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## LETTER

# Benchmarking fish biodiversity of seaports with eDNA and nearby marine reserves

Stéphanie Manel<sup>1,7</sup> | Laetitia Mathon<sup>1</sup> | David Mouillot<sup>2,7</sup> | Morgane Bruno<sup>1</sup> | Alice Valentini<sup>3</sup> | Gilles Lecaillon<sup>4</sup> | Anais Gudéfin<sup>4</sup> | Julie Deter<sup>2,5</sup> | Pierre Boissery<sup>6</sup> | Alicia Dalongeville<sup>3</sup>

<sup>1</sup>CEFE, Univ Montpellier, CNRS, EPHE-PSL University, IRD, Montpellier, France

<sup>2</sup>MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

<sup>3</sup>SPYGEN, Le Bourget-du-Lac, France

<sup>4</sup>Ecocean, Montpellier, France

<sup>5</sup>Andromède Océanologie, Mauguio, France

<sup>6</sup>Agence de l'Eau Rhône-Méditerranée-Corse, Délégation de Marseille, Marseille, France

<sup>7</sup>Institut Universitaire de France, Paris, France

## Correspondence

Stéphanie Manel, CEFE-CNRS, UMR 5175- CEFE, Campus CNRS 1919 route de Mende, 34293 Montpellier cedex 5 France. Email: [Stephanie.manel@ephe.psl.eu](mailto:Stephanie.manel@ephe.psl.eu)

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## Abstract

Coastal areas offer a diversity of habitats providing refugia and nursery for fish, promoting their biodiversity and associated contributions to people. Yet, natural coastlines are replaced by artificial infrastructures such as seaports and the influence of this artificialization on fish biodiversity remains poorly known. Here, we assessed fish biodiversity indicators using environmental DNA metabarcoding inside seaports and adjacent natural habitats including no-take marine reserves. We found that species assemblages within seaports were primarily influenced by their area and habitat. We detected a similar species richness in seaports and reserves during lockdown, but seaports host more threatened species than natural habitats. Yet, species turnover between seaports was lower than between natural areas, reflecting biotic homogenization. Seaport managers should consider that complexifying artificial infrastructures could increase habitat diversity and coastal fish biodiversity. Our study illustrates that eDNA-based indicators can be integrated in management and policy applications toward greener marine artificial infrastructures.

## KEYWORDS

artificial habitat, environmental DNA, indicator, IUCN, marine fish, marine-protected areas, metabarcoding

## 1 | INTRODUCTION

Coastal areas offer some of the most diverse ecosystems on Earth (Williams et al., 2022). These coastlines in their natural state offer a great diversity of habitats (seagrass meadows, estuaries, rocky reefs, etc.) that are refuges for many species, and nurseries essential for the settlement and growth of juveniles, especially harvested fishes

(Cheminée et al., 2021). This diversity of habitats and species underpins many contributions to people, including 90% of exploited marine resources (Barbier, 2017). Yet, these coastal areas concentrate today more than one-third of human population (Barbier, 2017) and few coastlines are left without anthropogenic pressure worldwide (Williams et al., 2022). Elsewhere, human activities induced an artificialization of coastal ecosystems (Dafforn et al., 2015;

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Wenger et al., 2018) and the subsequent loss of natural habitats (Dafforn et al., 2015; Todd et al., 2019).

This artificialization impacts coastal biodiversity and the associated nature's contributions to people. The removal of natural fish nurseries may lead to the decline of adult populations that sustain local fisheries (Yan et al., 2021). Surprisingly, high juveniles abundances can be observed in shallow artificial habitats created by seaports (Ido & Shimrit, 2015), which could support a nursery function (Bouchoucha et al., 2016; Macura et al., 2019). Recent studies show that seaports host fish assemblages different from those present in natural habitats (Todd et al., 2019). Yet, fish biodiversity in seaports is still poorly known (Bouchoucha et al., 2016; Madon et al., 2023) with no consensus about the comparative level of biodiversity between natural and artificial habitats (Macura et al., 2019). This lack of knowledge and consensus partly comes from inappropriate or limited sampling designs and methods. The use of visual or video surveys may bias biodiversity comparisons between natural and artificial habitats because species are more challenging to detect in seaports than in natural habitats given water turbidity and access.

