



Communications

Ecological Applications, 0(0), 2021, e02273

© 2020 The Authors. *Ecological Applications* published by Wiley Periodicals LLC on behalf of Ecological Society of America

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

All is fish that comes to the net: metabarcoding for rapid fisheries catch assessment

TOMMASO RUSSO,¹ GIULIA MAIELLO,^{1,2} LORENZO TALARICO ¹, CHARLES BAILLIE,³ GIULIANO COLOSIMO,⁴
LORENZO D'ANDREA,¹ FEDERICO DI MAIO,^{5,6} FABIO FIORENTINO,⁶ SIMONE FRANCESCHINI,¹ GERMANA GAROFALO,⁶
DANILO SCANNELLA,⁶ STEFANO CATAUDELLA,¹ AND STEFANO MARIANI ^{7,8}

¹Department of Biology, University of Rome Tor Vergata, Rome, Italy

²PhD program in Evolutionary Biology and Ecology, University of Rome Tor Vergata, Rome, Italy

³School of Environment and Life Sciences, University of Salford, Salford, United Kingdom

⁴San Diego Zoo, Institute for Conservation Research, San Diego, California 92027 USA

⁵Department of Biological, Geological and Environmental Sciences, University of Bologna, Bologna, Italy

⁶National Research Council, Institute for Marine Biological Resources and Biotechnology, Mazara del Vallo, Italy

⁷School of Biological & Environmental Sciences, Liverpool John Moores University, Liverpool, United Kingdom

Citation: Russo, T., G. Maiello, L. Talarico, C. Baillie, G. Colosimo, L. D'Andrea, F. Di Maio, F. Fiorentino, S. Franceschini, G. Garofalo, D. Scannella, S. Cataudella, and S. Mariani. 2021. All is fish that comes to the net: metabarcoding for rapid fisheries catch assessment. *Ecological Applications* 00(00):e02273. 10.1002/eap.2273

Abstract. Monitoring marine resource exploitation is a key activity in fisheries science and biodiversity conservation. Since research surveys are time consuming and costly, fishery-dependent data (i.e., derived directly from fishing vessels) are increasingly credited with a key role in expanding the reach of ocean monitoring. Fishing vessels may be seen as widely ranging data-collecting platforms, which could act as a fleet of sentinels for monitoring marine life, in particular exploited stocks. Here, we investigate the possibility of assessing catch composition of single hauls carried out by trawlers by applying DNA metabarcoding to the dense water draining from fishing nets just after the end of hauling operations (hereafter “slush”). We assess the performance of this approach in portraying β -diversity and examining the quantitative relationship between species abundances in the catch and DNA amount in the slush (read counts generated by amplicon sequencing). We demonstrate that the assemblages identified using DNA in the slush satisfactorily mirror those returned by visual inspection of net content (about 71% of species and 86% of families of fish) and detect a strong relationship between read counts and species abundances in the catch. We therefore argue that this approach could be upscaled to serve as a powerful source of information on the structure of demersal assemblages and the impact of fisheries.

Key words: DNA metabarcoding; eDNA; environmental impacts; fisheries; marine biodiversity; trawling.

INTRODUCTION

Monitoring exploitation of marine resources and assessing the status of marine stocks and communities are key activities in fisheries science and biodiversity conservation. Achieving effective fisheries management is increasingly important as overfishing threatens fish stocks globally, reduces biodiversity, alters ecosystem

functioning, and jeopardizes food security and livelihoods of hundreds of millions of people worldwide (FAO 2020). Monitoring fishing activities and their impacts rely on two main sources of information: catch data (fishery dependent) and research surveys (fishery independent) (Dennis et al. 2015).

Since fisheries are key agents of disturbance for marine ecosystems, management requires as accurate as possible data about what, where, and how much of key species is caught. The lack of such reliable catch data could lead to uncertainty about stock status, impairing our perception of resource availability, and increasing the chances of overfishing.

The collection of fishery-dependent data is historically carried out by logbooks, which are often inaccurate

Manuscript received 19 June 2020; revised 4 November 2020; accepted 13 November 2020. Corresponding Editor: Matthew Barnes.

T. Russo, G. Maiello, and L. Talarico are an equal contribution author group, and S. Cataudella and S. Mariani are another equal contribution author group.

⁸ Corresponding Author. E-mail: S.Mariani@ljmu.ac.uk

(Sampson 2011), on-board observers, or self-sampling of catch done by fishers (Kraan et al. 2013). Unfortunately, several issues often prevent exhaustive data collection on catch composition. These include discarding of non-commercial species, vessel size, distribution and operational range of the fleet, and the fact that the analysis of catch is based on time-consuming procedures such as visual sorting, taxonomic classification, counting, measuring, weighing, and/or tissue sampling. For all these reasons, the collection of fishery-dependent data is often limited to subsets of the fleet, compromising the accuracy and representativeness of the results achieved (Vilas et al. 2019). On the other hand, fishery-independent data, which are mainly collected by scientific surveys explicitly designed to capture resource distribution and status, depend on huge operational ship-time costs (Dennis et al. 2015).

