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Underwater drone-based eDNA metabarcoding reveals regional differences in fish communities and early detection of alien species around the Korean Peninsula

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Coastal ecosystems surrounding the Korean Peninsula are undergoing rapid environmental changes driven by global climate warming, highlighting the need for efficient methods to monitor marine biodiversity. This study aimed to analyze fish communities across four coastal regions: the East Sea, South Sea, West Sea, and Jeju using environmental DNA (eDNA) metabarcoding. Underwater drones were employed to collect water samples. A total of 63 sampling sites were surveyed, detecting 167 fish species from 72 families, encompassing tropical, subtropical, temperate, boreal, polar, and deep-water taxa. The East Sea hosted a mix of cold- and warm-water species, while Jeju exhibited a relatively high proportion of tropical and subtropical fish. Additionally, 13 alien species were identified, underscoring the utility of eDNA for the early detection of non-native taxa expanding their ranges in response to ongoing warming trends. This study further validated that eDNA sampling using underwater drones offers a rapid, non-invasive approach to biodiversity assessments, effectively addressing many of the limitations associated with traditional survey techniques. Collectively, these findings highlight the potential of eDNA to generate critical and timely data on fish assemblages the emergence of alien species, providing valuable insights to inform proactive resource management, and climate change research in marine ecosystems.

Keywords Environmental DNA, Metabarcoding, Alien species, Early detection, Fish habitat types

Coastal ecosystems are dynamic environments shaped by complex interactions among biological, physical, and climatic factors, serving as essential habitats for fish. The spatial distribution of fish assemblages in temperate coastal waters is particularly influenced by geographic and environmental factors such as water temperature, ocean currents, and depth[1,2]. For instance, *Gadus chalcogrammus*, a cold-water fish species that once represented a major commercial resource in the East Sea, has experienced a rapid population decline due to rising sea temperatures[3]. Similarly, in the European Atlantic, ocean warming has led to the disappearance of species that previously dominated (e.g., *Echinorhinus brucus*, *Squatina squatina*, *Raja batis*, *R. brachyura*, *R. clavata*, *Dasyatis pastinaca*, *Myliobatis aquila*, *Galeorhinus galeus*, *Mustelus asterias*). Concurrently, the distribution of tropical fish species has expanded northward[4].

To conserve and sustain biodiversity in these rapidly changing ecosystems, precise surveys and continuous monitoring of marine biota are critical for generating data to inform effective management and conservation strategies. Fish assemblage data, in particular, are extensively utilized to understand and interpret changes in aquatic ecosystems, serving as critical data for environmental assessments, biodiversity conservation, and ecosystem management[5,6]. Fluctuations in the abundance of specific species may indicate alterations in habitat conditions, disruptions in food web structures, or anthropogenic disturbances, making them valuable indicators for evaluating ecosystem stability and health[7]. Additionally, such data provide a foundation for effective fisheries management, aiding in the prevention of resource depletion caused by overfishing or habitat destruction and ensuring the sustainable use of fish resources.

As in many coastal regions worldwide, the composition and diversity of coastal fish assemblages are influenced by geographic, environmental, and anthropogenic factors. South Korea is bordered by the sea on

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three sides, with a coastline exceeding 15,000 km[8] and spanning more than 500 km from north to south (Fig. 1). This encompasses a broad climatic range from subtropical to subarctic zones. Warm currents move northward along the southern coast, whereas cold currents flow southward along the eastern coast, converging in the East Sea to form highly productive coastal fishing grounds[9]. Historically, the East Sea supported a mix of cold-water and warm-water fish species. However, rapid changes in fish assemblages have occurred in recent years due to rising sea temperatures. Tropical fish species are increasingly prevalent in Jeju, whereas cold-water fish species in the East Sea have declined, and subtropical species have become more dominant. Predicting future changes in fish assemblages under these rapid environmental shifts remains a significant challenge.

Environmental DNA (eDNA) metabarcoding is a novel, simple, efficient, and highly sensitive method for surveying biodiversity by analyzing the total DNA extracted from environmental samples[10]. This tool is particularly suitable for monitoring fish populations across large areas under environmental changes. Furthermore, eDNA offers a non-invasive method for detecting species presence without the need to capture organisms or disrupt habitats, making it effective for identifying rare and invasive species. Recent studies have demonstrated the efficacy of eDNA in monitoring fish assemblages across diverse aquatic environments, including rivers, lakes, estuaries, and coastal regions[11,12]. Compared to traditional sampling methods, eDNA provides a more effective approach to studying fish distributions and diversity. For instance, a study conducted on the Qinghai-Tibet Plateau in China identified 90 fish species from water samples collected across 63 river basins, providing new ecological insights and highlighting potential invasive species[13]. Similarly, a study conducted in the mangrove estuaries of Malaysia utilized COI and 12 S rRNA markers to detect 178 fish species, showcasing eDNA's practicality for assessing complex and diverse tropical fish assemblages within a short sampling period[7].

eDNA studies typically rely on water sampling methods using plastic buckets, bottles, or plankton nets, either from the shore or boats[14–16]. Surface water sampling commonly employs plastic buckets, whereas deep-water sampling often utilizes Niskin bottles or rosette samplers[17,18]. Plankton nets can also be deployed vertically or horizontally to concentrate plankton DNA into plastic bottles[19,20]. However, shore-based sampling is limited to a radius to about 1 m from the land, and boat-based sampling is both time-consuming and labor-intensive. Moreover, these traditional methods are prone to contamination, potentially leading to complex challenges. To address these issue, uncrewed vehicles such as uncrewed aerial vehicles, drones, autonomous surface vehicles, and autonomous underwater vehicles have been developed to reduce the risk of DNA contamination during water sampling[21–23]. Underwater drones, in particular, offer significant advantages by enabling controlled sampling at specific distances and depths from the shore while minimizing the risk of contamination.