Environmental DNA metabarcoding (eDNA) overcomes some shortcomings and biases of visual and video surveys to characterize marine fish assemblages across habitats by retrieving DNA naturally released by organisms in their environment (Polanco Fernández et al., 2021). This method improves detection of elusive, rare, and cryptobenthic fish species that are missed by classical surveys (Mathon et al., 2022). To our knowledge, eDNA metabarcoding has been applied to only a few seaports (> 3) (Lacoursière-Roussel et al., 2018; Rey et al., 2020) without comparison to natural habitats, whereas eDNA surveys have the potential to benchmark the level of fish biodiversity in seaports and nearby habitats that are more or less protected from human activities. We took advantage of eDNA detection capacity and an unprecedented sampling design inside and outside seaports to test whether seaport infrastructures (i) influence fish biodiversity, (ii) have an equivalent level of biodiversity than natural habitats under various human pressures, and (iii) homogenize regional biodiversity.

Here, we investigated fish biodiversity in seven seaports located in the Mediterranean Sea (Figure 1) and tested the influence of environmental factors, artificial infrastructure, and European clean harbors certification. We then compared fish biodiversity within seaports to those of 40 samples collected in nearby natural areas, located within and outside nearby no-take marine reserves, before and during the Covid-19 human lockdown to provide a benchmark assessing the extent to which seaports may contribute to regional fish diversity. We found that

species assemblages within seaports were mainly influenced by their habitat and area. We detected a similar species richness in seaports and reserves during lockdown, which was higher than in fished areas. We also reported that seaports host significantly more threatened species than natural habitats including reserves but that species turnover between seaports was lower than between natural habitats.