Overall, collection of fishery-dependent and/or -independent data is therefore complex, time-consuming, and costly. Yet, our understanding of distributions of the thousands of species caught by fisheries across the world's oceans remains incomplete (Seebens et al. 2016), especially in developing countries (Worm and Branch 2012, Pauly and Zeller 2016), while sustainability targets, in the face of increasing climatic instability, still require spatially and temporally accurate, widespread, and affordable monitoring approaches (Bradley et al. 2019).

The rise of new technologies for the collection, management and analysis of fishery-dependent data is providing a suite of possible solutions to update and modernize fisheries data collection systems and greatly expand data collection and analysis (Bradley et al. 2019, Plet-Hansen et al. 2019). In this context, DNA metabarcoding is one major innovation that is revolutionizing the way we assess biological diversity, by rapidly generating vast species inventories from trace DNA retrieved from water samples (Thomsen et al. 2012, Djurhuus et al. 2018, Stat et al. 2019), sediment cores (Fonseca et al. 2010), and bulk DNA from animal organs and tissues (McInnes et al. 2017). Different environmental media (water, sediment, gastric contents, etc.) exhibit varying levels of DNA concentration, affecting sampling, laboratory and data interpretation phases (Siegenthaler et al. 2019). However, some fishing gears, such as the nets used for bottom trawling, capture and concentrate in the net cod-end a large number of specimens in a reduced water volume. Since the amount of cells and tissues in a given volume of water should be positively associated with both animal abundance and volume of collected water (Lacoursière-Roussel et al. 2016), the high biomass concentration conditions generated by trawling should lead to much greater DNA concentration of target species compared to the highly diluted conditions in seawater commonly sampled for environmental DNA studies. This “surplus” DNA effect is expected to be even greater when animals are pressed against each other and/or wounded, losing blood or other fluids, as it typically happens during fishing operations.

In this study, we therefore asked the following questions: (1) Can the concentrated DNA from water collected from fishing nets provide a readily available DNA source? (2) Can DNA metabarcoding of such collections be effectively used to reconstruct catch composition? Specifically, we extracted DNA from samples of water draining from the net cod-end, just after it was hauled on board, while suspended above the deck (Fig. 1A). This water is a naturally concentrated “slush”, containing tissue and other cell material, which we hypothesized would yield DNA data reflecting species composition in the net. We used a DNA metabarcoding approach to reconstruct the species composition of several hauls across different locations in the Strait of Sicily (Mediterranean Sea, Fig. 1B) and compared them directly to concurrent assessments by on-board researchers based on visual sorting and classification. Additionally, we investigated the quantitative relationship between species abundances in the catch (number and biomass of individuals) and DNA amount in the slush (read counts generated by amplicon sequencing), as, despite the enormous potential of metabarcoding, there still is little consensus on the extent to which reads correspond to the actual abundance of species (Lamb et al. 2019).

We demonstrate that the assemblages identified using DNA in the slush adequately mirror those returned by visual inspection of catch both qualitatively and quantitatively. We therefore argue that this promising approach could be upscaled to serve as a powerful source of information on the composition of demersal assemblages and fishery impact on target stocks.

MATERIAL AND METHODS

Collection of DNA samples and catch data

Samples were obtained from nine sites (Appendix S1: Table S1; Fig. 1B), between July and August 2018, during a bottom trawl survey within the Mediterranean International Trawl Survey (MEDITS) framework (Bertrand et al. 2002). Sampling sites covered three depth layers (10–50, 51–100, and 101–200 m). For each site, water was collected in triplicate from the dripping net cod-end just after it was hauled on board (hereafter referred to as “slush”), while suspended above the deck (Fig. 1A), then stored in three 50-mL sterile tubes (i.e., field replicates; Fig. 1C). Contextually, to account for “baseline environmental DNA contamination”, seawater was sampled nearby the vessel during hauling procedures in six out of nine sites (i.e., seawater blanks). All samples were first frozen at -40°C on board and successively transferred in the laboratory and stored at -20°C until DNA extraction.

