Regional Studies in Marine Science 91 (2025) 104536

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Unveiling deep-sea fish communities through eDNA analysis during extreme upwelling events in Pantar Strait, Indonesia

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ARTICLE INFO

Keywords:
eDNA
Deep-sea fish
Pantar Strait
Extreme upwelling

ABSTRACT

Extreme upwelling in the Pantar Strait is a unique phenomenon that occurs for 1–3 days between August and November, with a frequency of twice per day, each event lasting approximately one hour. Due to the dramatic drop in sea surface temperature (SST) by more than 10°C brought on by tidal forces, this phenomenon is known as an extreme upwelling event (EUE). The cold water is estimated to originate from a depth of approximately 1000 m. One of the major questions regarding this phenomenon is its biological aspect, specifically the composition of the deep-sea fish community that ascends along with the cold water. This study uses an environmental DNA (eDNA) analysis approach during EUEs to determine the abundance, distribution, diversity, and dominance of these fish communities. Given that morphological identification is challenging, eDNA offers an advantage by detecting DNA released into the environment (water) and carried to the surface during upwelling. This study found 323 different kinds of fish at three different times: before, during, and after the upwelling, by focusing on the 12S rRNA gene and sequencing using Third Generation Sequencing (TGS) methods. The study successfully identified several deep-sea fish species, such as *Benthosema fibulatum, Dasyscopelus asper, Diaphus garmani, Diaphus suborbitalis, and Diaphus watasei*, likely originating from depths of approximately >1000 m. This provides an indication of the source of the upwelled cold water. In the future, further research focusing on different taxa, such as invertebrates and microorganisms, could complement our understanding of the EUE phenomenon.

1. Introduction

Upwelling is one of the most significant physical processes driving primary production (Setiawan et al., 2019). It is an oceanographic phenomenon in which deep, cold water rises to the surface over an extended period (days to weeks), lifting water masses by 100 m or more (Kämpf and Chapman, 2016). Upwelling primarily occurs when winds blow across the ocean surface, pushing surface waters away from the coast and inducing Ekman transport. To compensate for the displaced surface water, deeper, nutrient-rich water rises from below. This upwelled water is typically colder, lowering sea surface temperatures (SST) by 2–4 °C along the coastline (Wirasatriya et al., 2019; 2020).

Upwelling has both positive and negative effects on fish communities. On the positive side, enhanced productivity is one of the most significant benefits. On the positive side, nutrient-rich waters stimulate phytoplankton growth, supporting zooplankton and larger fish populations, and attracting migratory and commercially important species (Sommer et al., 2002; Chang et al., 2023; Wen et al., 2023). However, rapid changes in temperature, salinity, and oxygen can stress non-adapted species, while excess nutrients may trigger algal blooms and hypoxia, with dissolved oxygen levels falling below critical thresholds (Lubitz et al., 2024; Marium et al., 2023; Walter et al., 2024).

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https://doi.org/10.1016/j.rsma.2025.104536
Received 24 June 2025; Received in revised form 9 September 2025; Accepted 2 October 2025

These conditions alter species composition, community structure, and trophic dynamics, ultimately affecting ecosystem stability (Block et al., 2011; Dabuleviciene et al., 2023).

Unlike typical upwelling events, the upwelling in the Mulut Kumbang Strait is classified as an extreme upwelling event (EUE) due to the extreme SST reduction by more than 10 °C, generated by tidal force (Wirasatriya et al., 2023;2025). The Mulut Kumbang Strait is part of Pantar Strait, with a narrow water channel, approximately 300 m wide and 900 m long (Fig. 1). During EUE, sea surface temperatures (SST) can drop drastically to below 15°C. This phenomenon occurs over a short duration, lasting 1–3 days between August and November, with a frequency of twice per day, each event lasting about one hour. This phenomenon effectively acts as a natural pump, transporting deep-sea water masses to the surface in one of the only known tropical locations with such drastic SST reductions.

Deep-sea fish communities consist of long-lived, slow-growing species with late maturity, low resilience to human impacts, and very slow recovery capacity (FAO, 2009; Huvenne et al., 2016; Clark et al., 2019; O' Hea et al., 2020; Grinyó et al., 2022). Physical factors such as temperature and bathymetry play a crucial role in shaping the distribution of marine species (Queirós et al., 2024), such as distribution and abundance (Jobling, 2010). The latest study related to deep-sea fish communities was conducted by Triay-Portella et al. (2023), who examined the structure and composition of deep-sea fish communities in the Canary Islands and found that the fish communities inhabiting the seabed of these islands are characterized by a high number of species. However, little is still known about deep-sea fish species.

Environmental DNA (eDNA) analysis offers a cost-effective, user-friendly, and environmentally friendly alternative for studying deep-sea biodiversity. eDNA is a highly effective tool for deep-sea exploration, especially given the high costs and difficulty of physical sampling. One key advantage is its non-invasive nature, allowing biodiversity assessment without capturing or disturbing organisms. By analyzing genetic material present in water or sediment, researchers can detect various species with minimal ecosystem disruption. eDNA consists of genetic traces released by organisms into their environment via urine, feces, mucus, skin, or degraded tissue (Bohman et al., 2014). Miya et al. (2020) and Thomsen and Willerslev (2015) highlight its reliability in biodiversity studies. Thomsen et al. (2012) further demonstrated that metabarcoding of seawater eDNA can detect marine fish diversity as effectively as, or better than, conventional survey methods. However, the challenge for deep-sea fish exploration using eDNA analysis is related to the sampling procedure that should take the water or sediment sample from the deep sea.

Deep-sea exploration presents significant challenges due to the vast ocean volume, depth, and distance from land. Traditional sampling methods rely on advanced remotely operated vehicles, underwater vehicles, and cable observatories (Rogers, 2015), which are costly. Taking advantage of EUE, the deep-sea water sample from the Pantar Strait can easily be taken from the surface water of the Mulut Kumbang Strait. We assume that the deep-cold water mass transported to the Mulut Kumbang Strait during EUE also brings the genetic materials from the deep basin of Pantar Strait.