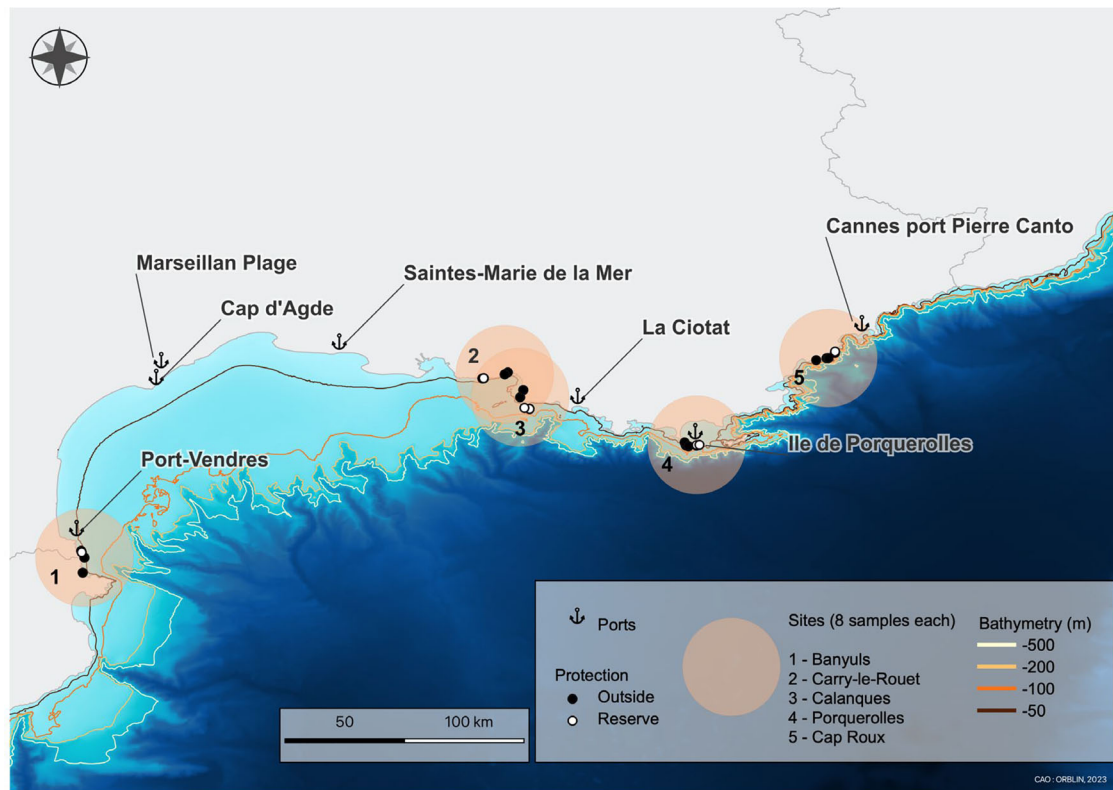
## 2 | METHODS

### 2.1 | Sampling

The study area includes seven seaports (Table S1) in the Mediterranean Sea (Figure 1) inside which 28 eDNA samples were collected at two seasons (spring and autumn) with two replicates. Each sample consists of 30 liters of seawater filtered during 30 min from a kayak along a transect (Figure S1). We covered the largest possible area in each seaport. We avoided approaching fishing boats and fish market (<50 m) in order to limit the risk of false positives resulting from eDNA released from bycatch, fishing gears, or market waste waters (Supporting Information). We collected seawater 1 m below the sea surface using a sterile tube and a peristaltic pump and filtered through a VigiDNA 0.2  $\mu$ M cross-flow filtration capsule.

To compare fish biodiversity within and outside seaports, we reanalyzed 40 eDNA samples collected in adjacent regions of five seaports (Figure 1).

Immediately after filtration, the capsule was emptied from the remaining water and filled with 80 mL of CLI conservation buffer and stored at room temperature until extraction (Polanco Fernández et al., 2021). eDNA extraction was performed in a dedicated room for water DNA sample extraction (Dalongeville et al., 2022). We carried out Polymerase Chain Reaction (PCR) amplification using the teleo primer pair, targeting a 64 bp fragment of the mitochondrial DNA 12S rRNA gene, specific to teleost fishes and elasmobranchs (Valentini et al., 2016). We sequenced in parallel 12 replicate PCRs per sample, six negative extractions, and three PCR controls. Five High throughput sequencing (HTS) libraries were finally sequenced using Miseq paired-end sequencing (2  $\times$  150 bp) runs (Supporting Information). Sequences were analyzed following a bioinformatic pipeline (Supporting Information) to produce a list of species per sample taking advantage of a quasi-exhaustive genetic reference database for Mediterranean fish species (Dalongeville et al., 2022). No rarefaction analysis was applied in our study because we found no correlation between the number of species and the number of reads (Supporting Information).



**FIGURE 1** Sampling design in the north-western Mediterranean Sea (France). Sampling locations in seven ports (28 samples) and five areas outside seaports (40 samples).

## 2.2 | Data analyses

We first performed a hierarchical and variation partitioning canonical analysis (HVPCA) using the *rdacca.hp* R package (Lai et al., 2022) to disentangle the effects of environmental factors (season, habitat, depth), artificial infrastructures (seaport area), and the European certification clean port (Supporting Information) on fish species composition using the Jaccard distance, an index of  $\beta$ -diversity, between eDNA samples. To visualize the results, we performed a distance-based redundancy analysis (dbRDA) on the Jaccard distance matrix keeping only factors detected significant in the HVPCA.

We estimated four biodiversity indicators in each seaport: the richness of all fish species, but also that of threatened species, harvested species, and cryptobenthic species. These species richness indicators were mapped after pooling eDNA field replicates and seasons by seaport. We used a mixed linear model with the R package *spaMM* (Rousset & Ferdy, 2014) to test the effect of seaport versus natural habitat on fish richness considering the interaction between the two factors and the geographic coordinates as a random effect. To compare species compositional change among seaports and among natural habitats outside seaports, we calculated the turnover and nestedness components of the Jaccard  $\beta$ -diversity index using the R

