

Sargasso Sea Report

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Environmental DNA (eDNA) refers to genetic material obtained directly from environmental samples, such as water, soil, or air, without direct observation of the target organisms (Taberlet *et al.*, 2012). This DNA originates from cellular material shed by organisms into their surroundings through processes like excretion, reproduction, skin sloughing, or decomposition (Thomsen & Willerslev, 2015). Once collected, eDNA can be extracted through a variety of methods and analysed using molecular techniques such as quantitative PCR (qPCR) or high-throughput sequencing (HTS), enabling researchers to identify the presence of individual species or assess entire communities.

The use of eDNA has revolutionised biodiversity monitoring across terrestrial, freshwater, and marine ecosystems. Evidence suggests that eDNA reflects the organisms present in proximity, both in time and space, to the location sampled (Shelton *et al.*, 2019), while its non-invasive nature makes it particularly valuable for detecting rare, elusive, or endangered species with minimal disturbance (Goldberg *et al.*, 2016). eDNA surveys are often more sensitive than traditional methods such as visual surveys, netting, or trapping, especially in aquatic environments where detecting low-density or cryptic species is difficult (Bohmann *et al.*, 2014; Deiner *et al.*, 2017). Furthermore, eDNA metabarcoding allows for simultaneous detection of multiple taxa from a single sample, offering a cost-effective and scalable tool for ecological research, conservation, and biosecurity (Pawlowski *et al.*, 2018; Pochon *et al.*, 2025).

The Sargasso Sea is a globally significant, yet understudied, high seas ecosystem characterised by its clear, oligotrophic waters and floating *Sargassum* mats (Fig. 1), which provide essential habitat for a wide range of marine organisms (Laffoley *et al.*, 2011). It serves as a nursery, migratory corridor, and spawning ground for ecologically and economically important species, including humpback whales, tuna, eels, billfish, and sea turtles (Freestone, 2021; Laffoley *et al.*, 2011; Miller *et al.*, 2015; Roe *et al.*, 2022). However, the remoteness and vastness of the region pose major challenges for traditional biodiversity assessments. eDNA offers a transformative solution for marine biodiversity monitoring in the Sargasso Sea by providing a non-invasive, efficient, and high-resolution approach to detecting species and assessing community composition across spatial and temporal scales (Adams *et al.*, 2023). By applying eDNA metabarcoding in the Sargasso Sea, researchers can detect a broad spectrum of taxa. These data are essential for supporting conservation and management in areas beyond national jurisdiction, particularly under frameworks such as the Sargasso Sea Commission and emerging BBNJ (Biodiversity Beyond National Jurisdiction) treaty negotiations (Tilot *et al.*, 2021; Dunn *et al.*, 2022). As pressures from climate change, shipping, and high seas fisheries intensify, eDNA can help fill critical knowledge gaps and inform evidence-based strategies for the protection of this unique oceanic ecosystem.



Figure 1. The Arctic Sunrise above a bed of floating *Sargassum* mat.

Methods

Study Site

eDNA sampling was conducted during a Greenpeace research expedition in the Sargasso Sea from 2-5 May 2024. Water samples were collected from ten offshore sampling stations along an ~810 km transect between Bermuda and the Bahamas (Figure 2; Table 1). At each station water was sampled at both the surface (0 m) and 30 m depth, except for Station 1, which was sampled only at 30 m. A total of three replicate water samples were collected per depth, resulting in a total of 57 discrete water samples.