Environmental DNA (eDNA) persistence varies across ecosystems, with its detectability influenced by environmental conditions. In aquatic systems, eDNA can remain present for several days to a week, especially

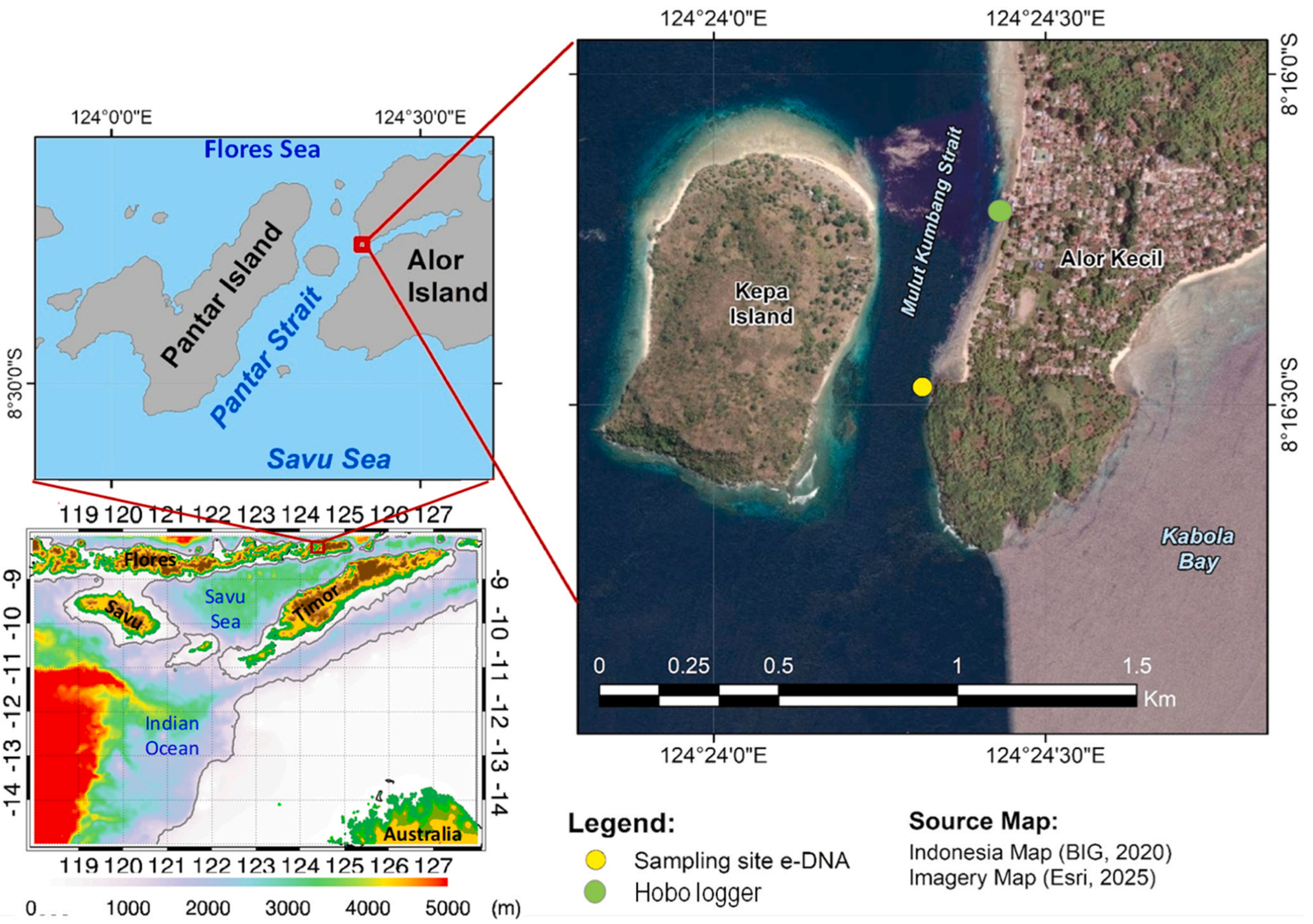

Fig. 1. Sampling sites for environmental DNA and HOBO logger deployment at Mulut Kumbang Strait, Alor Island, East Nusa Tenggara, Indonesia. The bathymetry data is taken from the ETOPO, with the gray contour representing 1000 m depth.

in colder waters where degradation processes are slower. Considering the relatively short timeframe of environmental upwelling events (EUE), these dynamics suggest that eDNA from deep-sea fish may still be detectable at the surface, providing a valuable opportunity to trace biological signals associated with upwelling (Allan et al., 2021; McCartin et al., 2022). Therefore, this study firstly examines the structure of deep-sea fish communities in the Pantar Strait using environmental DNA (eDNA) analysis during EUE to determine their abundance, distribution, diversity, and dominance.

2. Material and methods

2.1. Field sampling

This study was conducted at the coordinates of 8°16'27"S - 124°24'20"E (yellow circle at Fig. 1). Sampling was carried out on August 20, 2024 (before EUE), August 24, 2024 (during EUE), and August 25, 2024 (after EUE) and was repeated three times in each sampling time. Each replicate consisted of 1 L of seawater collected independently at the same site with a total of 9 samples that consisted of three times sampling (Before, During, and After EUE event). Then a total of 1 L of water was taken from the surface to a depth of approximately 10 cm from the surface with a sterile Nalgene bottle. The water was then filtered with filter paper with a pore diameter of 0.45 μm using a vacuum pump (Cahyani et al., 2024). The filter paper was then stored in an RNA shield and taken to the Diponegoro Biodiversity Project laboratory for further analysis.

For observing EUE, we deploy tide logger HOBO U20L to record tidal and temperature data minutely at Alor Kecil Pier (green circle in Fig. 1) from 21 to 25 August 2024 at 0.5 m below the lowest water. In addition, we also conducted in situ measurements by using a water quality checker to measure temperature and salinity at the same time and position as the water sampling point.

2.2. DNA extraction and amplification

DNA extraction from decanted samples was carried out using the ZymoBIOMICS™ Fecal and Soil Kit following the protocol provided. The extraction results were then quantified using a NanoDrop spectrophotometer before continuing with library preparation and sequencing (Cahyani et al., 2024).

We used PCR to get a specific DNA locus target (12S) for universal fish (Miya et al., 2015), with forward (5′-GTCGGTAAAACTCGTGCCAGC-3′) and reverse (5′-CATAGTGGGGTATCTAATCCCAGTTTG-3′) directions. The amplification process was carried out for 25 cycles consisting of pre-denaturation at 95°C for 10 min, denaturation at 94 °C for 30 s, annealing at 50°C for 30 s, extension at 72 °C for 1.5 min, and final extension at 72 °C for 10 min.

The PCR amplicon product was sequenced using the Oxford Nanopore Technology (ONT) MinION sequencing template. The DNA library was prepared following the manufacturers' protocols for Native Barcoding Kit 96 V14 (SQK-LSK114). Sequencing was done using the R10.4 flow cell (FLO-MIN114; Oxford Nanopore Technologies) for a total of 24 h.

2.3. Bioinformatics

The signal results from the sequencing process with Nanopore are basecalled using Dorado 0.8.1 (Wang et al., 2025) so that they can be translated into base sequences in FASTQ format. In this study, the Diponegoro Biodiversity Project (DBP) metabarcoding pipeline for nanopore data (Malik et al., 2025) was used. This pipeline is a single-script data processing workflow that begins with processing raw FASTQ data from nanopore basecalling. It includes several steps, such as quality filtering with NanoFilt, primer removal and length trimming with Cutadapt, dereplication, artifact detection from chimera, building an Operational Taxonomic Unit (OTU) table with VSEARCH, taxonomic assignment with Blastn, and building OTU and taxon tables for phyloseq objects.