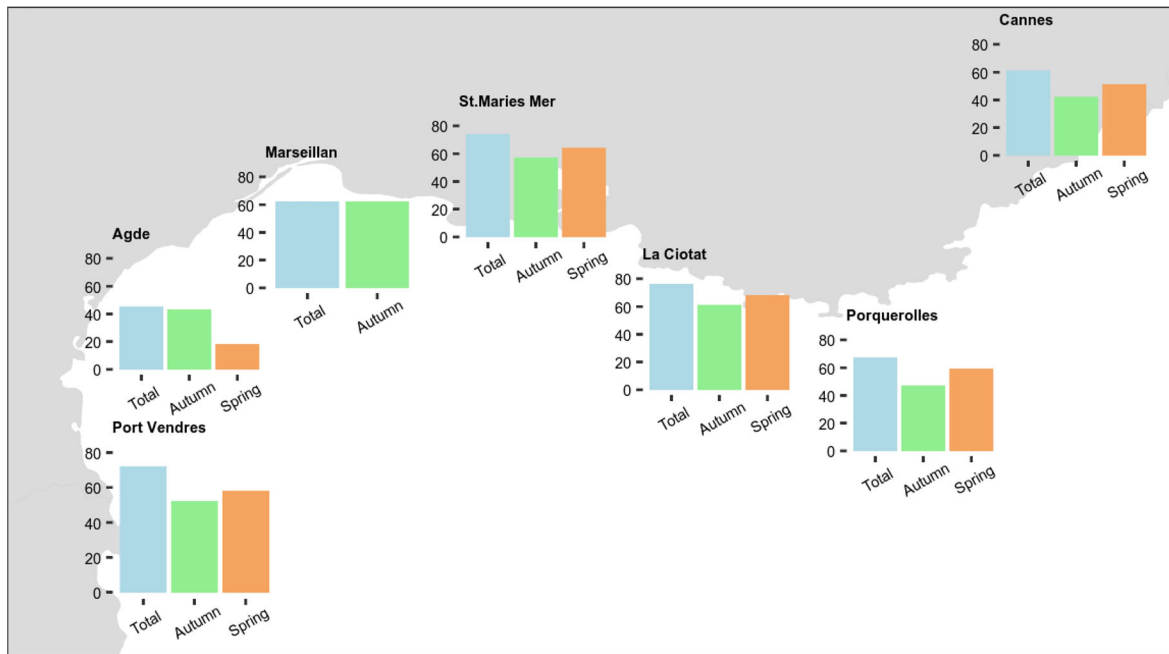
package *betapart* (Baselga & Orme, 2012). To account for potential false positives—species detected in seaports but not present (e.g., wastes)—all analyses were performed on the full list of species detected in seaports (main results) and on a reduced list of species after removing species not expected to naturally occur in seaports (Supporting Information).

## 3 | RESULTS

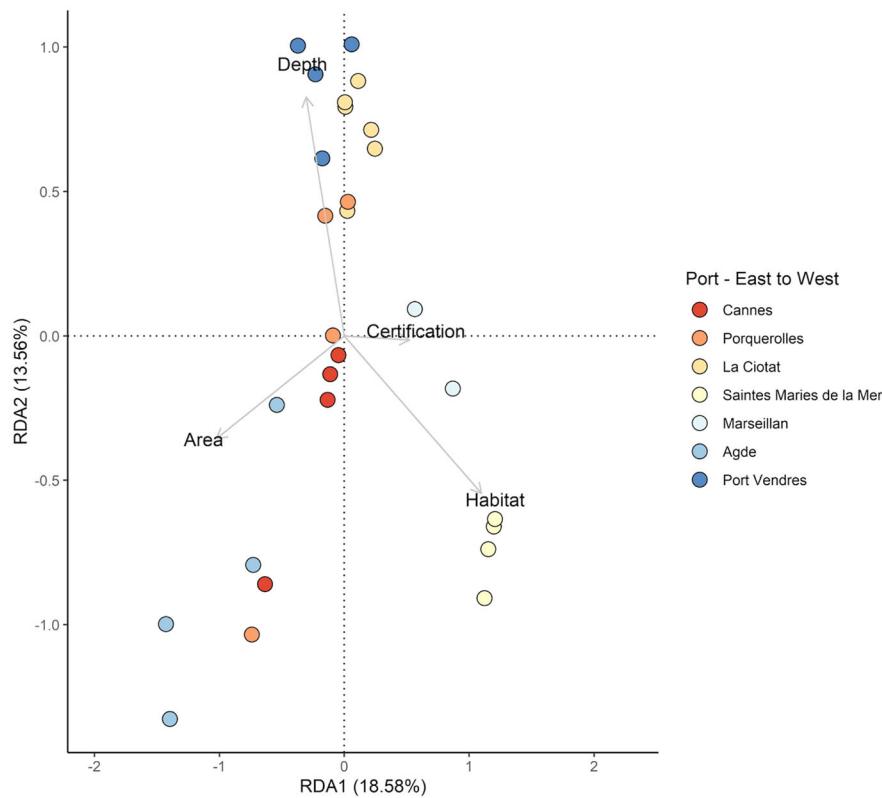
### 3.1 | Fish biodiversity within seaports

