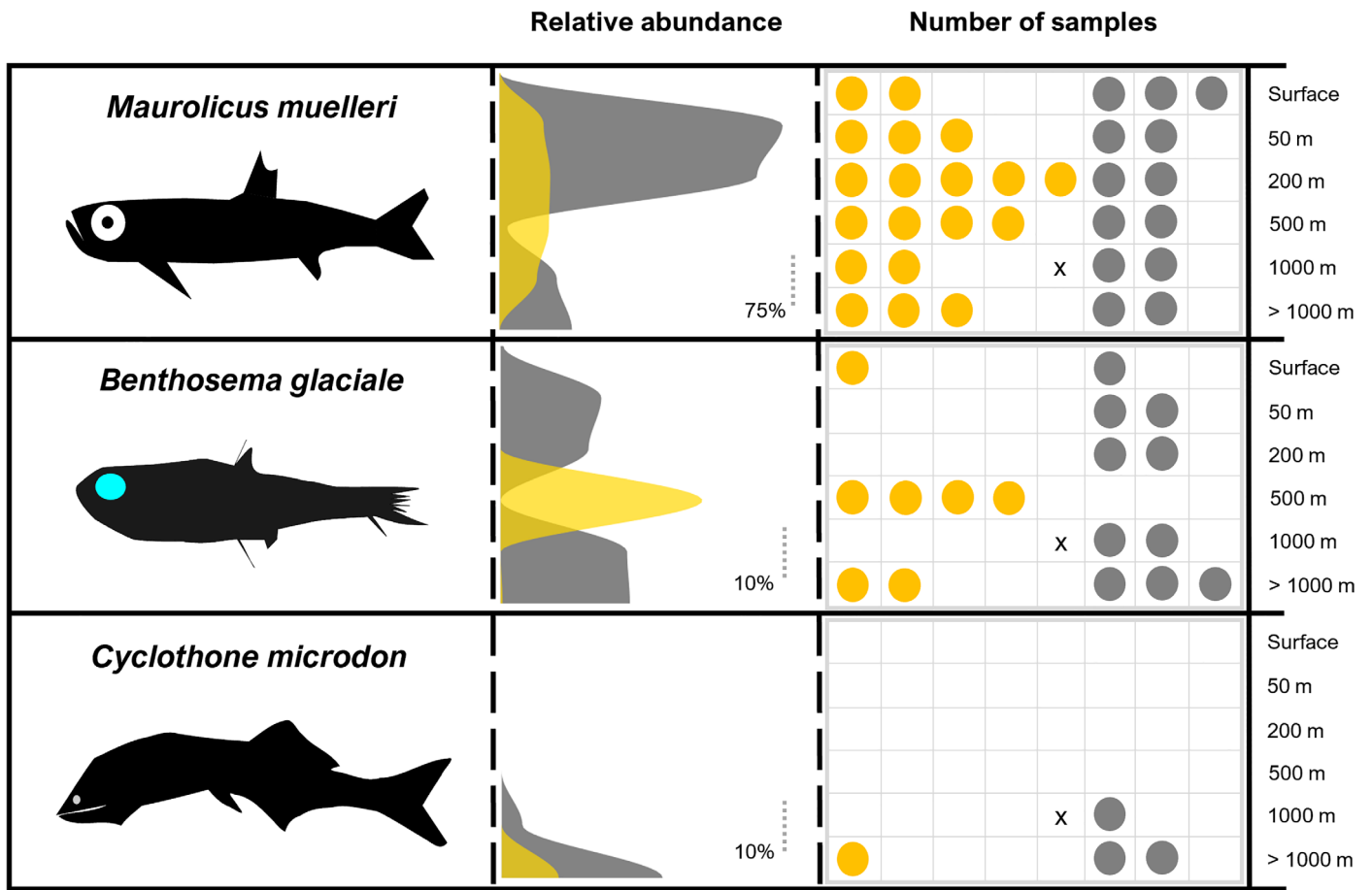
Fig. 5. Vertical distribution of *Maurolicus muelleri*, *Benthosema glaciale*, and *Cyclothone microdon* during day (gold) and night (gray) inferred from the percentage of reads (relative abundances) of each species at each depth studied, and the number of samples per depth at which these species were detected. A cross indicates that no data are available for that sample. Note that the depth gradient represented in the *y*-axis of the figures is not linear.