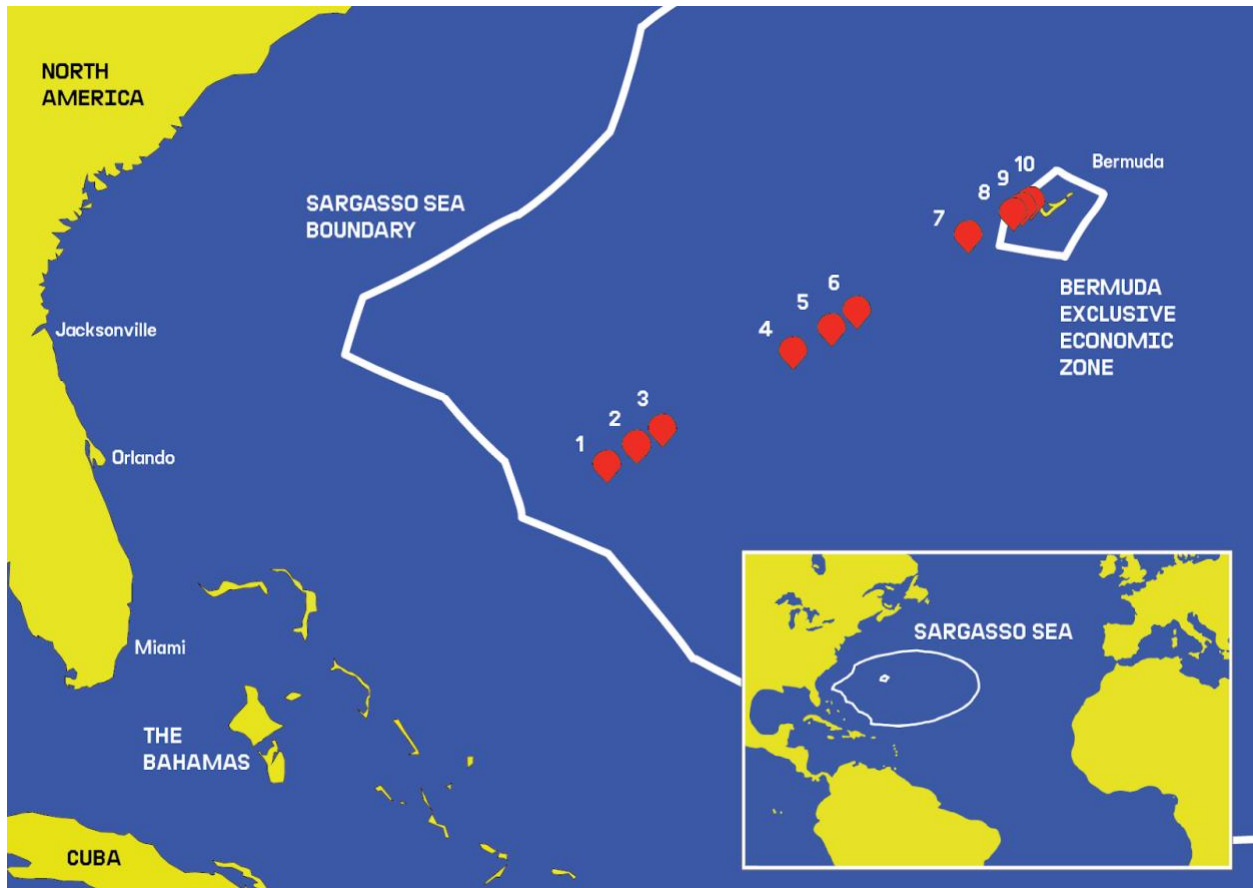


Figure 2. Map of sampling station locations within the Sargasso Sea.

Table 1. Latitude and longitude co-ordinates for each of the 10 sampling stations.

Station Number	Latitude	Longitude
1	28° 10.411' N	072° 13.294' W
2	28° 27.114' N	071° 45.049' W
3	28° 41.647' N	071° 19.413' W
4	29° 48.022' N	069° 11.633' W
5	30° 09.1' N	068° 32.2' W
6	30° 23.178' N	068° 07.940' W
7	31° 29.213' N	066° 03.260' W
8	31° 58.78' N	065° 12.2' W
9	32° 07.956' N	065° 02.015' W
10	32° 02.560' N	065° 05.252' W

eDNA Sample Collection

At each station, seawater was collected using a handheld niskin bottle deployed either from the Greenpeace vessel Arctic Sunrise (Fig. 3a) or from a rigid inflatable boat (RHIB) (Fig. 3b). Subsamples of seawater (300 mL per replicate) (Fig. 4) were filtered onsite using sterile 0.22 µm Sterivex filter cartridges (Fig 5). After filtration, filters were sealed, labelled, and immediately frozen (Fig 6). Samples were stored at -20°C during transit and later at -80°C at the University of Edinburgh until DNA extraction.

(a)



(b)



Figure 3. eDNA water sampling being conducted by handheld niskin aboard (a) the Arctic Sunrise and (b) an inflatable RHIB.



Figure 4. Seawater collection from the handheld niskin after deployment.



Figure 5. Seawater being passed through a Sterivex 0.22 mm filter unit by hand using a 300 ml syringe.