The 28 samples collected in the seven seaports yielded on average 496,674 reads per eDNA sample. After assigning reads to the reference database, we obtained a total of 50 fish families (Figure S2) and 122 fish taxa detected in seaports (Table S2). No species was detected in any of the negative controls (PCR nor extraction controls). On average, 45 taxa were detected by sample ( $sd = 12.6$ ;  $min = 15$ ,  $max = 63$ ). After pooling field replicates and seasons, the mean taxa richness per seaport was 65 ( $sd = 10.64$ ) (Figure 2a). The highest species richness was detected in La Ciotat (76) and the lowest in Agde (45). When considering seasons separately, on average, 52 fish taxa were detected per seaport and per season ( $sd = 13.64$ ). Except in Agde,

(a)



(b)



**FIGURE 2** Fish biodiversity patterns across seaports. (a) Map of total fish species richness in each port (blue), fish richness in autumn (green), and at spring (orange). (b) Plot of location scores following a partial distance-based redundancy analysis testing the effect of certification, habitat, port area, and depth conditioned by longitude on Jaccard pairwise distances between ports. Variables were selected from the hierarchical and variation partitioning canonical analysis.

and in Marseillan (not sampled in June), fish richness was always higher in spring than in autumn (Figure 2a). Following expert recommendations, we reduced this list of taxa to 96 fishes (Table S2) producing similar patterns of species richness in seaports (Figure S3a). Species removed by experts were mainly freshwater species (e.g., *Acipenser* sp., Leuciscinae, *Rutilus rutilus*, *Gambusia holbrooki*).

The HVPCA indicated that the total variation in fish species composition among eDNA samples explained by environmental and infrastructure factors was high ( $R^2_{\text{adj}} = 56.5\%$ ). Forty percent of this variation was unique to each factor (Table S3). Five factors had a significant individual contribution to the explained variation among seaports with habitat (sandy vs. rocky) having the highest individual contribution (26%), and then seaport area (20%), longitude (18%), depth (16%), and certification (11%) (Figure S4).

The two first axes of the dbRDA conditioned by the longitude explained 32% of species dissimilarity among samples with four significant factors (habitat, area, depth, certification) (Figure 2b). Saintes-Maries-de-la-Mer (SMM) and Marseillan, the two sandy ports, are positively associated with habitat on axis 1. Seaport area is negatively associated with axis 1 and discriminates le Cap d'Agde, having the highest area, from SMM, one of the smallest seaports (Table S1). This first axis also discriminated SMM on the positive side from Port-Vendres and Cap d'Agde, two seaports with no certification. Axis 2 clearly discriminated seaports with the greatest depth, Port-Vendres (−10 m) (Table S1) from the shallowest ports, SMM (−2.5 m) or le Cap d'Agde (−3.35 m). The patterns were unchanged with the corrected list of species (Figure S3b,c).

### 3.2 | Biodiversity comparison between seaports and natural habitats

We found a total of 101 fish families and 168 taxa across our 68 eDNA samples. We found that 75 taxa were common to seaports and natural habitats, whereas 27 were unique to seaports, 10 to reserves, 11 to fished areas (Figure 3a), and 30 to human lockdown (Figure 3b). When considering the reduced list of species, the number of unique taxa in seaports dropped down to 14 (Table S2).