During this process, all parameters were used, such as a Phred score greater than 10 (q-10) to remove low-quality bases (Delahaye and Nicolas, 2021; Liu-Wei et al., 2024) and sequence lengths between 150 bp and 200 bp in NanoFilt (De Coster et al., 2018; Maggini et al., 2024), as well as primer trimming in Cutadapt (Martin, 2011). The clustering process on the base sequence results was carried out with VSEARCH, achieving an Operational Taxonomic Unit (OTU) similarity level of 95 %. OTU results passed the taxonomy assignment process from the NCBI database using BLAST (blastn algorithm) with query coverage and taxon identification of 90 % each (Truelove et al., 2019; Munian et al., 2024; Malik et al., 2024) aligned with the Crux database (Gold et al., 2023).

The OTU results that have been identified are then filtered again with the rfishbase package (Boettiger et al., 2012) in RStudio so that the results are only animal species from Indonesian marine waters. The data visualization stage in RStudio begins with combining taxonomic results and OTU data with metadata into a phyloseq object with the phyloseq package (McMurdie and Holmes, 2013), then creating bar plots containing the composition of species that appear in conditions before, during, and after EUE, as well as creating alpha diversity (Shannon and Simpson) and beta diversity (Jaccard and Bray-Curtis). We employed these two approaches in beta diversity to cover species presence-absence data (Jaccard) and species abundance data (Bray-Curtis). The main purpose of the visualization is to view animal species composition when under conditions before, during, and after EUE. Statistical tests were also conducted in the form of ANOVA to compare alpha diversity, and PERMANOVA at locations with upwelling conditions.

3. Results

3.1. Extreme upwelling event during water sampling time

On 20 August 2024 at 14.00 WITA (local time, Eastern Indonesian Time Zone), the first water sampling was conducted to represent before the EUE. Despite the absence of HOBO logger data, in-situ measurement shows that on 20 August 2024, temperature and salinity were 27.2°C and 33 PPT, respectively (Fig. 2). On the 21st, the HOBO logger started to record, and the minimum temperature was 23.58°C at 12.40 WITA. On 22 August 2024, a significant temperature drop occurred twice at 00.51 WITA and 13.10 WITA, with the minimum temperatures of 16.71°C and 17.67°C, respectively. However, according to Wirasatriya et al. (2023), these temperature drops have not yet exceeded the EUE threshold, which is 15°C.

EUE occurred four times for 2 days on 23 and 24 August 2024. The minimum temperatures on 23 August 2024 were 12.79°C, and 13.75°C

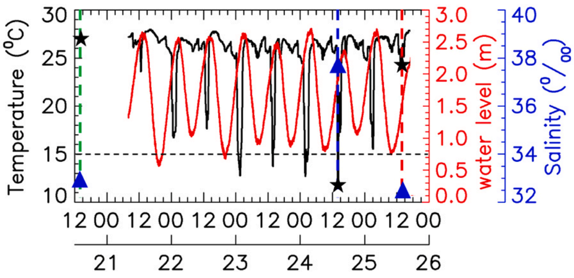

Fig. 2. Water sampling time in August 2024 along with the variation of temperature and tidal data recorded by the HOBO logger. Dashed green, blue, and red lines denote the sampling time for the period before EUE, during EUE, and after EUE, respectively. As stated by Wirasatriya et al. (2023), the dashed black line represents the EUE temperature threshold. The black star and blue triangle denote the in-situ measurement for temperature and salinity, respectively.

at 01.40 and 13.48 WITA, respectively. While on 24 August 2024, the minimum temperatures were 12.69°C and 11.72°C at 01.55 WITA and 14.06 WITA, respectively. The minimum temperature on 24 August 2024 at 14.06 WITA became the lowest temperature during EUEs in August 2024. Therefore, the second water sample was taken at this time to represent the EUE period. In situ measurement also shows that the temperature and salinity were 12°C and 37.5 PPT, respectively. The increasing salinity during EUE indicates that the cold water mass comes from the deeper layer, evidence of an upwelling event. Furthermore, tidal data shows that the EUE occurred twice a day during high tide following the semidiurnal cycle of the tide. These findings are in accordance with Wirasatriya et al. (2023). On 25 August 2024, the minimum temperatures were above the EUE threshold, and we took the third water sample at 14.00 WITA when the temperature and salinity were 26°C and 32.5 PPT to represent post-EUE period.

3.2. *eDNA analysis*

In general, the data generated from this study were 855,705 OTUs and a total of 6124,890 reads from a total of nine samples analyzed. The data consisted of the phylum Chordata and also Echinodermata. The phylum Chordata here also consists of the Actinopteri, Mammalia, and Chondrichthyes classes. This study focused on the Chordata classes, including dolphins and whales, because Sawu has the potential for migration routes for whales and dolphins. Thus, data filtering was carried out, which then produced a total of 859,146 reads.

3.3. *Taxa composition*

This study identified 36 orders, 71 families, 174 genera, and 323 species of class Actinopteri and Chondrichthyes from Pantar Strait. The number of species detected before EUE was 210, during EUE was 177, and after EUE was 215. The taxonomic composition at the order level is shown in Fig. 3, while the family-level composition (including only reads with an abundance greater than 2 %) is presented in Fig. 4.

Acanthuriformes, Perciformes, and Tetraodontiformes dominated taxa composition at the order level across upwelling conditions (Fig. 2). Before the EUE, Acanthuriformes (24.78 %) and Blenniiformes (24.01 %) were the most abundant. Acanthuriformes continued to be dominant during the EUE, with a relative abundance of 48.18 %, ahead of Clupeiformes (20.69 %) and Perciformes (11.60 %). After EUE, Perciformes became the most dominant order, increasing to 26.71 %, while Acanthuriformes declined to 21.09 %.

Acanthuridae and Anthiadidae are the families that consistently dominate during each EUE. Before EUE, the highest relative abundance was observed in Acanthuridae (23.84 %) and Blenniidae (22.48 %). During EUE, Acanthuridae (47.94 %) and Spratelloididae (20.57 %) showed the highest relative abundances. After EUE, the relative abundance was more evenly distributed among several families, including Anthiadidae (12.28 %), Epinephelidae (10.62 %), Muranidae (9.71 %), and Pomacentridae (8.25 %), with Acanthuridae (20.19 %) maintaining the highest relative abundance among the four families. Based on presence-absence data, Acanthuridae and Anthiadidae were the most commonly observed families across all upwelling conditions.