In this study, underwater drones were employed to collect water samples, and eDNA technology was applied to estimate fish diversity along the Korean coasts (Fig. 1). This approach aimed to early detection of previously unrecorded species in Korean coastal waters. This study underscores the composition and diversity of fish assemblages across different regions were analyzed, revealing spatial distribution patterns of fish communities. This study highlights the potential of eDNA as an effective tool for investigating fish diversity in Korea, a region at the forefront of climate change impacts.

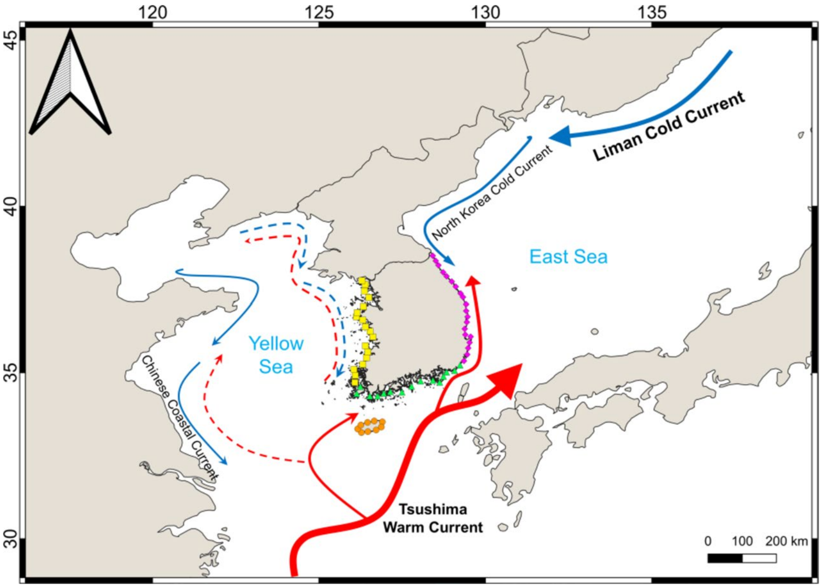

Fig. 1. Map of the 63 water sampling sites used for eDNA analysis in the Korean Peninsula. Sites are grouped into four regions: the East Sea, the South Sea, the West Sea, and Jeju. Each site is represented by a unique shape and color: pink diamonds for the East Sea, green triangles for the South Sea, yellow squares for the West Sea, and orange circles for Jeju.

Results

Sea surface temperature trends around the Korean Peninsula (2012–2020)

Sea surface temperature (SST) trends around the Korean Peninsula were analyzed using SST data collected by the NIFS from 2012 to 2020, comparing the East Sea, South Sea, West Sea, and Jeju waters (Fig. S1). During this nine-year period, the average SSTs for each region were as follows: 16.23 °C in the East Sea, 16.39 °C in the South Sea, 14.35 °C in the West Sea, and 18.87 °C in Jeju waters. While Jeju, located at the lowest latitude, recorded the highest average temperature, whereas the West Sea exhibited relatively lower temperatures than the other regions. All regions showed a consistent warming trend, with the West Sea and East Sea in particular experiencing higher rates of temperature increase compared to the others.

Sequencing analysis and fish species composition

Mitochondrial 12 S rRNA (12 S rRNA) sequencing and diversity analyses were conducted on 63 water samples collected from Korea's coastal waters, specifically the East Sea, South Sea, West Sea, and Jeju (Fig. 1). After sequence-length filtering, denoising, and chimera removal on the dataset obtained from the Illumina MiSeq dataset, a total of 4,675,239 ASVs (Amplicon Sequence Variants) were retained. The number of reads per region was as follows: East Sea:1,679,838 ASVs, West Sea: 1,230,757 ASVs, South Sea: 1,122,676 ASVs, and Jeju: 641,968 ASVs.

A total of 167 species representing 136 genera and 72 families were identified. The East Sea (20 sites) exhibited the highest species richness, with 108 species recorded (13–48 species per site). The South Sea (15 sites) accounted for 82 species (8–34 species per site), the West Sea (19 sites) recorded 69 species (5–28 species per site), and Jeju (9 sites) exhibited 94 species (15–38 species per site). The most dominant species was *Engraulis japonicus*, with 1,208,499 ASVs, accounting for 25.8% of the total fish detected. This was followed by *Takifugu* sp. (407,427 ASVs, 8.7%), *Paralichthys olivaceus* (263,482 ASVs, 5.6%), *Nuchequula nuchalis* (260,180 ASVs, 5.6%), *Acanthopagrus schlegelii* (222,549 ASVs, 4.8%), and *Pennahia argentata* (217,467 ASVs, 4.7%) with all of these species exhibiting ASV values exceeding 200,000, underscoring their dominance in Korean coastal waters.

Biodiversity in each coastal region

The fish species detected in each coastal region were categorized into six characteristics (tropical, subtropical, temperate, boreal, polar, and deep-water) based on FishBase[48]. Temperate species, such as *E. japonicus*, *N. nuchalis*, and *P. argentata*, were predominant across all regions, accounting for 49.1% in the East Sea, 42.7% in the South Sea, 56.5% in the West Sea, and 42.6% in Jeju, reflecting their high prevalence along the Korean Peninsula coast (Table 1).

Tropical species, such as *Johnius grypotus*, *Trachurus japonicus*, and *Sebastiscus marmoratus*, and subtropical species, including *P. olivaceus*, *A. schlegelii*, and *Mugil cephalus*, were more prevalent around the waters of Jeju. In contrast, temperate, boreal (e.g., *Gadus macrocephalus*, *Acentrogobius virgatulus*, and *Alcichthys elongatus*), polar (e.g., *Limanda sakhalinensis*, *Icelus spatula*, and *Gadus chalcogrammus*), and deep-water species (e.g., *Benthosema pterotum*, *Lycodes tanakae*, and *Malacocottus zonurus*) were primarily identified in the East Sea.