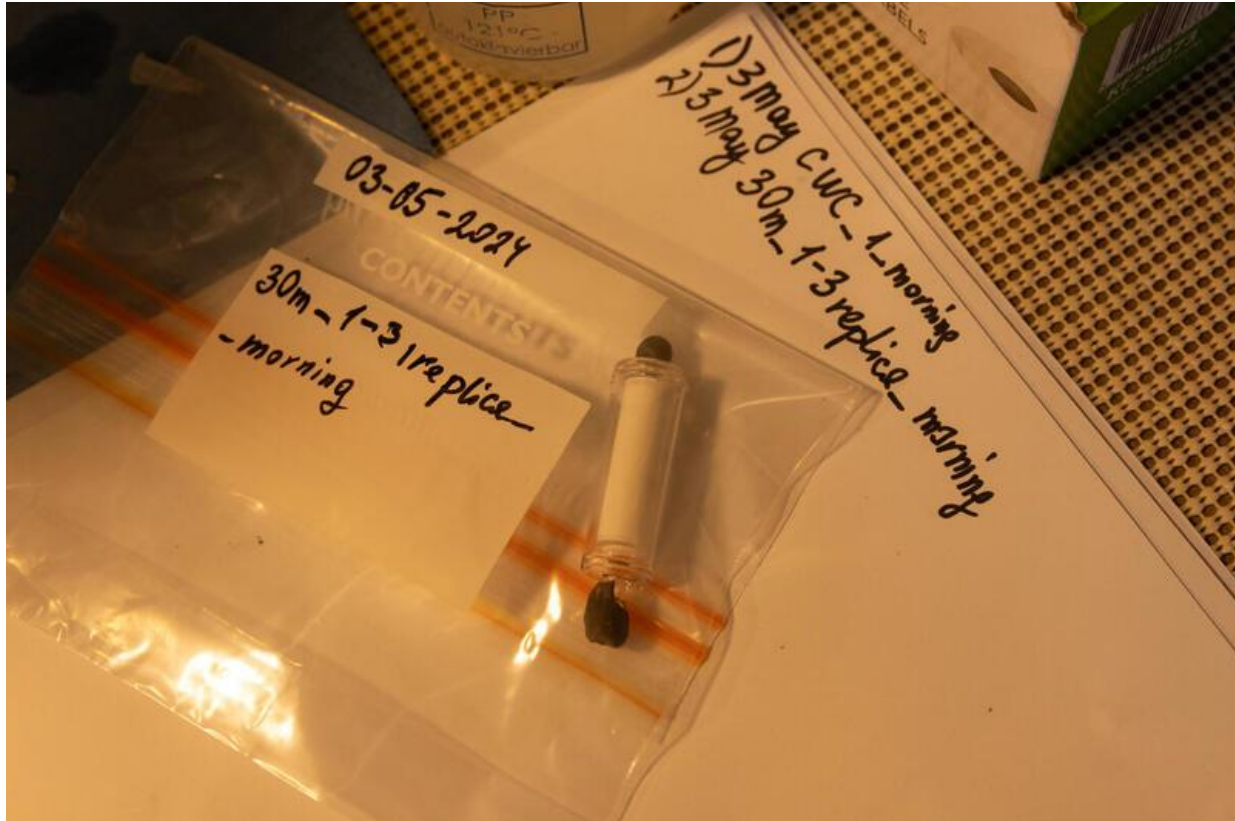


Figure 6. Final sealed Sterivex 0.22 mm filter unit in a labelled bag ready for freezing at -20°C .

Laboratory Processing

DNA was extracted from filters using a modified protocol based on the Qiagen DNeasy Blood & Tissue Kit. Extraction steps were conducted under sterile conditions to minimise contamination, and DNA yields were quantified using a Qubit fluorometer. To maximise species detection, three different mitochondrial 12S primer sets were used which targeted teleosts, mammals, and general vertebrates (Table 2). Each sample underwent PCR amplification for all three markers, and resulting amplicons were pooled in equimolar volumes to create an indexed DNA library. The library was quality-checked and sequenced on an Illumina MiSeq platform (2×150 bp paired-end) by Edinburgh Genomics.

Table 2. Primer pairs used along with target gene region, target group, primer sequence (5'-3'), and reference.

Primer name	Gene region	Target group	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	Reference
Tele01	12S	Teleosts	ACACCGCCCGTCACTCT	CTTCCGGTACTTACCATG	Valentini <i>et al.</i> , 2016
Mamm01	12S	Mammals	CCGCCCGTCACYCTCCT	GTAYRCTTACCWTGTTACGAC	Taberlet <i>et al.</i> , 2018
Vert01	12S	Vertebrates	TAGAACAGGCTCCTCTAG	TTAGATACCCCACTATGC	Taberlet <i>et al.</i> , 2018

Bioinformatics and Data Analysis

Raw sequencing data were quality-checked with FastQC (v0.12.1), trimmed using the truncLen parameter in DADA2 (v1.16), and denoised with DADA2 (v1.16). Amplicon sequence variants (ASVs) were assigned taxonomy using BLAST searches against the NCBI nucleotide (nt) database (downloaded on 15 August 2024), with supplementary checks against the Ocean Biodiversity Information System (OBIS) to ensure ecological plausibility. Species-level assignments were made for sequences with $\geq 98\%$ similarity, with more conservative thresholds used for higher taxonomic ranks. A strict filtering pipeline was applied to remove low-confidence reads, potential contaminants, and non-marine taxa. Only ASVs with ≥ 100 reads and not detected in any control samples were retained for analysis. Diversity metrics, including Shannon and Simpson indices, were performed in Phyloseq (v1.50) to assess variation in community structure by site and depth.

Results

A total of approximately 40 unique vertebrate taxa were detected across the stations sampled using the selected primer pairs (Table 2). These included a diverse array of teleost fishes from at least 14 families, such as Exocoetidae (flying fish), Myctophidae (lanternfish), Carangidae (jacks and trevallies), and Scombridae (tunas and mackerels). Species-level assignments were achieved for many taxa, including *Histrio histrio* (Sargassum frogfish), *Thunnus albacares* (yellowfin tuna), and *Cyclothone microdon* (veiled anglemouth), while others could only be resolved to genus or order level (e.g. *Exocoetus sp.*, *Anguilliformes indet.*). Marine mammals were also detected, with ASV's matching five cetacean and pinniped species including *Tursiops truncatus* (bottlenose dolphin) and *Otaria byronia* (South American sea lion). Most species were categorised as Least Concern on the IUCN Red List, although detections of *Caranx hippos* (Data Deficient) and *Salmo salar* (Near Threatened) highlight the value of eDNA for monitoring conservation-relevant taxa.

Table 2. List of taxa detected using primer pairs Vert01, Tele01, and Mamm01. The abbreviation “indet.” stands for indeterminate. In these cases, the lowest taxonomic rank that could be assigned was order. * denotes only one known occurrence in the area according to the species IUCN profile.

