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BRIEF COMMUNICATION

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Enhancing fish community monitoring at a cabled observatory by combining environmental DNA and imaging analysis

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Abstract

Cabled multiparametric observatories are sustaining ecological monitoring by collecting long-term real-time biological and environmental data. Here, we investigated fish communities by sampling environmental DNA (eDNA) over 4 days near the multiparametric cabled video-observatory OBSEA (Northwestern Mediterranean Sea). The multi-marker eDNA metabarcoding approach resulted in an increased species detection and helped provide a more comprehensive view of the local fish community when combined with imaging data. These results underline the potential of omics methods in long-term monitoring of economically and ecologically important fish species.

KEYWORDS

cabled observatories, ecological monitoring, eDNA metabarcoding, fish communities, omics, visual census

1 | INTRODUCTION

Effective marine monitoring of economically and ecologically important fish communities requires multiparametric time series of continuous and high-frequency data (i.e., biological, oceanographic and geochemical). Video-cabled observatories are sustaining such data collection in a long-term (from weeks to years) and continuous (i.e., near-real or real-time) manner, therefore, allowing the establishment of in situ ecological laboratories in both continental margins and abyssal plains (Danovaro et al., 2017). Most of these observatories are equipped with cameras that provide long-term imaging data, favouring the compilation of local species' richness and biodiversity lists (Francescangeli et al., 2025; Stefanni et al., 2022). Moreover, the environmental and multidisciplinary data collected facilitate the fine-scale monitoring of shifts in species presence, as well as their behaviour, at different temporal scales (Cuvelier et al., 2017; De Leo et al., 2018; Matabos et al., 2014). For instance, changes in the observed fish communities were related to tidal and day/night cycles at the OBSEA underwater cabled observatory off the coast of Catalonia (Spain) (Aguzzi et al., 2013), as well as at the SmartBay multiparametric platform in Galway Bay (Ireland) (Aguzzi, López-Romero, et al., 2020). Over a longer time scale, imaging data from underwater cameras in the Mediterranean Sea were used to detect

seasonal shifts in both commercially and ecologically important species (Aguzzi, Iveša, et al., 2020; Condal et al., 2020).

The main disadvantage of the data collected by imaging systems at cabled observatories is the limited field of view (Aguzzi, Chatzievangelou, et al., 2020; Rountree et al., 2020; Stefanni et al., 2022). Other limitations include the taxonomic expertise required for fish identification and environmental conditions, such as water turbidity, light diffusion and fouling (Marini et al., 2018), that can affect image quality. Issues deriving from low spatial representativeness of cameras and laborious species recognition could be mitigated by the integration of new omics monitoring approaches at cabled observatories, namely environmental DNA (eDNA) metabarcoding analysis.

In fact, in recent years, a growing need to achieve a global characterisation of biodiversity in marine ecosystems has motivated the development of omics technologies as complementary tools to imaging methods (Aguzzi et al., 2019). Beyond research in ecology, eDNA analysis has also been proven to be a useful approach for biodiversity monitoring purposes (Deiner et al., 2017), with the potential to impact practices and policies in management and conservation (Bohmann et al., 2014; Creer et al., 2016). Although promising, eDNA analysis faces many challenges, including amplification bias, inhibition and incomplete reference databases (Thomsen & Willerslev, 2015), as well as uncertainty on the temporal and spatial persistence of DNA in aquatic systems (Mauvisseau et al., 2022). Therefore, integrating eDNA with imaging data from cabled observatories will lessen each method's limitations and has the potential to improve biodiversity monitoring of fish communities. In recent years, studies have detected more species when using eDNA in combination with imaging data from a cabled observatory (SmartBay in Ireland; Mirimin et al., 2021) and Baited Remove Underwater Systems (BRUVs deployed in Western Australia; Stat et al., 2019). Both studies also identified eDNA false negatives (i.e., species detected by video but missed by molecular data), highlighting current gaps and areas for methodological improvement, as well as the complementarity of eDNA and imaging data.