An UpSet plot was used to visualize the overlap and exclusivity of fish species among regions, (Fig. 2). Of the 167 fish species detected along the Korean coast, 34 species were common across all regions. These included dominant species such as *E. japonicus*, *Takifugu* sp., *P. olivaceus*, *N. nuchalis*, *A. schlegelii*, and *P. argentata*, as well as subdominant species such as *Hyporhamphus sajori*, *M. cephalus*, *Konosirus punctatus*, *Muraenesox cinereus*, *Hexagrammos agrammus*, *Pseudopleuronectes yokohamae*, *Scomber japonicus*, and *J. grypotus*. Species exclusively detected in specific regions included 32 species in the East Sea, 26 in Jeju, 12 in the South Sea, and seven in the West Sea. Among these, subtropical and temperate species were observed along all coastal regions, whereas tropical species were restricted to Jeju and the South Sea, with 13 and four species, respectively. Boreal and polar species were exclusively identified in the East Sea, and deep-water species were found in the East Sea and South Sea, with eight and one species, respectively.

To complement the regional species composition analysis, we calculated alpha diversity using Shannon and species richness indices and compared fish communities across coastal regions. Jeju exhibited the highest average Shannon diversity (3.29 ± 0.11), which was significantly higher than that of the other regions ($p < 0.01$), followed by the East Sea (3.05 ± 0.08), South Sea (2.70 ± 0.12), and West Sea (2.63 ± 0.11) (Fig. 3a). Similarly, Jeju

Region	Habitat types						
	No. of species	**Tropical**	**Subtropical**	**Temperate**	**Boreal**	**Polar**	**Deep-water**
East Sea	108	15 (13.9%)	28 (25.9%)	53 (49.1%)	3 (2.8%)	5 (4.6%)	10 (9.3%)
South Sea	82	18 (22.0%)	28 (34.1%)	35 (42.7%)	1 (1.2%)	2 (2.4%)	4 (4.9%)
West Sea	69	9 (13.0%)	22 (31.9%)	39 (56.5%)	1 (1.4%)	1 (1.4%)	3 (4.3%)
Jeju	94	27 (28.7%)	29 (30.9%)	40 (42.6%)	0 (0.0%)	0 (0.0%)	4 (4.3%)

Table 1. Number of fish species and their corresponding habitat types detected in the East Sea, South Sea, West Sea, and Jeju waters of South Korea. The values in parentheses indicate the proportion of fish species detected in each sea area.

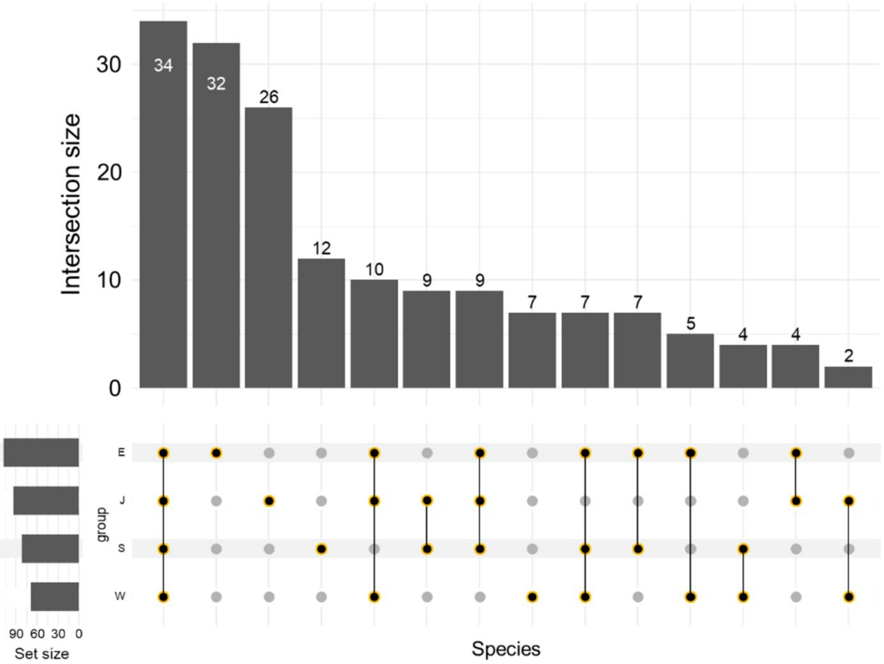

Fig. 2. UpSet plot of fish species detected by eDNA in the coastal waters of the Korean Peninsula (East Sea, South Sea, West Sea, and Jeju). The total number of fish species detected in each region is shown on the left. The yellow circles indicate the region(s) in which the species represented by the bars were detected, either exclusively or shared among multiple regions.

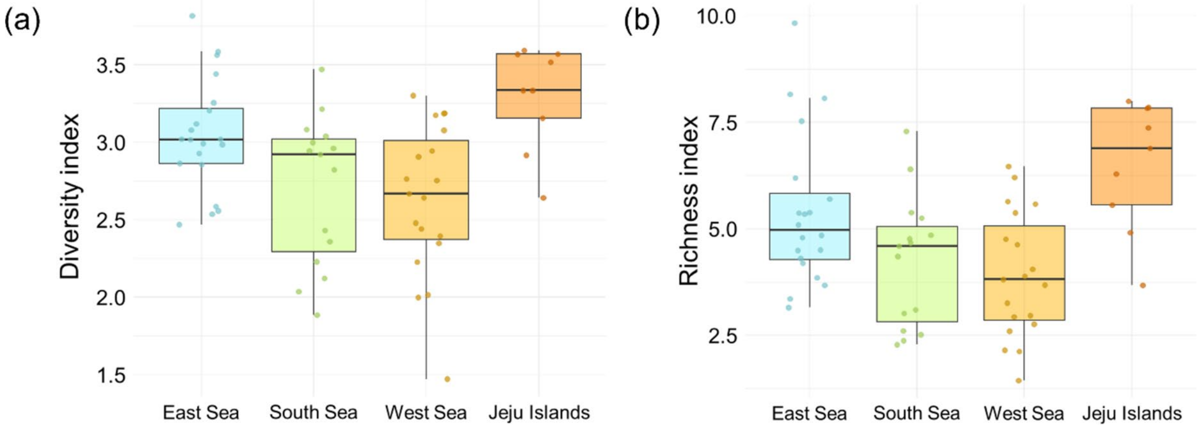

Fig. 3. Regional differences in (**a**) Shannon diversity index and (**b**) species richness index across four coastal areas of Korea: East Sea, South Sea, West Sea, and Jeju. Each box represents the interquartile range (IQR), with the horizontal line indicating the median. Jittered points denote individual sampling locations, illustrating spatial variability. Whiskers extend to 1.5 × IQR, and outliers beyond this range are represented as dots.