Scientific name	Common name	IUCN status
Actinopterii		
Antennariidae	Frogfishes	
<i>Histrio histrio</i>	Sargassum frogfish	Least Concern ¹
Bramidae	Pomfrets	
<i>Pteraclis carolinus</i>	Fanfish	Not Evaluated
Carangidae	Jacks, trevallies, and scads	
<i>Caranx hippos</i>	Crevalle jack	Data Deficient ²
Clupeidae	Herrings	

<i>Clupea harengus</i>	Atlantic herring	Least Concern ³
<i>Clupea sp.</i>		
Congridae	Conger and garden eels	
<i>Bathyrcongery vicinus</i>	Large-toothed conger	Least Concern ⁴
Emmelichthyidae	Bonnetmouths and rubyfishes	
<i>Plagiogeneion rubiginosum</i>	Rubyfish	Least Concern ⁵
Exocoetidae	Flyingfish	
<i>Exocoetus monocirrhus</i>	Barbel flyingfish	Not Evaluated
<i>Exocoetus volitans</i>	Tropical two-wing flyingfish	Least Concern ⁶
<i>Hirundichthys rondeletii</i>	Blackwing flyingfish	Least Concern ⁷
<i>Hirundichthys speculiger</i>	Mirrorwing flyingfish	Least Concern ⁸
<i>Exocoetus sp.</i>		
Gempylidae	Snake mackerels	
<i>Nealotus tripes</i>	Black snake mackerel	Least Concern ⁹
Gonostomatidae	Bristlemouths	
<i>Cyclothone microdon</i>	Veiled anglemouths	Least Concern ¹⁰
<i>Cyclothone sp.</i>		
Mullidae	Goatfishes	
<i>Pseudupeneus maculatus</i>	Spotted goatfish	Least Concern ¹¹
Myctophidae	Lanternfish	
<i>Ceratoscopelus warmingii</i>	Warming's lanternfish	Least Concern ¹²
<i>Ceratoscopelus sp.</i>		
<i>Hygophum hygomii</i>	Bermuda lanternfish	Least Concern ¹³
<i>Hygophum macrochir</i>	Large-finned lanternfish	Least Concern ¹⁴
<i>Lampanyctus sp.</i>		
<i>Nannobranchium sp.</i>		
Nemichthyidae	Snipe eels	

<i>Nemichthys curvirostris</i>	Pale threadtail snipe eels	Least Concern ¹⁵
Salmonidae	Salmons	
<i>Salmo salmar</i>	Atlantic salmon	Near Threatened ¹⁶
Scombridae	Tunas and mackerels	
<i>Acanthocybium solandri</i>	Wahoo	Least Concern ¹⁷
<i>Auxis thazard</i>	Frigate tuna	Least Concern ¹⁸
<i>Thunnus albacares</i>	Yellowfin tuna	Least Concern ¹⁹
Serranidae	Sea basses and groupers	
<i>Epinephelus sp.</i>		
Stomiidae	Dragonfishes	
Anguilliformes indet.	Eels	
Aulopiformes indet.	Grinners and lizardfishes	
Beryciformes indet.	Ridgeheads	
Labriformes indet.	Wrasses	
Myctophiformes indet.	Lanternfish	
Mammalia		
Cetacea		
Delphinidae	Dolphins	
<i>Stenella attenuata</i>	Pantropical spotted dolphin	Least Concern ²⁰
<i>Tursiops truncatus</i>	Bottlenose dolphin	Least Concern ²¹
Physeteridae	Pygmy and dwarf sperm whales	
<i>Kogia breviceps</i>	Pygmy sperm whale	Least Concern ²²
Ziphiidae	Beaked whales	
<i>Mesoplodon europaeus</i>	Gervais' beaked whale	Least Concern ²³
Carnivora		
Otariidae	Sea lions	
<i>Otaria byronia</i> *	South American sea lion	Least Concern ²⁴

Alpha diversity, measured using the Shannon and Simpson indices, varied notably across stations and between depths (0 m and 30 m) (Figure 7).

Alpha diversity is a measure of species diversity in a sample. It accounts for both the richness (the number of different species present) and evenness (distribution of species abundance). A higher alpha diversity value indicates a species rich and varied community, whereas low a value suggests fewer species or dominance by one or two taxa.

The Shannon index combines richness and evenness into a single value, with higher alpha diversity values indicating more diverse and evenly distributed communities. The Simpson Diversity index also measures diversity but gives greater weight to the most common species. It reflects the probability that two randomly selected individuals from a sample belong to different species, and therefore higher alpha diversity values means greater species diversity and lower dominance by one or two taxa.

At 0m, Shannon diversity was generally low, with near-zero values at stations 1, 2, 3, 8, 9, and 10 (Fig. 7). In contrast, stations 4-7 showed moderate to high Shannon values (Fig. 7), suggesting a richer and more evenly distributed community. Simpson values followed a similar trend but were slightly higher in some low-richness samples. This indicates that even when few taxa are present, the sample is not entirely dominated by a single species.

At 30 m depth, Simpson diversity values were consistently high across stations 3-4, 6, 8-10 (Fig. 7), indicating more evenly distributed communities overall. However, Shannon diversity at depth was more variable, with station 2 standing out as the most diverse (Fig. 7). Although 30 m samples consistently showed higher evenness across stations, as indicated by Simpson diversity, the total number of species detected was not always higher than at 0m.

These results suggest that deeper samples tend to support more evenly balanced communities, as indicated by high Simpson diversity values across stations. However, they were not consistently richer in species, with only station 2 showing higher taxonomic diversity (Fig. 7). This highlights clear vertical differences in community structure between surface and deeper waters.