3.4. *Number of species detected in different EUE phases*

The Venn diagram illustrates the number of species detected during different phases of the EUE in the Mulut Kumbang Strait (Fig. 5). Out of a total of 323 species identified in this study, 96 species were recorded

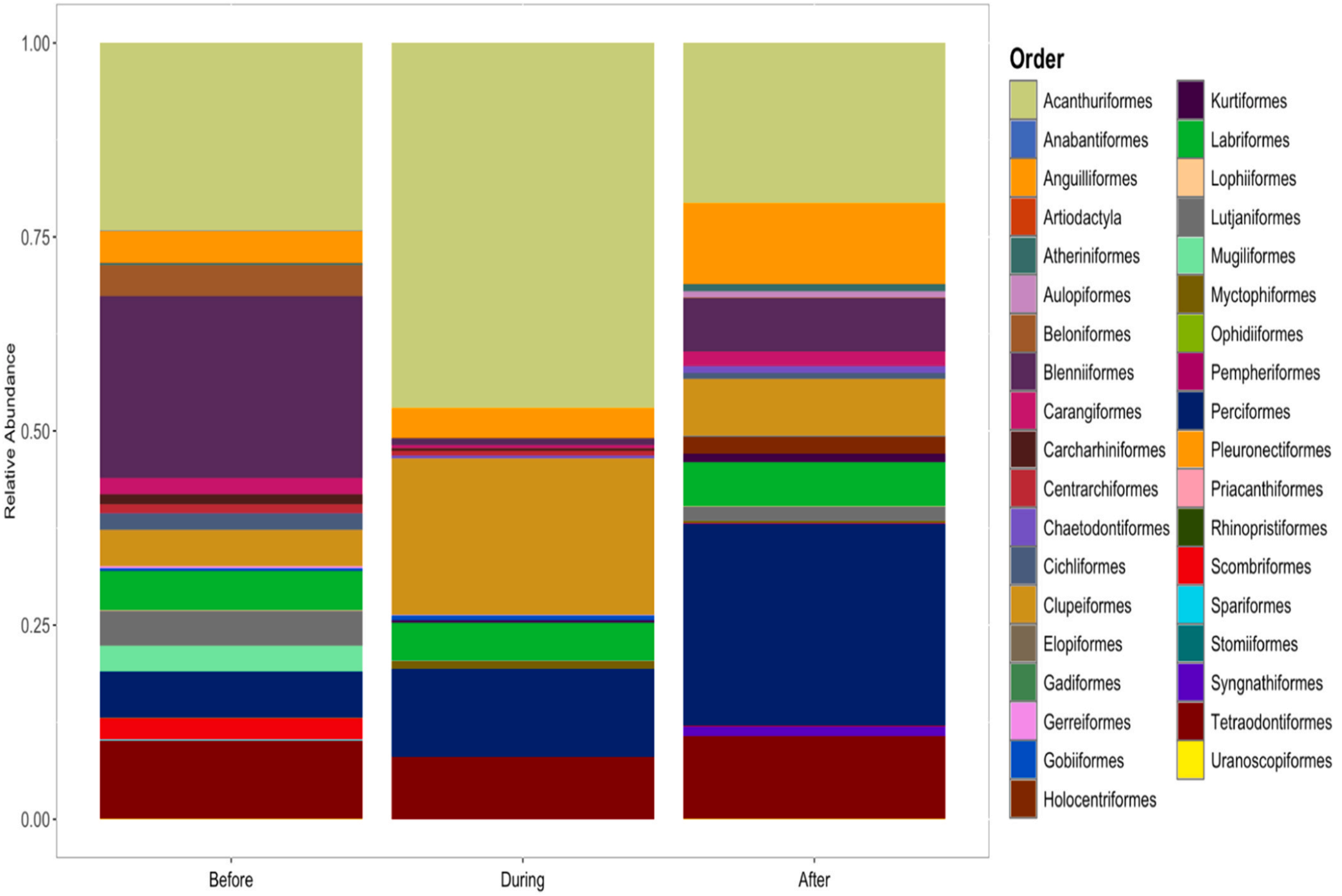

Fig. 3. Taxa composition at the order level based on read abundance from the EUE in Mulut Kumbang Strait, Alor Island, Indonesia.

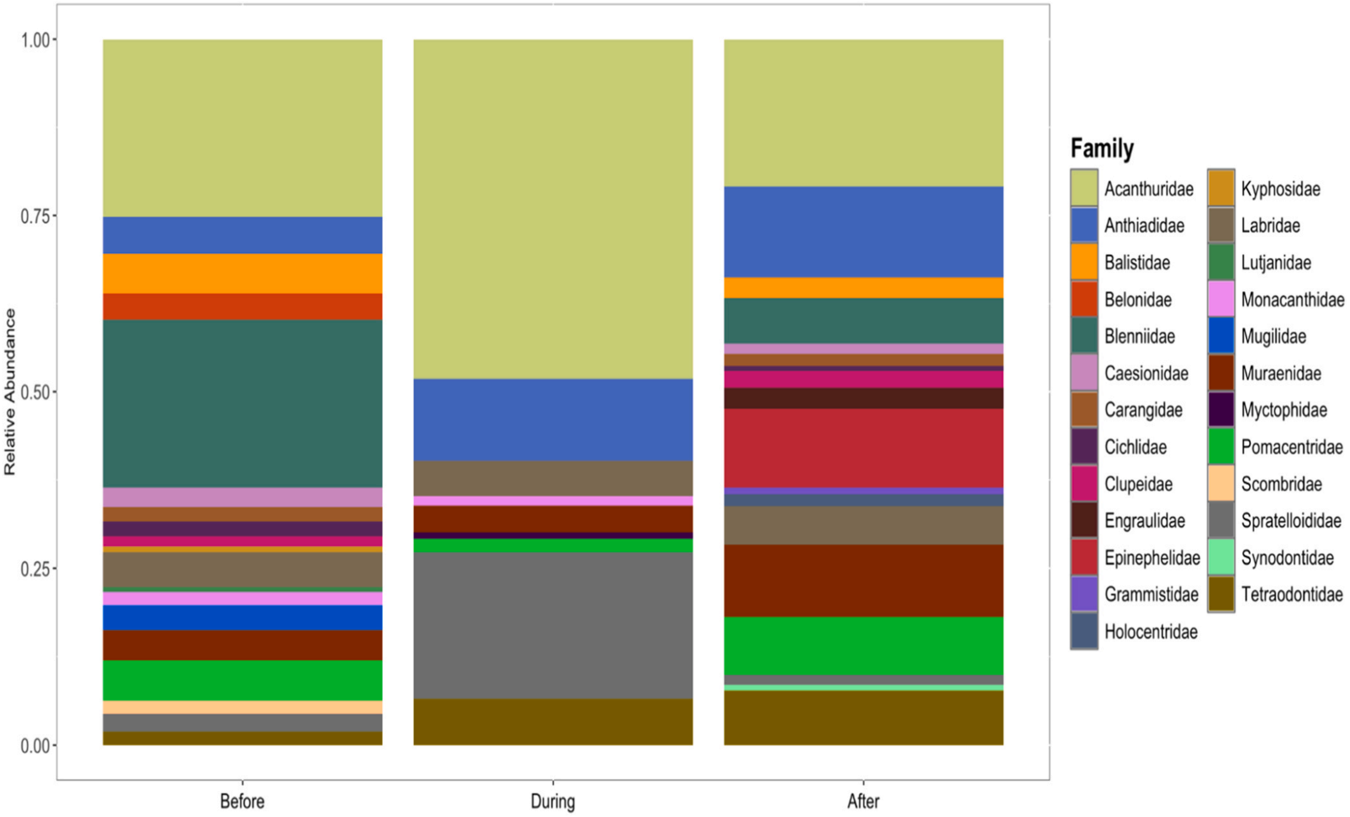

Fig. 4. Same as Fig. 3 but for taxa composition at the family level.