recorded the highest average species richness index (6.49 ± 0.50), with the East Sea, South Sea, and West Sea exhibiting values of 5.39 ± 0.39, 4.23 ± 0.40, and 3.91 ± 0.34, respectively (Fig. 3b).

Detection of alien species in Korean waters

Among the total fish species detected, 13 species were identified as alien species that had not been previously recorded in South Korean waters (Table 2). The distribution patterns of these species varied across the different study regions. The East Sea exhibited the highest occurrence, with eight alien species detected, followed by Jeju (four species), the West Sea (three species), and the South Sea (one species). In terms of habitat characteristics, these alien species were classified as four tropical species, two subtropical species, five temperate species, and two polar species based on FishBase classifications[48].

Regionally, the East Sea contained the largest number of alien species ($n = 8$), which included *Glyptocephalus zachirus*, *I. spatula*, *A. virgatulus*, *Gasterosteus nipponicus*, *Pseudorhombus arsius*, *Platycephalus cultellatus*,

Species	Read counts (ASV values)				Habitat types
	East Sea	South Sea	West Sea	Jeju	
Glyptocephalus zachirus	34,841	0	0	0	Temperate
Icelus spatula	24,085	0	0	0	Polar
Acentrogobius virgatulus	3,280	0	0	0	
Enneapterygius leucopunctatus	0	0	0	3,093	Subtropical
Gasterosteus nipponicus	2,721	0	0	0	Subtropical
Pseudorhombus arsius	5	0	2,099	0	Tropical
Platycephalus cultellatus	13	0	2,027	0	Temperate
Zoarchias neglectus	0	677	908	0	Temperate
Eviota punctulata	0	0	0	1,084	Tropical
Anisarchus medius	594	0	0	0	Polar
Hippoglossoides robustus	347	0	0	0	Temperate
Rastrelliger kanagurta	0	0	0	244	Tropical
Callogobius nigromarginatus	0	0	0	217	Tropical

Table 2. Read counts (ASV values) of alien fish species detected via eDNA across four coastal regions in South Korea (East Sea, South Sea, West Sea, and Jeju). Habitat types were classified according to FishBase.

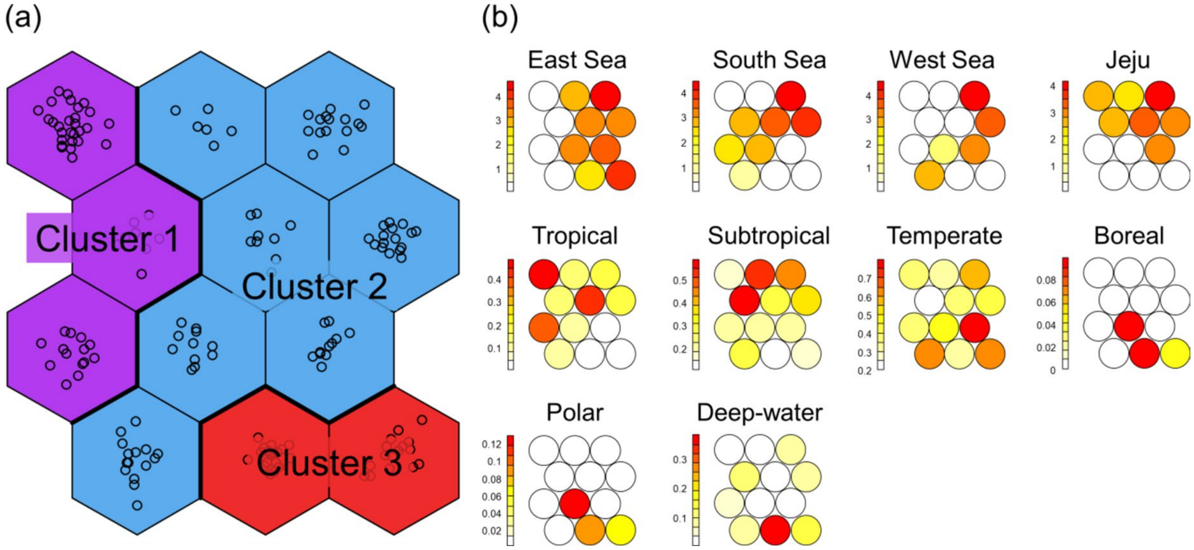

Fig. 4. (**a**) Fish species detected in the coastal areas of South Korea, projected onto a self-organizing map (SOM) with 12 output neurons arranged in a 4 × 3 grid. Hierarchical cluster analysis and U-matrix values were used to group the neurons into three clusters, which are indicated in the figure as follows: Cluster 1 (purple), Cluster 2 (blue), and Cluster 3 (red). (**b**) The component planes of the SOM illustrate the contribution of each variable to classifying coastal regions (East Sea, South Sea, West Sea, and Jeju) and fish habitat types (tropical, subtropical, temperate, boreal, polar, deep-water). Red neurons indicate high values for each variable, whereas white neurons indicate low values. These values were derived from the SOM learning process.