remarkable sinking-mediated vertical transport of fish eDNA along the oceanic water column, which would explain the consistent detection of epipelagic species up to the bathypelagic zone. These findings are in line with the global sinking process of particulate organic matter in the ocean, according to which fish-released DNA is expected to sink from upper to deeper layers—either attached to or being part of larger particulates such as feces, corpses, or scales, among others (Turner 2002). In this regard, previous studies have reported fish fecal pellets to present sinking rates ranging from hundreds to few thousands m d^{-1} (Saba and Steinberg 2012), that is, feces produced at the surface are expected to reach the mesopelagic zone in less than a day and, in some cases, even arrive to the bathypelagic realm in the same lapse of time. Hence, it may be deduced that the picture of fish community assessed by eDNA metabarcoding in the surface or at epipelagic depths is informative of the species present at the sampling point between the time of sampling and some hours before. The same principle could be also applied for deep-sea fish at least at the upper depth at which they are detected,

although this assumption should be further confirmed by performing diurnal and nocturnal vertical profiles from the same sampling site. It should be considered that other sources of eDNA, such as mucus or cells, may present different sinking rates than feces, and be differently affected by transportation and degradation processes. Further studies evaluating this issue are therefore needed for a better understanding of the eDNA behavior in the ocean. Noteworthy, following Preston et al. (2020), our findings open a lead to estimate fish species-specific contribution to the particulate carbon flux based on eDNA.

eDNA can infer fish diel vertical migratory behavior

Probably, the most relevant, unexpected, and promising finding of the present study is the observation that eDNA metabarcoding is not only able to detect shifts at the community level derived by the DVM phenomena as suggested (Easson et al. 2020), but to reveal species-specific diel vertical migratory patterns. Here, the most evident cases emerged for *B. glaciale* and *M. muelleri*. During daylight, *B. glaciale* DNA

was mainly found at 500 m depth and practically absent at upper layers, whereas at night, the molecular signal of *B. glaciale* moved upwards the water column, being widely detected at epipelagic depths. This clearly suggests DVM of *B. glaciale* from the deep sea to the epipelagic zone at dark, in agreement with previous knowledge on the ecology of this species (Olivar et al. 2012). *M. muelleri* DNA appeared highly spread along the water column during both day and night, but exhibited remarkable abundance peaks during dark-time at 50 and 200 m depth. Although it has been shown that the amount of eDNA shed into the environment by fish correlates with fish biomass (Klymus et al. 2015; Salter et al. 2019; Yates et al. 2021), interpreting metabarcoding data should consider that proportion of reads indicate relative and not absolute abundances, and that these relative abundances are influenced by changes in abundance of the whole community but also by technical factors such as PCR biases. Also, it has been demonstrated that eDNA shedding rates are influenced by diet, feeding activity—being up to 10-fold times higher in fed fishes than in starving ones—and life-cycle stage of the individuals (Klymus et al. 2015; Hansen et al. 2018). Thus, the noticeable high relative abundance of *M. muelleri* DNA at epipelagic depths during night may not only indicate a higher biomass but also elevated feeding activity, in line with the existing ecological knowledge on this species; *M. muelleri* is a very abundant sternoptychid in the Bay of Biscay, well known to perform DVM in big, compact schools to feed close to the surface after the sunset (Sobradillo et al. 2019).

Considerations for eDNA-based studies in oceanic vertical profiles

Notably, our eDNA-based data did not display vertical migratory patterns for some species known to migrate, that is, *Lampanyctus crocodilus* or *Notoscopelus kroyeri*. Also, some species were detected only once or twice in the data set (e.g., *Notacanthus chemnitzi*) and/or exclusively in day-collected vertical profile samples (*Stomias boa*). Since most of these species presented very low abundances, the inconsistencies in their diel vertical migratory patterns and/or their scarce detection is probably explained by the relative low number of reads of these species in comparison to the highly abundant ones. In light of these findings, further studies should focus on enriching for deep-sea species or on increasing sequencing depths to obtain insightful information about low abundant species. In addition, natural processes such as biological interactions or geological events can influence eDNA-derived inferences on fish vertical distribution. For instance, (1) the predation of sharks, dolphins, and other non-diel vertical migratory species on deep-sea fish, whose activity and high mobility could lead to detection of deep-sea fish DNA at shallow depths, (2) the effect of active convective cells, which can increase the vertical mixing of the water column (and therefore of fish eDNA) up to 700 m depth (Backhaus et al. 2003), and (3) the release of accumulated

DNA in marine sediments—where DNA is more concentrated and much longer conserved (Turner et al. 2015)—during submarine landslides (Canals et al. 2006). Finally, the completeness and accuracy of the reference database (Jerde 2019) and, in some cases, the limited taxonomic resolution of the *tele01* barcode (Collins et al. 2019) may also influence fish eDNA-based assessments; for instance, the detection of *Argentina silus* in our study instead of or in addition to the more abundant *Argentina sphyraena* due to the lack of references in public repositories of the latter.