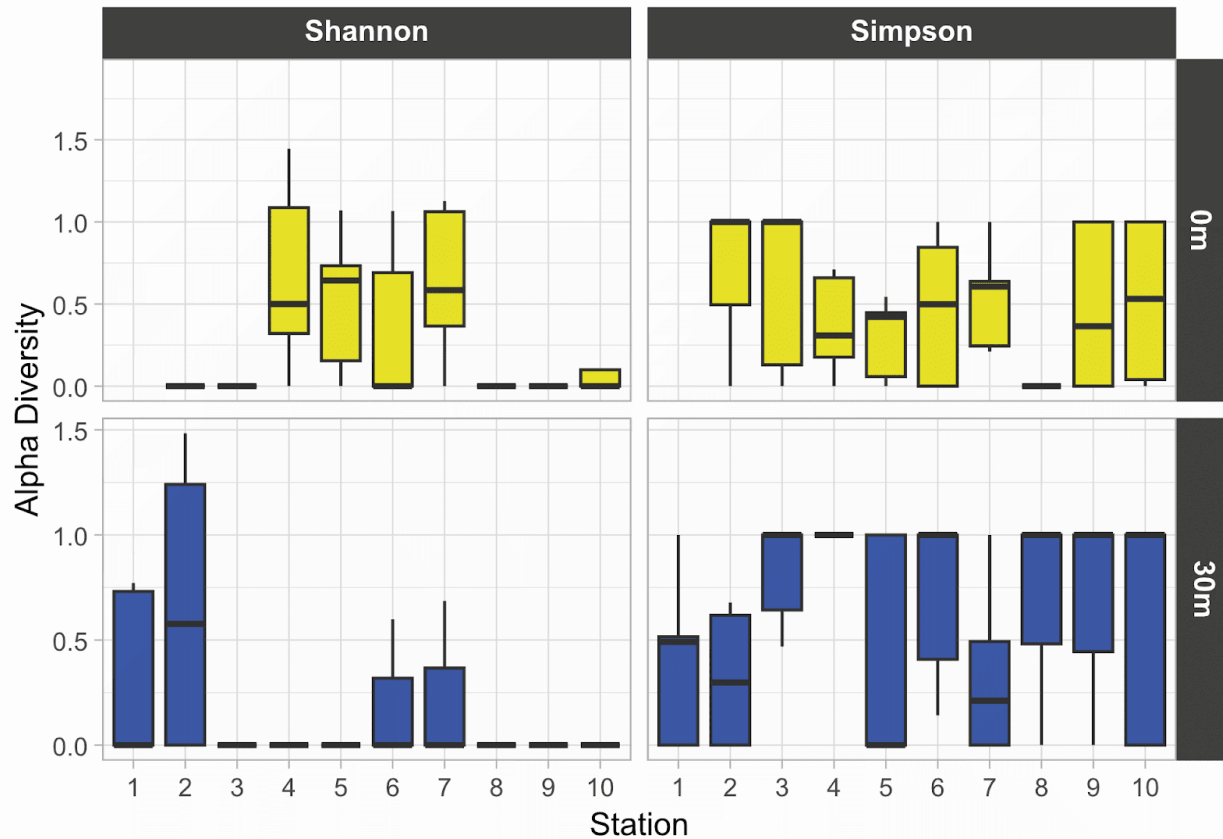


Figure 7. Shannon and Simpson Alpha Diversity for each station along the transect and separated by depth for all three primer pairs combined.

Discussion

eDNA metabarcoding revealed a broad range of marine vertebrate species. In total, approximately 40 unique vertebrate taxa were identified, representing a diverse range of ecological niches and trophic levels from surface-associated flying fish to deep-sea lanternfish (Table 3). The detection of marine mammals such as the bottlenose dolphin (*Tursiops truncatus*), pantropical spotted dolphin (*Stenella attenuata*), and Gervais' beaked whale (*Mesoplodon europaeus*) further highlights the sensitivity of this method for detecting both widespread and elusive species in marine environments.

Many detected taxa were resolved to species level, however instances where assignment was limited to genus or order level was often a result of limitations in reference databases or genetic similarity between closely related taxa. This continues to underscore an ongoing need to expand genetic databases, especially for oceanic regions like the Sargasso Sea, to improve taxonomic resolution and thus interpretability of subsequent eDNA datasets.

Several species detected are ecologically and economically important within the region. Groups such as tunas (*T. albacares*) and flying fish (*Exocoetidae* sp.) play critical roles in oceanic food webs and support commercial and subsistence fisheries throughout the Atlantic. Additionally, the detection of species listed by the IUCN as Near Threatened (*S. salar*) or Data Deficient (*Caranx hippos*) also highlights the value of eDNA for early-warning conservation monitoring in areas where traditional biological surveys are difficult or infeasible. Some species detected such as the South American sea lion (*O. byronia*) fall outside of known geographic ranges and therefore should be interpreted with caution. However, they may indicate the

presence of close relatives or insufficient representation in current reference data for the region rather than an incorrect detection. Such findings reflect the need for local taxonomic validation alongside eDNA methods.