Anisarchus medius, and *Hippoglossoides robustus*. These species were categorized into three temperate, two polar, and one each for tropical, subtropical, and unknown categories. In Jeju waters, four alien species were detected: *Enneapterygius leucopunctatus*, *Eviota punctulata*, *Rastrelliger kanagurta*, and *Callogobius nigromarginatus*. Among them, three were tropical species, while one (*E. leucopunctatus*) was subtropical. The West Sea exhibited the presence of three alien species: *P. arsius*, *P. cultellatus*, and *Zoarchias neglectus*. These species were categorized as two temperate species and one tropical species. The South Sea had the lowest occurrence of alien species, with only one species (*Z. neglectus*), detected. This species is classified as temperate.

Habitat characteristics of fish species by region

A self-organizing map (SOM) analysis was used to classify associations between sampling regions and fish habitat types, using presence-absence data of species and habitat traits derived from FishBase. The SOM training resulted in 12 output neurons (3 × 4 hexagonal grid), and hierarchical clustering grouped these into three distinct clusters (Fig. 4a). The robustness of clustering was statistically validated through a Multi-Response Permutation Procedure (MRPP), which showed significant differences among clusters ($A = 0.2575$, $p = 0.001$),

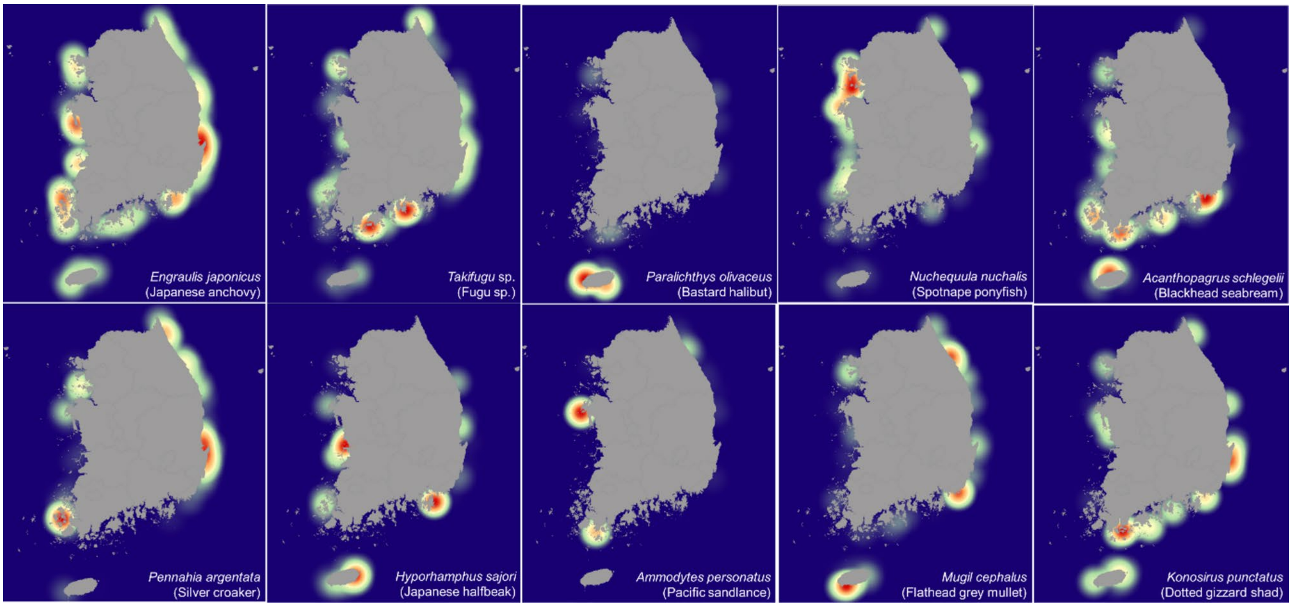

Fig. 5. Distribution patterns of the top 10 species detected by eDNA along the coast of the Korean Peninsula, visualized using GIS. ASV values were log-transformed, with red indicating higher detection values and white indicating lower detection values.

indicating that the observed associations were not due to chance. Each SOM cluster reflected region-specific species composition: Cluster 1 primarily included species associated with the South Sea and Jeju; Cluster 2 contained species broadly distributed across all regions; and Cluster 3 grouped species specific to the East Sea. These distributions corresponded to distinct habitat preferences and oceanographic conditions.

The relative contribution of each input variable (sampling region and habitat characteristic) to fish clustering is visualized on the SOM map (Fig. 4b). Neurons located in the upper left of the SOM map corresponded to tropical and subtropical species inhabiting warmer waters, whereas neurons in the lower right corresponded to boreal, polar, and deep-water species inhabiting colder environments. When comparing sampling regions and habitat types, the South Sea and Jeju exhibited neurons associated with tropical, subtropical, and temperate species. The West Sea displayed neurons corresponding to temperate and subtropical species, whereas the East Sea encompassed all habitat types, including certain tropical species. These clustering patterns highlight the diversity of habitats and species composition across the regions studied.

Spatial distribution of dominant species detected by eDNA

The spatial distribution patterns of the top 10 fish species detected along the Korean coastal waters were visualized using QGIS-generated heatmaps (Fig. 5, Table S1). Although these species were detected across all sea regions, distinct variations in regional distribution densities among species were observed.

Engraulis japonicus and *Takifugu* sp. were widely distributed across all regions, including the East Sea, South Sea, West Sea, and Jeju. *Paralichthys olivaceus* exhibited high densities around Jeju, whereas *N. nuchalis* exhibited high densities in the West Sea, particularly at site W4. *Acanthopagrus schlegelii* was abundant in the South Sea and around Jeju but had lower eDNA detection levels in the East Sea. *Pennahia argentata* was primarily concentrated along the southern coasts of the East Sea and West Sea, and *H. sajori* was evenly distributed across Jeju, the South Sea, and the West Sea. *Ammodytes personatus*, a species known to inhabit the West Sea, exhibited high densities in that region. *Mugil cephalus* was widely distributed in the East Sea. Lastly, *K. punctatus* exhibited high densities in the southern part of the East Sea, as well as around Jeju and the South Sea.