Time series of environmental and biological data are important to understand past community shifts and forecast future trends (Dornelas et al., 2013); therefore, efforts should be placed into increasing the amount of time series data available. The ease and standardisation of eDNA sampling procedures make this technique an optimal candidate to be implemented in long-term sampling. Additionally, the taxonomic breadth (i.e., the possibility to target multiple taxonomic groups and individual species from a single sample) represents a great advantage of eDNA compared to more traditional methods (Bálint et al., 2018; Djurhuus et al., 2020). The collection and analysis of eDNA time series have been increasing. For instance, Carvalho et al. (2024) used a 1-year eDNA time series to monitor seasonal dynamics of fish in a fjord; Bista et al. (2017) explored annual trends of biodiversity in a lake ecosystem; Djurhuus et al. (2020) surveyed marine biodiversity across taxonomic groups with an 18-month eDNA dataset; and Chrismas et al. (2023) studied intra- and inter-annual diversity patterns in fungi over 17 years.

Cabled observatories can act as testing hubs for methodological and technological advances that could increase ecological monitoring efficiency (Favali et al., 2010; Gaughan et al., 2019). In particular, by having access to real-time continuous data from the observatory's imaging system, the species detected using eDNA can be cross-referenced with video-sampled species (when water collection occurs nearby the cameras, synchronously with imaging). Additionally, knowledge on occurrence and fish community composition obtained from long-term imaging-based monitoring at these sites could help identify gaps in reference DNA data. Previous studies have presented the advantage of collecting eDNA samples at cabled observatories (Mirimin et al., 2021), but to validate the transferability of the eDNA/imaging approach, the latter requires to be tested in different environments and on different fish community types. In this framework, the present study aimed to assess the effectiveness of eDNA metabarcoding to describe the diverse fish community composition of OBSEA cabled observatory area by comparing molecular and underwater imaging data.

Sampling was carried out at the OBSEA cabled observatory, located 4 km off the coast of Vilanova i la Geltrú (41.193° latitude,1.754° longitude, Catalunya, Spain) at a depth of 20 m in an area protected from illegal fishing with artificial biotopes (Aguzzi et al., 2011; Del-Rio et al., 2020), between 14 and 17 September 2021. Imaging data were collected by the Underwater IP Camera (OPT-06, OpticCam; resolution 640 × 680 pixels) every 30 min continuously during day and night ($n = 48$ images per day). For every image, all individuals of each species were identified following Francescangeli et al. (2025), and only individuals classified to the species level were retained for the scope of this study. Species identified in the images were used to cross-validate species detected using omics markers. Further cross-validation was carried out using data extracted from images captured by OBSEA's camera during the month of September from 2012 to 2021 and processed as detailed above. This 10-year dataset was used to supplement the records of species that were not detected during the 4-day sampling period.

Seawater eDNA samples were collected at the OBSEA location at 8:00 AM in the morning and at 8:00 PM in the evening, using a Niskin bottle (5-L volume). For each sampling event, three independent replicates were collected at the site ($n = 20$ in total) by lowering the open Niskin in the water and closing it at 20 m of depth. For each replicate, 1 L of seawater was filtered on sterile Sterivex filters (pore size of 0.45 μm) using sterile syringes. During the filtering procedures, one negative control was collected by filtering 1 L of distilled water alongside the field samples. All laboratory steps were carried out by a third-party service provider (Applied Genomics, UK). DNA was extracted using the DNeasy Blood and Tissue extraction kit (QIAGEN), including an extraction negative control. Extracts were amplified targeting two commonly used mitochondrial genes, cytochrome B (CytB) and 12S ribosomal RNA, using fish-specific universal markers: L14841/H15149c for CytB (Evans et al., 2016) and MiFish-U-F/MiFish-U-R for 12S ribosomal RNA (Miya et al., 2015). Amplicon libraries were sequenced on an Illumina MiSeq sequencer using MiSeq version 2 2 × 250 bp reagent kit. Bioinformatic processing steps are detailed