Notwithstanding, applying eDNA metabarcoding still offers remarkable advantages for the study of deep-sea ecosystems: it surpasses the depth-related orographic limitations imposed to trawling, and provides (1) a higher diversity detection capability (Fraija-Fernández et al. 2020; Stoeckle et al. 2021), facilitating the monitoring of threatened, invasive, elusive, and rare species (Jerde 2019; Bani et al. 2020)—for instance, we detected here six fish species considered at least as vulnerable by the International Union for Conservation of Nature (Supporting Information Table S3)—(2) a lower dependency on taxonomic expertise (Hansen et al. 2018), (3) a reduction in costs (Jerde 2019; Bani et al. 2020), and (4) a minimum habitat destruction and organism disturbance (Valentini et al. 2016; Bani et al. 2020).

Outlook

Protecting and preserving the remote deep-sea ecosystem is crucial to secure the global biogeochemical functioning of the planet, but techniques to monitor this environment, which is the basis of the development of protective measures, are lacking (St. John et al. 2016; Martin et al. 2020). Our findings support eDNA-based monitoring as a ready-to-use approach to acquire insights on this underexplored yet threatened environment as they provide evidence that the routine eDNA sample collection could lead to a significant advance in the understanding of deep-sea organismal ecology, including diversity, vertical structuring, and species-specific diel vertical migratory behavior. The incorporation of eDNA sampling to fisheries surveys has been shown to be economically affordable (Stoeckle et al. 2021), and the collection of vertical profile samples should not imply any important delay or reschedule of onboard activities (taking vertical profile samples with a rosette down to 2000 m depths takes between 1.5 and 2 h long). This extra effort certainly constitutes a small price to pay in exchange of acquiring the urgently needed knowledge on deep-sea environments to establish the basis of a sustainable extraction of its resources.

References

Backhaus, J. O., E. N. Hegseth, H. Wehde, X. Irigoien, K. Hatten, and K. Logemann. 2003. Convection and primary production in winter. Mar. Ecol. Prog. Ser. **251**: 1–14. doi: 10.1016/bs.aecr.2020.01.011

Bani, A. & others 2020. Chapter ten—informing marine spatial planning decisions with environmental DNA, p. 375–407. In A. J. Dumbrell, E. C. Turner, and T. M. Fayle [eds.]. Advances in ecological research: Academic Press.

Canals, M., P. Puig, X. D. de Madron, S. Heussner, A. Palanques, and J. Fabres. 2006. Flushing submarine canyons. Nature **444**: 354–357.

Collins, R. A., and others. 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. Methods Ecol. Evol. **10**: 1985–2001. doi:10.1111/2041-210X.13276

Davison, P., D. Checkley Jr., J. Koslow, and J. Barlow. 2013. Carbon export mediated by mesopelagic fishes in the Northeast Pacific Ocean. Prog. Oceanogr. **116**: 14–30. doi:10.1016/j.pocean.2013.05.013

DiBattista, J. D., and others. 2019. Digging for DNA at depth: Rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. PeerJ **7**: e6379–e6379. doi:10.7717/peerj.6379

Duarte, C. M., and others. 2020. Rebuilding marine life. Nature **580**: 39–51. doi:10.1038/s41586-020-2146-7

Easson, C. G., K. M. Boswell, N. Tucker, J. D. Warren, and J. V. Lopez. 2020. Combined eDNA and acoustic analysis reflects diel vertical migration of mixed consortia in the Gulf of Mexico. Front. Mar. Sci. **7**: 552. doi:10.3389/fmars.2020.00552

Food and Agriculture Organization (FAO). 2018. The State of World Fisheries and Aquaculture 2018—Meeting the sustainable development goals (Report No. ISBN 978-92-5-130562-1). Report by United Nations (UN).

Fraija-Fernández, N., and others. 2020. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. Ecol. Evol. **10**: 7560–7584. doi:10.1002/ece3.6482

Gilbey, J., and others. 2021. Life in a drop: Sampling environmental DNA for marine fishery management and ecosystem monitoring. Mar. Policy **124**: 104331. doi:10.1016/j.marpol.2020.104331

Govindarajan, A. F., and others. 2021. Exploring the use of environmental DNA (eDNA) to detect animal taxa in the mesopelagic zone. Front. Ecol. Evol. **9**: 574877. doi:10.3389/fevo.2021.574877

Hansen, B. K., D. Bekkevold, L. W. Clausen, and E. E. Nielsen. 2018. The sceptical optimist: Challenges and perspectives for the application of environmental DNA in marine fisheries. Fish Fish. **19**: 751–768.

Irigoien, X., and others. 2014. Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nat. Commun. **5**: 1–10.

Jerde, C. L. 2019. Can we manage fisheries with the inherent uncertainty from eDNA? J. Fish Biol. **98**: 341–353.