Discussion

This study demonstrates that eDNA analysis using underwater drones can be an effective method for assessing fish species diversity and distribution patterns in coastal waters around the Korean Peninsula. Compared to traditional water collection methods, such as using baskets or boats for water collection, underwater drones offer significant advantages, including improved time efficiency, standardized quantitative sampling, and a reduced risk of contamination. While previous studies have highlighted challenges such as high labor demands and contamination risks associated with boat-based sampling[7,24], the use of underwater drones in this research minimized these limitations and enabled consistent sampling across diverse marine environments, such as shorelines, breakwaters, and beaches. Future applications of underwater drones in eDNA studies could further enhance biomonitoring efforts, particularly in ecologically sensitive or hard-to-access areas.

Recent data reveal a clear increase in SST around the Korean Peninsula, with the West Sea experiencing a higher rate of warming compared to the East Sea, South Sea, or Jeju[25]. This trend is likely influenced by the West Sea's geographical characteristics, such as its relatively shallow depth and semi-enclosed waters. Marine heatwaves driven by global climate change pose significant threats to marine ecosystems, such as coral bleaching and fluctuations in fish populations, as documented in previous studies[26–28]. Furthermore, rising sea temperatures and marine heatwaves have been linked to large-scale fish die-offs[29]. Concurrently, the occurrence of alien species continues to be reported each year[30–47]. Over the past five years, several alien species have been reported in the waters around the Korean Peninsula. Four species were identified in 2024 (*Ostorhinchus fleurieu*, *Lepidotrigla longifaciata*, *Symphurus longirostris*, *Lophiodes triradiatus*), four in 2022 (*Echidna nebulosa*, *Chlorophthalmus nigromarginatus*, *Oncorhynchus tshawytscha*, *Synodus kaianus*), three in 2021 (*Heniochus diphreutes*, *Cryptacanthodes bergi*, *Albula glossodonta*), and eight in 2020 (*Lepidocybium flavobrunneum*, *Glossogobius olivaceus*, *Strophidon sathete*, *Peristedion liorhynchus*, *Festucalex erythraeus*, *Lumpenopsis pavlenkoi*, *Hexanchus griseus*, *Alopias superciliosus*).

In this study, we identified a total of 13 alien species, most of which were native to neighboring countries, including Taiwan, China, Japan, and Russia[48]. Notably, species known to inhabit Russian waters, particularly the Bering Sea and the Sea of Okhotsk (*H. robustus*, *G. nipponicus*, *I. spatula*, *G. zachirus*), were exclusively detected in the East Sea. In contrast, tropical and subtropical species (*C. nigromarginatus*, *R. kanagurta*, *E. punctulata*, *E. leucopunctatus*) originating from southern regions like Taiwan and Japan, were predominantly found around Jeju. The detection of these non-native species in Korean waters may be influenced by multiple factors, yet oceanic currents are likely the primary driver of their dispersal. For example, the Kuroshio Current, which transports warm tropical and subtropical waters northward, may have facilitated the introduction of tropical and subtropical fish species into Korean waters. Conversely, the North Korean Cold Current, flowing southward along the eastern coast, could have contributed to the presence of polar fish species in the East Sea. Because eDNA degrades rapidly in seawater, typically within a few days, with an estimated empirical turnover rate of approximately 10 h—significantly faster than in freshwater—long-distance dispersal is improbable[49,50]. However, it is important to note that these detections are based on eDNA signals collected at a single time point and may not necessarily indicate the presence of established populations[51]. Although taxonomic assignments were made using well-curated databases, there remains a possibility of false positives, especially in the absence of additional bioinformatic validation or morphological confirmation. Furthermore, all species identified as ‘alien’ in this study have native distributions within the North Pacific Ocean, and their occurrence in Korean waters can be plausibly explained by natural dispersal mechanisms rather than anthropogenic introduction. Nonetheless, our findings highlight the potential of eDNA as a sensitive tool for the early detection of non-native species, offering valuable insights for long-term biodiversity monitoring and future marine ecosystem management.

The spatial distribution patterns of fish detected via eDNA in this study appear to be closely linked to the climatic and oceanographic features of the waters surrounding the Korean Peninsula. The East Sea, characterized by its considerable deep and the interaction of cold and warm currents, supported the presence of polar, boreal, and deep-water species. For example, cold-water species such as *G. chalcogrammus* inhabit the East Sea; however, ongoing warming trends may eventually drive these species further north[3]. In contrast, the West Sea, characterized by its shallow depth and a semi-enclosed coastline, experiences significant temperature fluctuations and substantial freshwater inflow, such as from Yangtze River. These conditions make it particularly vulnerable to climate change[52,53]. Over approximately the last decade, the West Sea has exhibited the fastest warming rate among the regions studied[54]. Species detected in this region included coastal residents (e.g., *A. personatus* and *Chelon haematocheilus*) as well as migratory fish, suggesting that future habitat shifts driven by climate change could profoundly impact both residential and migratory species. Jeju, the southernmost region with higher proportion of tropical and subtropical fish, such as *C. nigromarginatus* and *R. kanagurta*, highlighting the potential for range expansion these species under continued warming. Moreover, these findings suggest that the ecosystem structure around Jeju—and, by extension, the broader Korean Peninsula—may undergo significant transformations, emphasizing the need for proactive resource management and ecological conservation measures to mitigate potential impacts.

The geographic isolation of Jeju from the Korean Peninsula may have contributed to its relatively high fish diversity. The surrounding waters are characterized by structurally complex reef systems, including hard corals and rocky substrates, which provide diverse microhabitats for fish species. Furthermore, Jeju is located more than 80 km from the mainland, resulting in lower levels of anthropogenic disturbances, such as dam construction, industrial complexes, and land reclamation. Similar patterns have been observed globally, where fish assemblages in remote, less disturbed island ecosystems tend to exhibit higher diversity compared to those in areas with high human activity[55,56]. Therefore, the combination of reduced human impact and the influence of ocean currents facilitating the dispersal of tropical and subtropical fish species may have played a crucial role in shaping the diverse fish communities observed in Jeju waters.