in Supplementary Materials. Briefly, the ObiTools3 pipeline (version 3.0.1b25, Boyer et al., 2016) was applied. Reads (R1/R2) were merged setting a minimum alignment score to 0.80. Sequences were dereplicated, and only clusters with more than 10 sequences were retained. Taxonomic assignment was performed using the MIDORI2 curated database (release 257, Leray et al., 2022) of 12S and CytB mitochondrial genes. Reads were taxonomically identified to a species based on a sequence length coverage of at least 150 bp and a percentage of identity above 97%. Statistical analysis and graphical outputs were obtained using R software (R Core Team, 2021) and R studio (Posit Team, 2023), with the package *VennDiagram* (Chen, 2022).

A total of 192 images were obtained from OBSEA's underwater camera across the four sampling days, and all were viable for analysis. A total of six species (*Chromis chromis*, *Coris julis*, *Dentex dentex*, *Diplodus cervinus*, *Diplodus sargus and Oblada melanura*) from three families (Labridae, Pomacentridae and Sparidae) were identified from the collected images (Figure S1 in Supplementary Materials). Out of all the images in which fish were detected, individuals were classified to species level in 51%, 30%, 18% and 33% of cases, respectively for 14, 15, 16 and 17 September.

The 12S marker sequencing run produced a total of 1081,519 reads for an average of 54,067 (±11,287) reads per sample. On average, 36% (±11%) of the raw reads were assigned to fish taxa. The CytB marker sequencing run produced a total of 1331,735 reads for an average of 70,091 (±83,071) reads per sample. On average, 1% (±2%) of raw reads were assigned to fish taxa. Further details are available in Tables S1 and S2 in Supplementary Materials. In total, eDNA detected 24 species across 13 families. Data from the 12S marker identified 22 species, whereas data from the CytB marker identified 5 species. Overall, 3 species were identified by both markers, 2 only by CytB and 19 only by 12S. These results show a clear difference between the two markers, with 12S outperforming CytB, confirming previous findings in fish eDNA metabarcoding studies (Bylemans et al., 2018). However, considering the species detected exclusively by the CytB data, these results indicate that a multi-marker approach can provide a more comprehensive view of the local biodiversity (e.g., Kumar et al., 2022; Mirimin et al., 2021; Stefanni et al., 2018).

Venn diagrams of the species detected by the eDNA analysis (data from both markers merged), 4-day imaging and 10-year imaging data are shown in Figure 1 (full species lists provided in Table S3). Imaging and eDNA collected during the four sampling days detected 3 species in common, with the former method detecting 3 species exclusively and the latter detecting 21 species exclusively. All fish species detected by the camera during the 4 days had been previously recorded in the long-term monitoring data collected over the previous 10 years during September. In contrast, eDNA identified 14 species that had not been recorded during the long-term monitoring.

Overall, 20 eDNA samples collected over 4 days detected more species than those detected in the 10-year-long effort of imaging analysis, underlining the potential of eDNA to monitor fish communities in a marine environment. Environmental DNA samples detected 4 times more taxa compared to the underwater camera for the 4-day

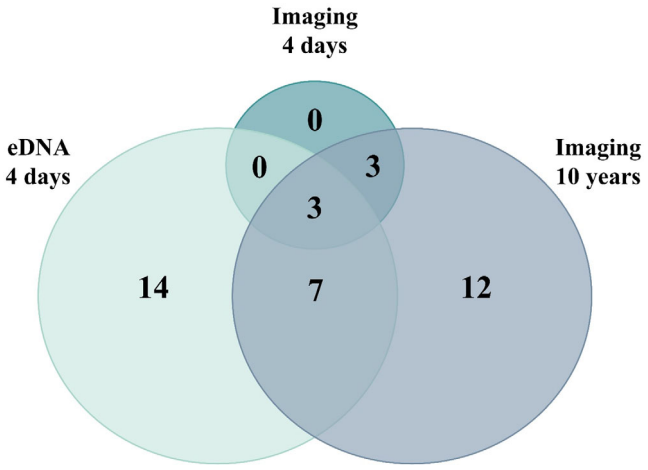

FIGURE 1 Venn diagrams representing the number of species detected by three methods: environmental DNA (eDNA) samples (data from 12S and CytB markers merged), imaging data collected over 4 days and long-term imaging data collected over 10 years (for the month of September).