Alpha diversity patterns differed notably between depths. At the surface (0 m), Shannon diversity was generally low, with near-zero values at stations 1–3 and 8–10 (Fig. 7). In contrast, stations 4–7 showed moderate to high Shannon values (Fig. 7), indicating a more species rich and evenly distributed community. Simpson diversity followed a similar trend at many stations but was notably higher at stations 2, 3, 5, 6, 9, and 10 (Fig. 7), suggesting that even when species richness was low, the few species present were more evenly represented, and no single species was dominating the community.

At 30 m depth, Simpson diversity values were consistently high at stations 1, 3, 4, 6, and 8–10 (Fig. 7), indicating that samples taken from deeper depth generally contained communities with more evenly distributed species. However, Shannon diversity at 30 m was highly variable. Only station 2 showed a clear increase in both species' richness and evenness, while most other stations at this depth exhibited relatively low Shannon values. These findings indicate that although samples from 30 m generally supported more evenly balanced communities, they did not consistently contain a greater number of taxa compared to surface samples.

This depth-related variation may reflect both ecological and environmental factors. Many pelagic and mesopelagic species occupy specific depths, and vertical stratification of the water column can influence how species and eDNA signals are distributed (Allan *et al.*, 2021). In addition, environmental conditions such as light, temperature (Andruszkiewicz Allan *et al.*, 2021; Barnes *et al.*, 2014; McCartin *et al.*, 2023), and UV (Collins *et al.*, 2018; Strickler *et al.*, 2015) can affect how long eDNA persists in the water column which subsequently determines how representative each sample is of the local community.

This study also demonstrates the effectiveness of multiple primer sets (Vert01, Tele01, and Mamm01) in capturing broad taxonomic coverage showcasing the utility of multi-marker approaches for biodiversity monitoring (Ferreira *et al.*, 2024). However, it is important to note that some primers may amplify non-target species or perform differently across taxa due to biases such as amplification efficiency during PCR (Kelly *et al.*, 2019). This reinforces the importance of method validation, primer evaluation, and marker selection tailored to the specific monitoring goals and taxonomic focus of the area of interest (Collins *et al.*, 2019).

These results highlight the value of eDNA as a non-invasive and practical tool for assessing biodiversity in open-ocean environments. In remote regions like the Sargasso Sea, where conventional surveys are costly, time-consuming, and spatially limited, eDNA can offer an efficient way to record species presence, detect rare or seasonal species, and monitor changes over time.

The application of eDNA in the Sargasso Sea contributes directly to the Socio-Ecosystem Diagnostic Analysis (SEDA) by helping to characterise which species are present and how marine life is distributed across the region. These findings are especially relevant for supporting the Sargasso Sea Commission's efforts to promote stewardship and protection of this globally significant high seas ecosystem. As the region continues to face increasing environmental pressures such as climate change, fishing, and maritime activity, tools like eDNA will be key to tracking biological responses, informing management decisions, and ensuring the long-term resilience of Sargasso Sea ecosystems.

In summary, this study highlights the value of incorporating eDNA into broader marine monitoring frameworks. eDNA complements traditional survey methods by capturing a wide range of species, including elusive or difficult-to-detect taxa, and strengthens our ability to monitor biodiversity in areas where traditional methods of data collection can be limiting. Applying eDNA in the Sargasso Sea illustrates its value for detecting species, potential for monitoring ecological change, and subsequently its ability to inform evidence-based policy, support regional conservation efforts, and improve long-term monitoring of ocean environments.

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References

- Adams, C. I. M., Jeunen, G.-J., Cross, H., Taylor, H. R., Bagnaro, A., Currie, K., Hepburn, C., Gemmill, N. J., Urban, L., Baltar, F., Stat, M., Bunce, M., & Knapp, M. (2023). Environmental DNA metabarcoding describes biodiversity across marine gradients. *ICES Journal of Marine Science: Journal Du Conseil*, *80*(4), 953–971. <https://doi.org/10.1093/icesjms/fsad017>
- Allan, E. A., DiBenedetto, M. H., Lavery, A. C., Govindarajan, A. F., & Zhang, W. G. (2021). Modeling characterization of the vertical and temporal variability of environmental DNA in the mesopelagic ocean. *Scientific Reports*, *11*(1), 21273. <https://doi.org/10.1038/s41598-021-00288-5>
- Andruszkiewicz Allan, E., Zhang, W. G., C. Lavery, A., & F. Govindarajan, A. (2021). Environmental DNA shedding and decay rates from diverse animal forms and thermal regimes. *Environmental DNA (Hoboken, N.J.)*, *3*(2), 492–514. <https://doi.org/10.1002/edn3.141>
- Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819–1827. <https://doi.org/10.1021/es404734p>
- Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*(6), 358–367. <https://doi.org/10.1016/j.tree.2014.04.003>
- Collins, R. A., Wangensteen, O. S., O’Gorman, E. J., Mariani, S., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology*, *1*, 185. <https://doi.org/10.1038/s42003-018-0192-6>
- Collins, R. A., Bakker, J., Wangensteen, O. S., Soto, A. Z., Corrigan, L., Sims, D. W., Genner, M. J., & Mariani, S. (2019). Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution*, *10*(11), 1985–2001. <https://doi.org/10.1111/2041-210X.13276>
- Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., de Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895. <https://doi.org/10.1111/mec.14350>
- Dunn, D. C., Crespo, G. O., Vierros, M., Freestone, D., & Halpin, P. N. (2022). The BBNJ agreement: Building a strong foundation for marine genetic resources and area-based management tools. *Marine Policy*, *135*, 104860. <https://doi.org/10.1016/j.marpol.2021.104860>
- Ferreira, A. O., Azevedo, O. M., Barroso, C., Duarte, S., Egas, C., Fontes, J. T., Ré, P., Santos, A. M. P., & Costa, F. O. (2024). Multi-marker DNA metabarcoding for precise species identification in ichthyoplankton samples. *Scientific Reports*, *14*(1), 19772. <https://doi.org/10.1038/s41598-024-69963-7>