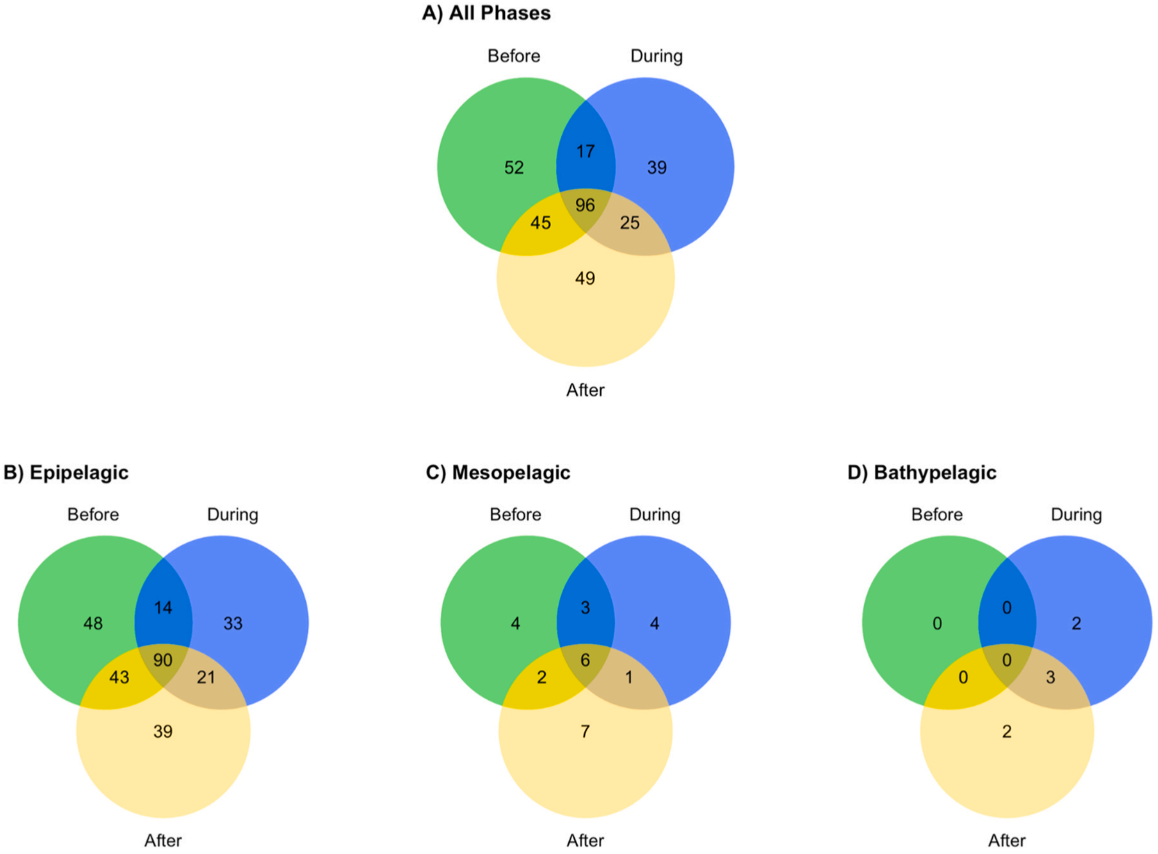

Fig. 5. Venn Diagram shows the species detected in each EUE phase, which are further grouped by ocean depth zones.

across all three phases (before, during, and after EUE). Additionally, 52 unique species were detected before the upwelling event, 39 during the event, and 49 after the event.

For further analysis, we classified the found species by the ocean depth zone (Bessey et al., 2025), i.e., epipelagic (0m-200m), mesopelagic (200m-1000m), and bathypelagic (1,000m-4000 m). Before the EUE, 48 epipelagic species and 4 mesopelagic species were uniquely recorded, with no bathypelagic species detected. During the EUE, 33 epipelagic species, 4 mesopelagic species, and 2 bathypelagic species were uniquely captured. After EUE, 39 epipelagic species, 7 mesopelagic species, and 2 bathypelagic species were detected exclusively.

3.5. *Alpha diversity*

A comparison of the diversity based on Shannon and Simpson results between events has a variety of results (Fig. 6; Table 1). The Shannon diversity index reveals that after EUE (8.75), before EUE (8.33), and during EUE (8.17) have the highest levels of richness. The Simpson index shows the opposite pattern, where the highest index is during the event (0.0147), followed by after EUE (0.0026) and before EUE (0.0019). The different test results from ANOVA of alpha diversity between events indicate no significant difference from the Shannon result (ANOVA-Shannon = 1.422, p-value $>$ 0.05) and Simpson results (ANOVA-Simpson = 4.659, p-value $>$ 0.05) (Appendix 1; Appendix 2).

3.6. *Beta diversity*

A Principal Coordinate Analysis (PCoA) plot that shows beta diversity from the composition of the fish community between events shows that the fish composition during EUE is clumped together (Fig. 7). In the Jaccard analysis (Fig. 7A), the data from before and after EUE clustered differently. However, analysis using Bray Curtis (Fig. 7B) shows that data from before and after EUE are clustered together. Moreover, the results from PERMANOVA and dispersion homogeneity tests (Betadisper) indicated significant differences among EUE phases (p-value $<$ 0.05) (Table 2; Appendix 1; Appendix 2).

Table 1
Shannon and simpson index values of each individual sample based on EUE phases.