study period (24 vs. 6). Although imaging data detected mainly rocky/reef-associated demersal and benthopelagic fish, eDNA detected demersal (e.g., *Mullus surmuletus*), benthopelagic (e.g., *D. cervinus*) and pelagic (e.g., *Trachurus mediterraneus*) species. Additionally, eDNA identified highly commercially valued fish (e.g., *Micromesistius poutassou*, *Pomatomus saltatrix*, *Scomber scombrus*, *Spicara maena*) that were not detected by the camera (Froese & Pauly, 2024). Many of the fish species ($n = 7$) detected by eDNA are known to be present in the area and had been previously recorded by OBSEA's camera (Francescangeli et al., 2025). However, 12 species detected by eDNA were not present in the 10-year time series, which is likely due to their behavioural/foraging/reproductive traits. In line with previous studies (Mirimin et al., 2021; Stat et al., 2019), these results showed increased species richness when combining multidisciplinary monitoring approaches, suggesting the transferability of this integrated approach to different geographical regions.

Conversely, eDNA incorrectly identified one Atlantic species (*Coris atlantica*). Upon examination of imagery data (short and long term), it was determined that the correct assignment was most probably the local species, *C. julis*. This highlights the importance of cross-referencing multidisciplinary data and suggests that using multiple genetic markers (more than two) may be necessary to accurately cross-validate species richness lists. In addition, eDNA did not detect three species that were picked up by the camera: *C. chromis*, *D. dentex* and *O. melanura*. For one of the three undetected species, *O. melanura*, the 12S reference database had an incomplete entry, whereas the other two had full entries. The CytB reference database had a full entry for all three species. Although incomplete databases are still a critical limitation for eDNA studies (Petit-Marty et al., 2023), the missed detection of these three species cannot be explained entirely by partial references. In fact, there must have been other aspects that prevented the detection of *C. chromis* and *D. dentex*, such

as low volumes of filtered water or heterogeneous shedding eDNA rates among species. During the 4 days of sampling, *C. chromis* and *D. dentex* were identified every day (during daylight hours), whereas *O. melanura* was detected in only one image, potentially indicating low abundance in the environment and consequently low probability of eDNA detection, which could explain the false negative. These missed detections show the need to address current eDNA limitations. In fact, cabled observatories could play a crucial role in advancing our understanding of the ecology of eDNA in relation to community data, obtained through cameras and environmental parameters (e.g., water temperature, salinity, pH, wind speed, currents), which are usually collected continuously by these infrastructure and available in near-real time.

Ultimately, given the ease of sample collection, possibility to standardise sampling procedures and enhanced species detection, we argue that it would be beneficial to introduce routine long-term eDNA sampling efforts at cabled observatories as a great asset to monitor ecologically and economically important fish species.

AUTHOR CONTRIBUTIONS

Luca Mirimin, Sergio Stefanni and Jacopo Aguzzi conceptualised the study. Joaquin Del Rio and Daniel Mihai Toma led the eDNA sampling. Maddalena Tibone led bioinformatic and statistical analysis. Marco Francescangeli led the imaging data analysis. Maddalena Tibone drafted the manuscript. All authors provided feedback and approved the final version of the manuscript. PhD supervision to Maddalena Tibone was provided by Luca Mirimin, Sergio Stefanni, Jacopo Aguzzi and Bernadette O'Neill.

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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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