Traditional methods for monitoring fish communities typically require considerable investment of personal, time, and the sacrifice of numerous fish, in addition to potential habitat damage caused by fishing gear. For instance, in marine environments, researchers commonly utilize various types of gear, including gill nets, fyke nets, and bottom trawls[57]. Gill nets and fyke nets typically require more than 24 h from deployment to retrieval, and bottom trawling can disturb benthic ecosystems[58]. Moreover, the choice of specific gear types can influence the composition of sampled fish assemblages, potentially leading to misperceptions regarding species dominance[59,60]. Consequently, even within the same study area, researchers may overestimate or underestimate fish community composition depending on their choice of sampling gear[61]. In contrast, eDNA sampling requires only a small water sample, is non-invasive, and can be analyzed rapidly[50]. In our study, water sample collection required about 30 min per site, enabling us to complete sampling at 63 sites within two weeks. Despite conducting a single round of sampling, we detected 167 fish species on a large scale across the Korean Peninsula. This finding underscores the feasibility of using eDNA to rapidly and effectively identify marine species, including alien species, in rapidly changing environments, providing critical data to inform strategic responses to environmental change.

Although this study successfully characterized large-scale fish communities and their distribution patterns along the Korean peninsula's coasts using underwater drones and eDNA analysis, several limitations were identified, highlighting the need for further refinements. Despite detecting a wide range of species at the species level, certain groups (e.g., *Takifugu*, *Sebastes*, *Thunnus*, *Platycephalus*, *Repomucenus*) could not be identified below the genus level. This limitation arises when employing a single 12 S rRNA marker, as closely related species may be difficult to distinguish due to insufficient base sequence variation or the lack of adequate reference sequences in genetic databases. Similar limitations have been reported in eDNA studies conducted in Japan[62]. To address this issues, future research could adopt multi-locus approaches (e.g., COI, 16 S rRNA) or metatranscriptome analysis, which may enhance species-level identification and provide insights into ecological functions and viability[63,64].

This study focused on a single time point in March 2024, conducting intensive surveys at 63 sites. However, marine ecosystems are subject to substantial temporal and spatial variability driven by environmental factors such as seasonal fluctuations in water temperature, salinity, and ocean currents[65]. Given the relatively rapid degeneration of eDNA in seawater, multi-seasonal or year-round monitoring would provide a more comprehensive understanding of species diversity and shifts in distribution patterns. Notably, integrating long-term eDNA datasets with temperature records could further enhance its utility as a foundational tool for climate change research and biodiversity conservation.

Despite being a highly efficient and innovative approach for monitoring biodiversity, eDNA metabarcoding still faces challenges that must be addressed to enhance monitoring accuracy and reliability. First, if a species lacks a reference sequence in public databases, species-level classification may be difficult. The absence of reference sequences can lead to misidentifications, where an organism is incorrectly assigned to a different species. Therefore, expanding reference databases is crucial for improving taxonomic resolution and ensuring more accurate species detection in future studies. Second, mutations in primer-binding sites can interfere with primer annealing, leading to inefficient amplification of certain taxa. As a result, some species that are present in the environment may be underrepresented or even undetected due to low amplification efficiency. This limitation can lead to an underestimation of biodiversity, affecting the overall reliability of eDNA-based assessments. Nonetheless, these biases can be mitigated through multi-locus approaches that incorporate multiple genetic markers or through rigorous primer validation and optimization. These strategies can improve amplification success and reduce primer bias, ultimately enhancing the robustness of eDNA-based biodiversity monitoring.

In this study, we considered geographical features, species' climatic zones, and long-term warming trends. However, we did not include detailed analyses of ocean current dynamics or transport estimates, which are critical factors in influencing the northward movement and range expansion of alien, tropical, and subtropical species, and can also influence eDNA distribution patterns[7]. Incorporating GIS and marine modeling to quantify ocean current patterns and eDNA degradation rates could provide researchers with more accurate predictions of potential habitat ranges and strengthen the ecological validation of newly detected species.

Methods

Study sites and water sampling

The coastal waters of the Korean Peninsula are bordered by the East Sea, Yellow Sea, and East China Sea. For this study, the coastal waters were categorized into four regions: the East Sea, South Sea, West Sea, and Jeju. To examine long-term trends in sea surface temperature (SST) around the Korean Peninsula, SST data collected by the National Institute of Fisheries Science (NIFS, https://www.nifs.go.kr/risa/main.risa) from 2012 to 2020 were analyzed to estimate the warming trends for each region.

To analyze fish communities using eDNA, water samples were collected from 63 locations across the coastal waters of the Korean Peninsula in March 2024. This included 20 sites in the East Sea, 15 in the South Sea, 19 in the West Sea, and 9 around Jeju, with sampling locations spaced approximately 20–40 km apart (Fig. 1).

Water samples were collected offshore using an underwater drone (FIFISH V6 EXPERT, QYSEA) from coastal areas or breakwaters. The drone collected 1 L of water at a depth of 1 m below the surface and 5–10 m from the shore, which was stored in sterilized collection bags. The bags were kept in an icebox under refrigeration during transportation. Subsequently, the water samples were filtered using mixed cellulose ester membrane filters (0.45 µm pore size, 47 mm diameter, Advantec, Japan). The filtered samples were stored at − 80 °C until DNA extraction.

DNA metabarcoding

The eDNA extraction process followed a modified protocol, utilizing glass beads and the DNeasy Blood and Tissue Kit (Qiagen, Germany). Extracted DNA was analyzed using a microplate reader (Thermo Fisher, USA), which provided the total DNA concentration as well as the 260/230 and 260/280 ratios for each sample. Ratios outside the optimal range of 1.8–2.2 typically indicate the presence of contaminants, such as dissolved proteins, which can interfere with the polymerase chain reaction (PCR).