Samples	Observed	Shannon	Simpson	EUE Condition
DBP014204	8070	8.120481	0.001365	Before
DBP014205	36531	9.024097	0.00118	Before
DBP014206	10641	7.845045	0.003023	Before
DBP014222	56124	7.866349	0.025641	During
DBP014223	23333	8.158627	0.011073	During
DBP014224	32188	8.496040	0.007244	During
DBP014241	19314	8.743833	0.001215	After
DBP014242	27586	8.476700	0.005026	After
DBP014243	34401	9.014948	0.001545	After

4. Discussions

The biological aspect of upwelling events is generally characterized by increased biodiversity in marine epipelagic fish due to nutrients that rise with deep sea water (Messié et al., 2023). However, one interesting aspect related to environmental DNA is whether eDNA from the deep sea can also be detected during upwelling events. The study of fish diversity using the eDNA approach conducted in Alor, East Nusa Tenggara, Indonesia, showed that at three different times, fish diversity in Alor was very high. However, the composition of fish DNA found at the 3 times was very different. At the time of the upwelling event, there were several deep-sea fish detected, including Spinycheek Lanternfish (*Benthosema fibulatum),* Prickly Lanternfish *(Dasyscopelus asper),* Garman's Lanternfish *(Diaphus garmani),* Lanternfish *(Diaphus suborbitalis), and* Watase's Lanternfish *(Diaphus watasei).*

High biodiversity is a characteristic of Alor waters, an area rich in marine biodiversity. This is because Alor's geographical location is in the heart of the Coral Triangle, an area that is globally known as the center of the highest marine biodiversity in the world and is the main foundation of species richness in this region (Veron et al., 2009, and Sherman, 2023). Various studies and surveys have documented the extraordinary diversity of coral reef ecosystems in Alor, which are habitats for thousands of species of reef fish, invertebrates, and algae

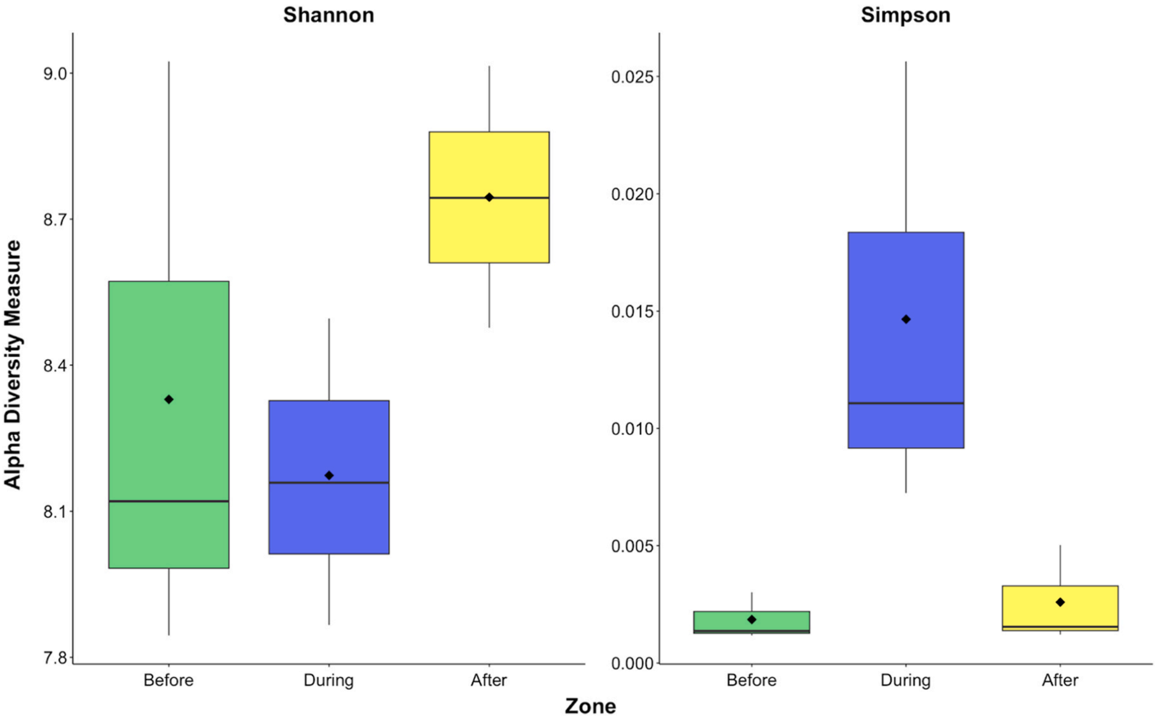

Fig. 6. The richness plot shows the richness and diversity of operational taxonomic units (OTUs) in each EUE phase. The black diamond inside the box represents the mean of the data, while the horizontal line inside the box represents the median of the data.

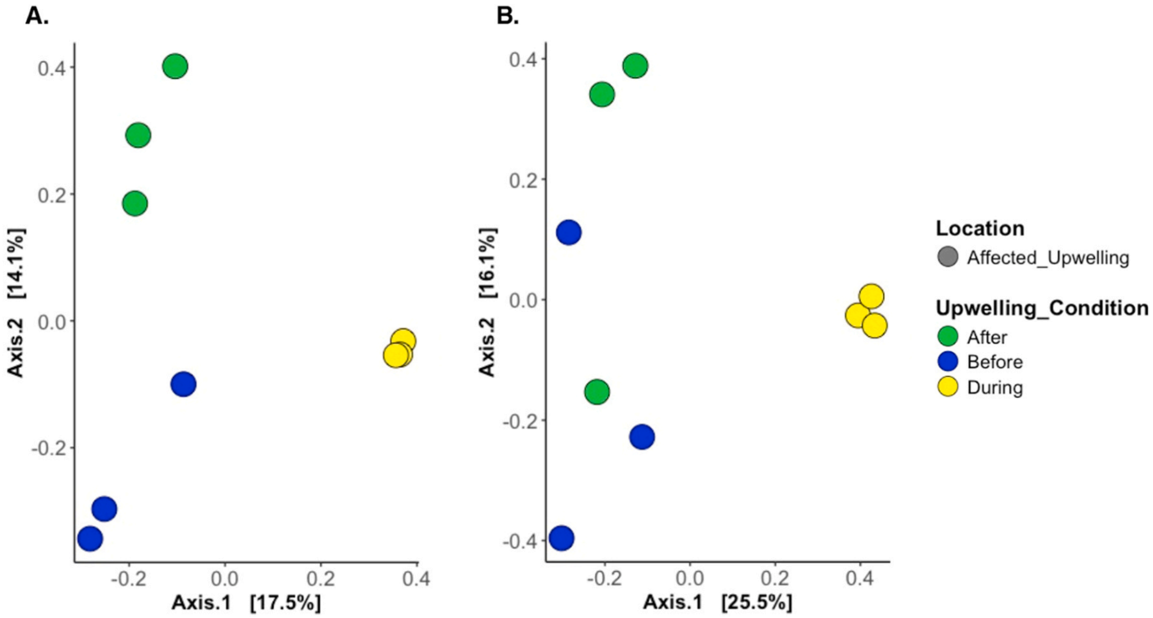

Fig. 7. PCoA plots using Jaccard (A) and Bray-Curtis (B) analysis showing the fish composition in each EUE conditions.

Table 2
PERMANOVA test results on Jaccard and Bray-Curtis analysis.