Species richness per sample detected within seaports (mean = 45, sd = 12.6) was similar to that detected in samples collected during the lockdown period within and outside marine reserves (mean = 46, sd = 12) but higher to that detected in fished areas (Figure 3c). For cryptobenthic fishes, the lockdown significantly increased species richness outside seaports (Figure 3d, Table S4). Seaports had significantly more threatened and harvested species than natural habitats outside seaports including

marine reserves (Table S4). The interaction between protection and lockdown was not significant. Those patterns remained unchanged with the reduced list of species (Figure S3d,e,g), except the loss of significance for threatened species (Figure S3f).

### 3.3 | Biotic homogenization among seaports

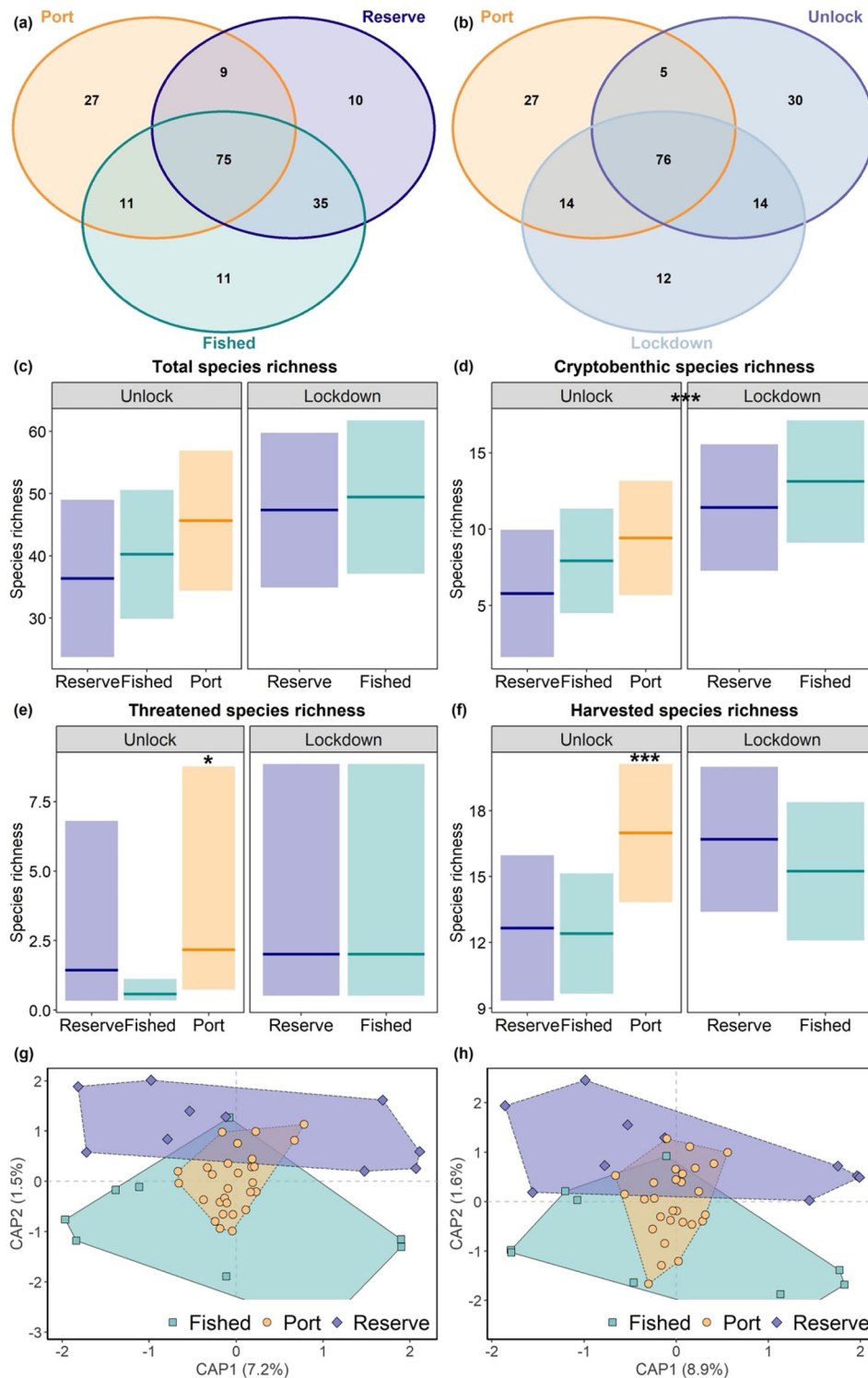
A partial dbRDA conditioned by the longitude on all samples showed that fish assemblages within seaports were less differentiated than assemblages outside seaports with a narrower distribution of the site scores along axis 1 (Figure 3 g,h). The distribution of site scores on axis 2 indicates that seaports shared species composition with both reserves and fished areas. Finally, the turnover component of the Jaccard  $\beta$ -diversity was smaller among seaports than among natural habitats outside seaports (Figure S5), but the difference was not significant.