The 12 S rRNA gene NGS library was prepared using a two-step PCR process. In the first PCR, universal fish primers (Mifish-U-F: GTCGGTAAAACTCGTGCCAGC; Mifish-U-R: CATAGTGGGGTATCTAATCCCAGTTTG)[66] were employed, and the process was performed in triplicate for each sample and negative control. The reaction mixture consisted of 20 ng of DNA from field samples, DNA extraction blanks, and PCR blanks; 20 pM of each 12 S rRNA primer; and 10 mM Tris-HCl, in a total reaction volume of 20 µl. The thermal cycling protocol included an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 3 min. The amplified PCR products were purified and size-selected using AMpure XP beads (Beckman Coulter, USA), and their concentrations were quantified using the Qubit dsDNA HS kit with a Qubit 4.0 fluorometer (Thermo Fisher, USA). A second PCR was performed using the Nextera XT Index kit (Illumina, USA), followed by purification and concentration measurement under the same conditions as the first PCR products. The prepared library was diluted to a concentration of 2 nM, pooled to achieve a final dilution of 6 pM, and supplemented with a 10% PhiX control for sequencing. Paired-end sequencing was conducted on the Illumina Miseq platform using the MiSeq Reagent Micro Kit v2 (paired-end, 2 × 150 bp).

Bioinformatics

The 12 S rRNA read libraries generated on the MiSeq platform were processed using the Qiime2 framework and its associated plugins[67]. Prior to data analysis, primers were removed from the sequences using Cutadapt[68]. Additionally, the DADA2 error-filtering algorithm was employed to denoise the sequences by identifying and removing errors based on a sample-specific error model[69]. This process yielded 12 S rRNA amplicon sequence variants along with their corresponding read counts for each sample. Taxonomic assignments for the ASVs were obtained using the BLASTn algorithm and the MitoFish database[70,71]. Genus- or species-level classifications were provided if the sequence identity exceeded 97%. To mitigate the impact of false sequences and sequencing errors associated with low-abundance taxa, ASVs with 10 reads or fewer were excluded from further analysis.

Identification of alien species

We classified alien species if it was not listed in the National List of Korea[72]. This list serves as the official reference for fish species in Korean waters. Any species detected through eDNA analysis that was absent from this database was categorized as an alien species. Additionally, species identification was cross validated using FishBase[48] to confirm their known geographic distributions.

Statistical analysis

All detected fish species were categorized into six habitat types, tropical, subtropical, temperate, boreal, polar, and deep-water, based on information from FishBase[48]. For taxa identified only at the genus level (e.g., *Takifugu* sp., *Sebastes* sp., *Thunnus* sp., *Platycephalus* sp., and *Repomucenus* sp.), habitat data for species known to occur in Korean waters were used.

Statistical analyses were conducted using R software (2022.07.1). Shared fish species among the four coastal regions were visualized using an UpSet plot generated with the ComplexUpset package[73]. Shannon diversity and species richness indices were calculated for each sampling point and compared across regions using boxplots created with the ggplot2 package[74]. Self-organizing maps (SOM), an unsupervised artificial neural network comprising input and output layers connected by weight vectors, were utilized to classify the habitat characteristics of fish species detected in each region[75]. For SOM training, binary presence/absence data of fish species from each region, along with habitat information from FishBase, were used as input data. Twelve SOM output units ($N = 3 \times 4$) were employed to ensure biologically relevant clustering. The SOM analysis was performed using the kohonen package[76]. To evaluate the statistical significance of the SOM-derived clusters, a Multi-Response Permutation Procedure (MRPP) was performed using Bray–Curtis dissimilarity with the vegan package[77]. This analysis tested whether within-group dissimilarities were significantly smaller than between-group dissimilarities.

The geographic distribution characteristics of the 20 most dominant fish species in Korean waters were visualized using QGIS 3.26 (QGIS Geographic Information System, QGIS Association). ASV data for the detected fish species were log-transformed for analysis and visualized as a heat map to illustrate species distributions effectively.

Conclusion

Our research demonstrates that underwater drone-based eDNA metabarcoding provides a robust and high-throughput method for assessing fish diversity across extensive coastal regions under rapidly changing environmental conditions. By integrating nine years of sea surface temperature (SST) data with spatial patterns of fish assemblages, we identified significant warming trends in the West Sea and East Sea and documented shifts in fish community composition, including the northward expansion of warm-water species and the sustained presence of cold-water taxa in deeper areas. The early detection of alien species further underscores the pivotal role of eDNA in biological monitoring and resource management. However, several limitations remain, including the need for multi-marker approaches to resolve taxonomic ambiguities and the importance of multi-season sampling to capture temporal variability. Additionally, incorporating detailed ocean current modeling could provide deeper insights into how physical factors influence fish distributions and eDNA dispersal. Despite these challenges, our findings highlight the immense potential of eDNA as an essential tool for large-scale marine biodiversity monitoring, particularly in the context of ongoing climate change.

Data availability

The sea surface temperature (SST) data analyzed in this study were obtained from the National Institute of Fisheries Science (NIFS) and are publicly available at https://www.nifs.go.kr/risa/main.risa. The datasets generated during the current study are available in the NCBI BioProject repository under accession number PRJNA1223655. For access inquiries, please contact the corresponding author, Prof. Ihn-Sil Kwak.

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Author contributions

I.-S.K. and T.-S.Y. conceived and designed the study. T.-S.Y. and W.-S.K. conducted field sampling and eDNA extraction. W.-S.K. performed sequencing and bioinformatics analysis. T.-S.Y. and I.-S.K. analyzed the data and interpreted the results. T.-S.Y. conducted statistical analyses and visualization. T.-S.Y. wrote the first draft of the manuscript, and all authors contributed to manuscript revisions and approved the final version for submission.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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