Freestone, D. (2021). The Sargasso Sea Commission: An evolving new paradigm for high seas ecosystem governance? *Frontiers in Marine Science*, 8, 668253. <https://doi.org/10.3389/fmars.2021.668253>

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2016). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, 183, 1–3. <https://doi.org/10.1016/j.biocon.2014.11.040>

Kelly, R. P., Shelton, A. O., & Gallego, R. (2019). Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Scientific Reports*, 9, 12133. <https://doi.org/10.1038/s41598-019-48546-x>

Laffoley, D., Roe, H. S. J., Angel, M. V., Ardron, J., Cavanagh, R. D., Cheung, W. W. L., ... & Rogers, A. D. (2011). The protection and management of the Sargasso Sea: The golden floating rainforest of the Atlantic Ocean. *Sargasso Sea Alliance*.

McCartin, L. J., Vohsen, S. A., Ambrose, S. W., Layden, M., McFadden, C. S., Cordes, E. E., McDermott, J. M., & Herrera, S. (2022). Temperature controls eDNA persistence across physicochemical conditions in seawater. *Environmental Science & Technology*, 56(12), 8629–8639. <https://doi.org/10.1021/acs.est.2c01672>

Miller, M. J., Bonhommeau, S., Munk, P., Castonguay, M., Hanel, R., & McCleave, J. D. (2015). A century of research on the larval distributions of the Atlantic eels: A re-examination of the data. *Biological Reviews*, 90(4), 1035–1064. <https://doi.org/10.1111/brv.12144>

Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2018). Environmental DNA: What's behind the term? Clarifying the terminology and applications of eDNA. *Molecular Ecology*, 27(22), 4258–4264. <https://doi.org/10.1111/mec.14742>

Pochon, X., Bowers, H. A., Zaiko, A., & Wood, S. A. (2025). Advancing the environmental DNA and RNA toolkit for aquatic ecosystem monitoring and management. *PeerJ*, 13, e19119. <https://doi.org/10.7717/peerj.19119>

Roe, H. S. J., Freestone, D., & Sapsford, F. (2022). The Sargasso Sea high seas EBSA after ten years: Is it still relevant and how has it helped conservation efforts? *Frontiers in Marine Science*, 9, 821182. <https://doi.org/10.3389/fmars.2022.821182>

Shelton, A. O., Kelly, R. P., O'Donnell, J. L., Park, L., Schwenke, P., Greene, C., Henderson, R. A., & Beamer, E. M. (2019). Environmental DNA provides quantitative estimates of a threatened salmon species. *Biological Conservation*, 237, 383–391. <https://doi.org/10.1016/j.biocon.2019.07.003>

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, 183, 85–92. <https://doi.org/10.1016/j.biocon.2014.11.038>

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. *Molecular Ecology*, 21(8), 1789–1793. <https://doi.org/10.1111/j.1365-294X.2012.05542.x>

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press. <https://doi.org/10.1093/oso/9780198767220.001.0001>

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, 183, 4–18. <https://doi.org/10.1016/j.biocon.2014.11.019>

Tilot, V., Durussel, C., & Laur, R. (2021). Marine biodiversity and genetic resources of the high seas: Science, policy, and governance. *Marine Policy*, 128, 104466. <https://doi.org/10.1016/j.marpol.2020.104466>

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., ... & Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, 25, 929–942. <https://doi.org/10.1111/mec.13428>

References for taxonomic table

1. McEachran, J.D., Polanco Fernandez, A. & Russell, B. 2015. *Histrio histrio* (errata version published in 2017). The IUCN Red List of Threatened Species 2015: e.T190183A115312798. <https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T190183A16510502.en>. Accessed on 17 July 2025.

2. Di Natale, A., Bilecenoglu, M., Bariche, M. & Massuti, E. 2011. *Caranx hippos* (Mediterranean assessment). The IUCN Red List of Threatened Species 2011: e.T190458A8798641. Accessed on 17 July 2025.

3. Herdson, D. & Priede, I.G. 2010. *Clupea harengus*. The IUCN Red List of Threatened Species 2010: e.T155123A4717767. <https://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T155123A4717767.en>. Accessed on 17 July 2025.

4. Smith, D. 2010. *Bathuroconger vicinus*. The IUCN Red List of Threatened Species 2010: e.T154673A4602802. <https://dx.doi.org/10.2305/IUCN.UK.2010-4.RLTS.T154673A4602802.en>. Accessed on 17 July 2025.