## 4 | DISCUSSION

New vision of biodiversity is needed because wild nature disappears with growing human population and urbanization. Our study brings original results on fish biodiversity in seaports. Experts and organizations at the moment focus on increasing the coverage of protected areas that is essential (Isbell et al., 2022). Yet, alternative solutions should be promoted in urbanized areas that may host other forms of biodiversity. The goal is no longer to fully restore artificialized natural habitats or remove artificial infrastructures but to reinstall or maintain some ecological key functions like nurseries that sustain local fisheries and other contributions to people.

We globally found higher species richness in seaports than outside, but a lower species turnover, reflecting a smaller variation in taxonomic composition among seaports than among natural habitats. Seaport area and habitat, after controlling for geography using longitude, are the major drivers of species  $\beta$ -diversity between seaports. Those patterns are conserved when removing potential contamination (26 species removed, Table S2, Figure S3), resulting from DNA transported via freshwater intakes, wastewater releases from fish markets or fisheries, cleaning of fishing gears, or consumption of fish on recreational crafts at berth.

This relatively high species richness in coastal infrastructures compared to natural habitats has been observed in a meta-analysis of 471 time series spanning from 1962 to 2015 in artificial reefs across the world (Elahi et al., 2015). Our pattern is consistent with those reported in this study



**FIGURE 3** Comparison of fish biodiversity between seaports and nearby natural habitats. (a–b) Venn diagrams showing common and unique lists of fish species inside and outside seaports after pooling by habitat (a) and COVID-19 human lockdown (b). (c–f) Boxplots of fish richness representing mean and 95% confidence intervals. Results of the significance for the mixed linear models testing the binary effects of seaport versus outside, reserve versus nonreserve, and lockdown versus nonlockdown on species richness are reported with stars (\*  $p < 0.01$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ). Boxplots are for total species richness (c), cryptobenthic richness, (d) threatened richness, and (e) harvested richness (f). (g–h) Plots of location scores following the distance-based redundancy analysis testing the effect of habitat on the Jaccard distance estimated between samples including seaports and natural habitats for the total  $\beta$ -diversity (g) and only species turnover (h).

partly due to invasion by nonindigenous species in seaports, but in our case, other reasons can be highlighted. The role of nursery played by artificial structures such as ports and marinas offer sheltered productive areas for many species (Bouchoucha et al., 2016; Mercader et al., 2019). We cannot rule out that contamination by dead fished species or urban water releases artificially increase the number of detected species in eDNA traces, although we tried to be very cautious in our sampling. After validating our initial list of species by experts, the number of unique species in seaports drops from 27 to 14 (Table S2) and similar patterns were observed (Figure S3). Species unique to seaports and confirmed by experts are, for example, cryptobenthic species (*Zosterisessor ophiocephalus*, *Aphia minuta*, or *Pomatoschistus*). Seaports are submitted to more restrictive fishing rules and could be potential refuges for exploited threatened species (García-Gómez et al., 2015). Some species could also be present inside seaports at a juvenile stage, before spending their adult life in pelagic or deep waters.