PCoA	Df	Sum of Sqs	R2	F	Pr(>F)
Jaccard					
Upwelling_Condition	2	1.112	0.3111	1.351	0.004 **
Residual	6	2.469	0.6895		
Total	8	3.581	1		
Bray Curtis					
Upwelling_Condition	2	1.264	0.3949	1.958	0.009 **
Residual	6	1.937	0.6051		
Total	8	3.200	1		

** Significant value (p-value < 0.05)

(Allen and Adrim, 2003). The uniqueness of Alor's underwater topography creates a variety of habitats ranging from coastal coral reefs to volcanic slopes and deep waters, contributing to the high species diversity. One of the waters in Alor Regency that shows high biodiversity is the Pantar Strait, where this area is a cetacean migration route and home to various interesting marine megafauna such as whales, dugongs, dolphins, whale sharks, thresher sharks, manta rays, and sea turtles (Maro et al., 2021; IUCN-MMPATF, 2022; Hartoko et al., 2023). In 2002, a survey showed that there were around 3211 individuals from 11 cetacean species, consisting of 5 whale species and 6 dolphin species (Kahn, 2002).

This high diversity is also seen from the high alpha diversity of the locations we studied and also the number of species detected in all three events. There is no significant difference in richness, meaning that in each event the diversity detected is always high, whether or not there is an upwelling event. Several studies on fish diversity using the eDNA approach show the high number of fish OTUs that can be detected (Wang et al., 2022; Malik et al., 2025). Several studies show eDNA methods can detect species that are not found in the visual census (Roblet et al., 2024). Some of the reasons are fish DNA that is released into the environment; it could come from nocturnal fish that cannot be detected during the day when the visual census (Thomsen et al., 2016; Closek et al., 2019; Bessey et al., 2020) is carried out. Another reason, of course, is the influence of currents that may carry fish DNA from locations outside the data collection site. The high alpha diversity after the upwelling event shows a combination of high biodiversity in Alor waters plus residual DNA carried by deeper waters during the upwelling.

The high diversity during upwelling events is generally due to nutrients brought by water from deep waters, which then become a source of fish food (Dabuleviciene et al., 2023). In addition, the extreme temperature drop in Alor, reaching 12°C, likely induced cold shock in many fish. Cold shock is an acute stress response caused by rapid decreases in water temperature, which can impair swimming ability, disrupt physiological balance, and, in severe cases, increase mortality (Beitinger et al., 2000; Donaldson et al., 2008). Morphological identification of fish caught during the upwelling revealed individuals from the genera Chrysiptera and Abudefduf, along with several other species including Orangespine Unicornfish *(Naso lituratus),* Bignose Unicornfish *(Naso vlamingii),* Moorish Idol *(Zanclus cornutus),* Saddleback Puffer *(Canthigaster valentini),* Atlantic fangjaw *(Gonostoma atlanticum),* Sunburst butterflyfish *(Chaetodon kleinii),* Three-lined Rainbowfish *(Stethojulis trilineata),* Iridescent Cardinalfish *(Pristiapogon kallopterus),* Dusky-tailed Cardinalfish *(Taeniamia macroptera),* Ring-tailed Cardinalfish *(Ostorhinchus aureus),* Goldenstriped Soapfish *(Grammistes sexlineatus),* Crown Squirrelfish *(Sargocentron diadema),* Panatella Silverside *(Stenatherina panatela),* Honeycomb Grouper *(Epinephelus merra),* Blue-spotted Cornetfish *(Fistularia commersonii),* Giant Trevally *(Caranx ignobilis),* Butterfly Scorpionfish *(Dendrochirus bellus),* Blue-barred Parrotfish *(Scarus ghobban), and* Undulated Moray *(Gymnothorax undulatus)*. Most of the genus and species are also detected in the eDNA data.

The dominant fish during the study were epipelagic species, fish species that are generally found in shallow waters. The Simpson index reflects the degree of dominance within a community, where higher values indicate stronger dominance by one or a few taxa. In this study, the Simpson index values were higher during the upwelling period, suggesting the dominance of certain taxa at that time. When we looked at the fish community at the order level, we saw that Acanthuriformes were the most common before and during the upwelling period. This suggests that they were adapting to the unique conditions of that phase. However, after the upwelling, there was a shift in dominance to the Perciformes order, indicating a different response from the larger fish groups to post-upwelling oceanographic changes. Despite fluctuations in order dominance, the Acanthuridae family remained the most dominant group throughout the upwelling phase. This family's consistent dominance in the biodiversity-rich Alor Waters shows that they can adapt to changes in the environment caused by the upwelling phenomenon (Shaughnessy and Cortesi, 2024) or that there are plenty of resources to support them. These data strengthen the characteristics of Alor as an area with high biodiversity because they show the dynamic response of the fish community to oceanographic changes.

Significant beta diversity in fish composition under different upwelling conditions suggests that EUE plays a critical role in shaping fish community structures, particularly during these events. Certain taxa, such as Striated Frogfish (*Antennarius striatus)* (Meso) and Lanternfish (*Diaphus suborbitalis)* (Bathy), are found exclusively during EUE conditions and are typically associated with mesopelagic and/or bathypelagic zones. Upwelling significantly influences coastal community structures and dynamics by affecting taxon abundance, recruitment processes, dispersal patterns, and distribution (Lourenço et al., 2020). The impacts of this upwelling event may stem from several factors influencing the community taxon in the region. These include increased nutrient-richness (Escribano, Morales, 2011; Messié et al., 2023), enhanced dispersal of larvae (Satterthwaite et al., 2021), and the upward movement of genetic material from the ocean depths to the surface (Peluso et al., 2024).

In this study, the eDNA method was used to identify several deep-sea fish species. These include Smallscale Codlet (*Bregmaceros nectabanus),* Goldnose Lanternfish *(Diaphus chrysorhynchus),* Japanese Rubyfish (*Erythrocles schlegelii),* Deepwater Longtail Red Snapper (*Etelis coruscans),* Pale Snapper *(Etelis radiosus),* and Snake Mackerel (*Gempylus serpens)*. These species are known to have a relatively wide distribution. For example, *B. nectabanus* has been documented off Palmahim, Israel (Goren and Galil, 2008), in Izmir Bay, Türkiye (Özgül and Akyol, 2017), Syrian waters (Othman and Galiya, 2019), Korea (Jang and Kim, 2021), and again in Izmir Bay, northeastern Aegean Sea (Akyol, 2022). *D. chrysorhynchus*, also known as the Goldnose Lanternfish, inhabits the bathypelagic zone of the Pacific Ocean at depths ranging from 2130 to 5870 m. This species reaches a maximum length of 1.1 cm (De la Paz and Interior, 1979). The presence of *D. chrysorhynchus* has also been confirmed through eDNA analysis in the Bailong Pearl Bay Demonstration Area, Beibu Gulf (Jia et al., 2023). *E. schlegelii* has been found off the coast of the Philippines (Pastana et al., 2022) and Western Australia (Coulson and Moore, 2021). *E. coruscans* has been recorded off the coast of Kimberley (Western Australia), the Northern Territory, the northern Great Barrier Reef (Queensland), offshore from Byron Bay (New South Wales), Christmas Island in the eastern Indian Ocean, Taupo Seamount, and offshore from Lord Howe Island in the Tasman Sea. It occurs in tropical and central Indo-West Pacific regions at depths of 90–500 m and can reach a maximum length of 120 cm (Bray, 2017). *E. radiosus* has been recorded in Papua, Indonesia (Nurulludin et al., 2016), and along the coast of Pakistan (Zohra et al., 2020), with a total length ranging from 30 to 85 cm (Carpenter and Niem, 2001).