5. Al Abdali, F.S.H., Al Buwaiqi, B., Al Kindi, A.S.M., Ambuali, A., Borsa, P., Carpenter, K.E., Govender, A. & Russell, B. 2019. *Plagiogeneion rubiginosum*. The IUCN Red List of Threatened Species 2019: e.T123426979A123494667. <https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T123426979A123494667.en>. Accessed on 17 July 2025.

6. Collette, B.B., Singh-Renton, S., Robertson, R., Marechal, J., Aiken, K.A., Dooley, J., Oxenford, H., Pina Amargos, F. & Kishore, R. 2015. *Exocoetus volitans*. The IUCN Red List of Threatened Species 2015: e.T198566A15550246. <https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T198566A15550246.en>. Accessed on 17 July 2025.

7. Collette, B., Carpenter, K., Meliane, I. 2010. *Hirundichthys rondelarii*. The IUCN Red List of Threatened Species 2010: e.T183776A8175047. <https://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T183776A8175047.en>. Accessed on 17 July 2025.

8. Collette, B., Singh-Renton, S., Robertson, R., Marechal, J., Aiken, K.A., Dooley, J., Oxenford, H., Pina Amargos, F. & Kishore, R. 2015. *Hirundichthys speculiger*. The IUCN Red List of Threatened Species 2015: e.T190292A15551447. <https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T190292A15551447.en>. Accessed on 17 July 2025.

9.
Collette, B.B., Smith-Vaniz, W.F., Williams, J.T., Curtis, M., Pina Amargos, F. & Grijalba Bendeck, L. 2015. *Nealotus tripes*. The IUCN Red List of Threatened Species 2015: e.T16509242A16510887.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T16509242A16510887.en>. Accessed on 17 July 2025.
10.
Harold, A. 2015. *Cyclothone microdon*. The IUCN Red List of Threatened Species 2015: e.T190133A42691704.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T190133A42691704.en>. Accessed on 17 July 2025.
11.
Dooley, J., Aiken, K.A., Collette, B., Marechal, J., Pina Amargos, F., Kishore, R. & Singh-Renton, S. 2015. *Pseudupeneus maculatus*. The IUCN Red List of Threatened Species 2015: e.T16545086A16546282.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T16545086A16546282.en>. Accessed on 17 July 2025.
12.
Hulley, P. 2015. *Ceratoscopelus warmingii*. The IUCN Red List of Threatened Species 2015: e.T15598596A15603675.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T15598596A15603675.en>. Accessed on 17 July 2025.
13.
Hulley, P. 2015. *Hygophum hygomii*. The IUCN Red List of Threatened Species 2015: e.T198617A15583995.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T198617A15583995.en>. Accessed on 17 July 2025.
14.
Hulley, P. 2015. *Hygophum macrochir*. The IUCN Red List of Threatened Species 2015: e.T15598815A15603745.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T15598815A15603745.en>. Accessed on 17 July 2025.
15.
Tighe, K. 2015. *Nemichthys curvirostris*. The IUCN Red List of Threatened Species 2015: e.T199323A2582833.
<https://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T199323A2582833.en>. Accessed on 17 July 2025.
16.
Darwall, W.R.T. 2023. *Salmo salar*. The IUCN Red List of Threatened Species 2023: e.T19855A67373433.
<https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T19855A67373433.en>. Accessed on 17 July 2025.
17.
Collette, B.B., Graves, J. & Viera Hazin, F.H. 2023. *Acanthocybium solandri*. The IUCN Red List of Threatened Species 2023: e.T170331A46938521.
<https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T170331A46938521.en>. Accessed on 17 July 2025.
18.
Collette, B.B., Di Natale, A., Miller, S. & Pollard, D.A. 2023. *Auxis thazard*. The IUCN Red List of Threatened Species 2023: e.T170344A46651210.
<https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T170344A46651210.en>. Accessed on 17 July 2025.
19.
Collette, B.B., Boustany, A., Fox, W., Graves, J., Juan

- Jorda, M. & Restrepo, V. 2021. *Thunnus albacares*. The IUCN Red List of Threatened Species 2021: e.T21857A46624561.
<https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T21857A46624561.en>. Accessed on 17 July 2025.
- 20.
- Kiszka, J. & Braulik, G.T. 2018. *Stenella attenuata*. The IUCN Red List of Threatened Species 2018: e.T20729A50373009.
<https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T20729A50373009.en>. Accessed on 17 July 2025.
- 21.
- Wells, R.S., Natoli, A. & Braulik, G.T. 2019. *Tursiops truncatus* (errata version published in 2019). The IUCN Red List of Threatened Species 2019: e.T22563A156932432.
<https://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T22563A156932432.en>. Accessed on 17 July 2025.
- 22.
- Kiszka, J. & Braulik, G.T. 2020. *Kogia breviceps*. The IUCN Red List of Threatened Species 2020: e.T11047A50358334.
<https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T11047A50358334.en>. Accessed on 17 July 2025.
- 23.
- Pitman, R.L. & Brownell Jr., R.L. 2020. *Mesoplodon europaeus*. The IUCN Red List of Threatened Species 2020: e.T13245A50365198.
<https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T13245A50365198.en>. Accessed on 17 July 2025.
- 24.
- Cárdenas-Alayza, S., Crespo, E. & Oliveira, L. 2016. *Otaria byronia*. The IUCN Red List of Threatened Species 2016: e.T41665A61948292.
<https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T41665A61948292.en>. Accessed on 17 July 2025.