Seaports are characterized by a smaller variation in fish taxonomic composition than natural habitats (Figure 3 g,h). Seaports offer more redundant and simplified habitats to fishes, and cannot provide refuges for all species (e.g., highly mobile pelagic species). Seaports could be submitted to high stress like noise and chemical pollution (Todd et al., 2019) inappropriate for some species. These lower  $\beta$ -diversity and higher species richness levels along an urbanized gradient, already observed in seagrass beds (Kelly et al., 2016) and seagrass fish communities (Iacarella et al., 2018), suggest an homogenization of fish biodiversity with coastal artificialization. Artificial structures indeed establish redundant habitats worldwide making them poor substitutes for natural habitats (Airoldi et al., 2021; Momota & Hosokawa, 2021). They can facilitate the colonization of specific species (e.g., early-colonizing, opportunistic, and non-indigenous species) and support distinctive assemblages (Momota & Hosokawa, 2021). While further urbanization in the future is inevitable given continued population growth, the incorporation of ecological rules like complexifying artificial infrastructures can contribute to limit the degradation of habitats and the decline of marine species and their functions ultimately sustaining natural resources (Dafforn et al., 2015). Seaports illustrate the response of wild nature to anthropogenic environmental changes (Kueffer & Kaiser-Bunbury, 2014). This anthropogenic biodiversity should not be neglected, and more studies are needed to understand how to rewild this biodiversity.

Our results also suggest that eDNA is a powerful tool for uncovering such human–ecosystem interactions that

might otherwise remain hidden (Kelly et al., 2016). eDNA may contribute to provide quasi-exhaustive lists of species (Dalongeville et al., 2022), including early life stages that are difficult to observe. The same samples with an appropriate metabarcode can be used to target other taxonomic groups to test whether similar patterns are found, that is, higher richness but lower turnover. Overall, eDNA-based methods, as revealed by our study, pave the way toward the assessment of standardized and comparable biodiversity indicators (Cordier et al., 2020) needed to achieve emerging seaport green ambition (e.g., Goal 14—life below water—of the sustainable development goals promoted by the United Nations, <https://sdgs.un.org/goals>).

Given that seaports make the backbone of the economy accounting for around 80% of global trade in terms of weight (Dundas et al., 2020) and 50% in terms of value (Verschuur et al., 2022), the artificialization of coastlines may intensify in the next decades with a demand for seaports roughly doubling to quadrupling by 2050 (Hanson & Nicholls, 2020). In this context, effective universal and integrated environmental management in seaports becomes essential toward a greener blue economy, that is, that preserves ocean health while maintaining contributions to people (Winther et al., 2020). However, at the moment, initiatives for sustainable ports are heterogeneously distributed and depend on the country or continent but are poorly addressing the biodiversity crisis (Hossain et al., 2021). For example, EcoPorts, a European port-sector-based environmental initiative under the European Sea Ports Organization (ESPO, <https://www.espo.be/>), aims to cooperate and share environmental performance indicators (e.g., carbon footprint, waste management and consumption, wildlife protection, etc.) to evaluate the reach of sustainability targets. Green Marine is an environmental certification program for maritime companies in North America to establish sustainability in marine transportation (Hossain et al., 2019). Port authorities at Zhuhai port in China have developed their own “green port” indicators (Hua et al., 2020). Those examples illustrate the wish of port stakeholders to commit with seaport sustainability policies. Yet, organizational guidance like Ecoports of ESPO should be generalized at the international level to help seaports reaching environmental objectives (Hossain et al., 2021) including biodiversity targets. Our study reveals that eDNA-based species inventories and biodiversity indicators, in comparison with natural benchmarks like marine reserves, are relevant tools to track progresses toward greener seaports. We illustrate this application on the overcrowded Mediterranean coast, but it can be extended to any other marine artificial infrastructures that proliferate to face the ongoing energy and food crisis.



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## DATA AVAILABILITY STATEMENT

### Code and data repositories

The data repository associated with this work is located at [https://github.com/lmathon/Med\\_Port\\_eDNA](https://github.com/lmathon/Med_Port_eDNA). Codes are available at the following github (private access until paper acceptance): [https://github.com/lmathon/Med\\_Port\\_eDNA](https://github.com/lmathon/Med_Port_eDNA). Raw data are available at Zenodo (<https://zenodo.org/records/10401873>, <https://doi.org/10.5281/zenodo.10514755>, <https://doi.org/10.5281/zenodo.10515153>).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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