Klevjer, T. A., X. Irigoien, A. Røstad, E. Fraile-Nuez, V. M. Benítez-Barrios, and S. Kaartvedt. 2016. Large scale patterns in vertical distribution and behaviour of mesopelagic scattering layers. Sci. Rep. **6**: 19873.

Klymus, K. E., C. A. Richter, D. C. Chapman, and C. Paukert. 2015. Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp *Hypophthalmichthys molitrix*. Biol. Conserv. **183**: 77–84.

Laroche, O., O. Kersten, C. R. Smith, and E. Goetze. 2020. Environmental DNA surveys detect distinct metazoan communities across abyssal plains and seamounts in the western Clarion Clipperton Zone. Mol. Ecol. **29**: 4588–4604.

Littlefair, J. E., L. E. Hrenchuk, P. J. Blanchfield, M. D. Rennie, and M. E. Cristescu. 2021. Thermal stratification and fish thermal preference explain vertical eDNA distribution in lakes. Mol. Ecol. **30**: 3083–3096. doi:10.1111/mec.15623

Martin, A., and others. 2020. The oceans' twilight zone must be studied now, before it is too late. Nature **580**: 26–28. doi:10.1038/d41586-020-00915-7

McClenaghan, B., and others. 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. PLoS 1 **15**: e0236540. doi:10.1371/journal.pone.0236540

Mengerink, K. J., and others. 2014. A call for deep-ocean stewardship. Science **344**: 696–698. doi:10.1126/science.1251458

Olivar, M. P., and others. 2012. Vertical distribution, diversity and assemblages of mesopelagic fishes in the western Mediterranean. Deep-Sea Res. I Oceanogr. Res. Pap. **62**: 53–69. doi:10.1016/j.dsr.2011.12.014

Preston, C. M., C. A. Durkin, and K. M. Yamahara. 2020. DNA metabarcoding reveals organisms contributing to particulate matter flux to abyssal depths in the North East Pacific Ocean. Deep-Sea Res. II Top. Stud. Oceanogr. **173**: 104708.

Rodríguez-Ezpeleta, N. 2021. Metadata of "Vertical stratification of environmental DNA in the open ocean captures ecological patterns and behavior of deep-sea fishes". *Zenodo*. doi:10.5281/zenodo.4543255. Dataset accessed 16 February 2021.

Saba, G. K., and D. K. Steinberg. 2012. Abundance, composition and sinking rates of fish fecal pellets in the Santa Barbara Channel. Sci. Rep. **2**: 1–6.

Salter, I., M. Joensen, R. Kristiansen, P. Steingrund, and P. Vestergaard. 2019. Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. Commun. Biol. **2**: 461.

Sobradillo, B., G. Boyra, U. Martinez, P. Carrera, M. Peña, and X. Irigoien. 2019. Target strength and swimbladder morphology of Mueller's pearlside (*Maurolicus muelleri*). Sci. Rep. **9**: 17311.

St. John, M. A., and others. 2016. A dark hole in our understanding of marine ecosystems and their services: Perspectives from the mesopelagic community. Front. Mar. Sci. **3**: 31.

Stoeckle, M. Y., J. Adolf, Z. Charlop-Powers, K. J. Dunton, G. Hinks, and S. M. VanMorter. 2021. Trawl and eDNA

assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. ICES J. Mar. Sci. **78**: 293–304.

Sutton, T. T. 2013. Vertical ecology of the pelagic ocean: Classical patterns and new perspectives. J. Fish Biol. **83**: 1508–1527.

Thomsen, P. F., J. Kielgast, L. L. Iversen, P. R. Møller, M. Rasmussen, and E. Willerslev. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS One **7**: e41732.

Thomsen, P. F., P. R. Møller, E. E. Sigsgaard, S. W. Knudsen, O. A. Jørgensen, and E. Willerslev. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS One **11**: e0165252.

Thurber, A. R., A. K. Sweetman, B. E. Narayanaswamy, D. O. Jones, J. Ingels, and R. Hansman. 2014. Ecosystem function and services provided by the deep sea. Biogeosciences **11**: 3941–3963. doi:10.5194/bg-11-3941-2014

Turner, C. R., K. L. Uy, and R. C. Everhart. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol. Conserv. **183**: 93–102.

Turner, J. T. 2002. Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat. Microb. Ecol. **27**: 57–102.

Valentini, A., and others. 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol. Ecol. **25**: 929–942. doi:10.1111/mec.13428

Yates, M. C., D. M. Glaser, J. R. Post, M. E. Cristescu, D. J. Fraser, and A. M. Derry. 2021. The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. Mol. Ecol. **30**: 3068–3082.