Interestingly, bathypelagic fish were also found, including *Benthosema fibulatum, Dasyscopelus asper, Diaphus garmani, Diaphus suborbitalis, Diaphus watasei*, and *Howella cf. broidei.* Bathypelagic fish are fish from a depth of 1000–4000 m, while the Pantar Strait is <1000 m deep (Wirasatriya et al., 2023). So there is a possibility that the water mass carried during the EUE also came from the Sawu Sea, which is >1000 m deep (Setiawan et al., 2021). Extending over an area of 52,000 km2, the Savu Basin has a maximum depth of 3470 m (Kartaadiputra et al., 1982, Van Der Werff, 1995). This shows that there are indications that the deep sea water that rises into the Pantar Strait during upwelling also comes from deeper surrounding areas, for example, from the Savu Sea. Water mass analysis based on CTD observations must still examine this indication, though.

Evidence from other systems also suggests that eDNA can migrate vertically in the water column. In the Gulf of Mexico, shifts in eDNA composition closely mirrored diel vertical migration of scattering layer communities, indicating that eDNA signals can track organismal movement through depth (Easson et al., 2020). Similarly, a mechanistic model of the mesopelagic zone showed that eDNA released at depth can be redistributed vertically by advection, dispersion, and particle settling, resulting in detection at depths different from where it originated (Allan et al., 2021). These findings support the interpretation that deep-sea taxa detected during the Alor upwelling may reflect vertical transport of genetic material from mesopelagic or bathypelagic sources.

However, our study relied on surface-only eDNA collected before, during, and after the EUE, with a single replicate at one site, providing a first overview of eDNA detection during this event in Alor. Future work should include sampling at multiple site locations and with larger water volumes to enable cross-site comparisons and improve the detection of diverse taxa (Schabacker et al., 2020; Bessey et al., 2020). Additionally, in short-term events like an EUE, less than one hour (Wirasatriya et al., 2023), eDNA may not reflect only the organisms present at that exact moment. Because DNA can persist in seawater for hours to days (Li et al., 2019; Yao et al., 2022), the signals that were detected in this study could be a mix of fresh DNA released during the upwelling and older DNA that has remained in the water or been carried in by currents. This means some deep-sea species detected in surface waters might represent immediate upwelling transport, while others could come from earlier conditions. Our findings demonstrate that upwelling in Alor reveals deep-sea taxa in surface waters, underscoring the connectivity between oceanographic processes and community composition through eDNA usage. This has potentially direct implications for conservation practice, as the Pantar Strait functions as both a coral reef hotspot and a corridor linking shallow and deep-water ecosystems, reinforcing the need for its protection within marine spatial planning. Additionally, detecting commercially valuable species from shallow and deep-sea in this study highlights that upwelling events may generate temporary hotspots of fish abundance, increasing risks of overexploitation if fisheries are not carefully managed.

5. Conclusion

This study shows the potential of eDNA to explore the diversity of deep-sea fish collected in Alor during upwelling. The types of fish that dominate before, during, and after upwelling show different compositions, and the presence of deep-sea fish is detected during and after upwelling. This shows the ability of eDNA to detect DNA of living things released into the environment. The use of eDNA provides a cost-effective method for learning about biodiversity in various habitats. However, the eDNA method still needs to be supported by conventional data collection and genetic identification of deep-sea biodiversity to later become a database for rapid monitoring activities, for example, with the eDNA method.

CRediT authorship contribution statement

Yulita Dwi Astuti: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Nining Nursalim:** Writing – review & editing, Data curation. **Muhammad Danie Al Malik:** Writing – review & editing, Formal analysis. **Ekitia Reyza Syakal:** Writing – review & editing, Formal analysis. **Lilik Maslukah:** Writing – review & editing, Supervision. **Yusuf Jati Wijaya:** Writing – review & editing, Supervision. **Rizki Taqwa Putranto:** Writing – review & editing, Data curation. **Heru Nur Krisna:** Writing – review & editing, Data curation. **Ikhsan Mustaqim:** Writing – review & editing, Data curation. **Azza Fidyani Sintania Susanti:** Writing – review & editing, Data curation. **R. Dwi Susanto:** Writing – review & editing, Supervision. **Ni Kadek Dita Cahyani:** Writing – review & editing, Supervision, Conceptualization. **Anindya Wirasatriya:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethics in publishing statement

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

This manuscript represents entirely original works and or if work and/or words of others have been used, that this has been appropriately cited or quoted and permission has been obtained where necessary.

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All authors have been personally and actively involved in substantive work leading to the manuscriptand will hold themselves jointly and individually responsible for its content.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This research is funded by Diponegoro University through World Class Research UNDIP scheme with contract number: 357-11/UN7.D2/PP/IV/2024. This work is also supported by the Physical Oceanography program of the National Science Foundation (NSF; grant #2242151) through the University of Maryland for R. Dwi Susanto. Special thanks to Diponegoro Biodiversity Project Laboratory and Team for generously providing the facility and time for conducting this study, creating a conducive environment for the successful completion of this research. Special thanks to UPTD Pengelola Taman Perairan Kepulauan Alor dan Laut Sekitarnya for the research permit and Mr. Rachmad Marweki and his family for their hospitality in providing the accommodation during the field survey.

Appendix

Alpha diversity
Appendix 1

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
ANOVA-Shannon					
Upwelling_Condition	2	0.5235	0.2617	1.422	0.312
Residuals	6	1.1043	0.1840		

Appendix 2

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
ANOVA-Simpson					
Upwelling_Condition	2	0.0003097	1.548e−04	4.659	0.0601
Residuals	6	0.0001994	3.324e−05		

Beta diversity
Appendix 1
Betadisper test result on Jaccard analysis

	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Jaccard						
Groups	2	0.0016547	0.00082737	1.5284	999	0.234
Residuals	6	0.0032479	0.00054132			

Appendix 2
Betadisper test result on bray-curtis analysis

	Df	Sum Sq	Mean Sq	F	N.Perm	Pr(>F)
Bray-Curtis						
Groups	2	0.012092	0.0060461	0.8008	999	0.481
Residuals	6	0.045300	0.0075500			

Data availability

Data will be made available on request.

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