In parallel, the species composition of each catch was determined by on-board processing of net content. All the individuals in the net were identified at the species level by visual inspection of external morphology and, if needed, analysis of meristic characters. For each species

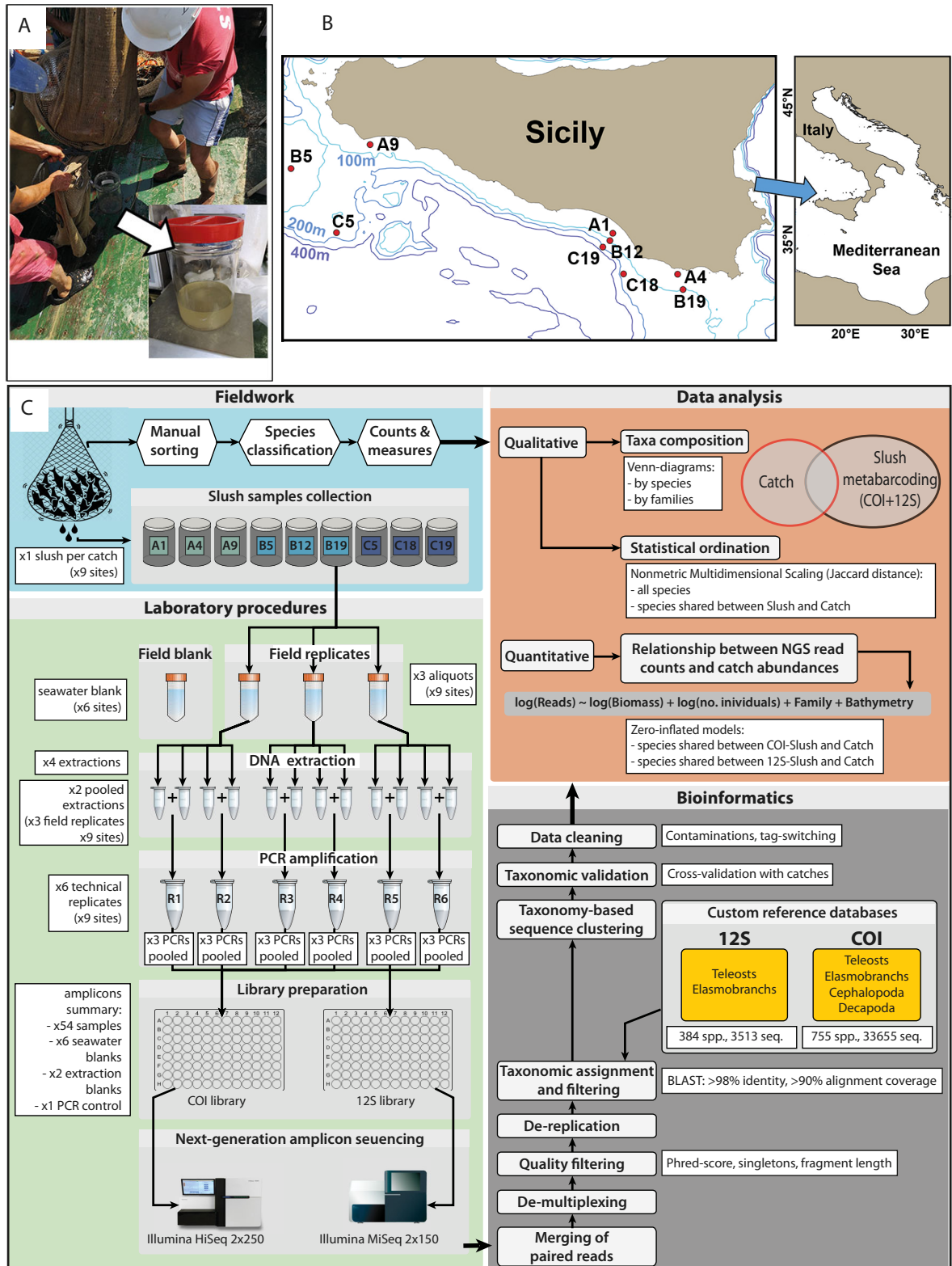


Fig. 1. Sampling of water draining from net cod-end and study technical procedures: (A) collection of water at the end of the hauling phase, (B) maps identifying the sampling locations along the southern coast of Sicily, Mediterranean Sea; (C) graphical schematic illustrating the key analytical steps: fieldwork, laboratory, bioinformatics, and data analyses. Colors of slush samples refer to those in Fig. 3A and B (according to their depth stratum). Sequences is abbreviated “seq.”

at each site, we recorded the overall number of individuals and their total mass.

DNA extraction, amplification, library preparation, and Illumina sequencing

After centrifuging the slush tubes, the supernatant was removed, and 500 μL of slush pellet was collected, 100 μL at a time, and lysed for 2 h in a ThermoMixer (Eppendorf, Hamburg, Germany) at 50°C and 1,500 rpm, with the lysis solution from the Mu-DNA (Sellers et al. 2018) protocol for tissue. Following lysis, the rest of the protocol followed the Mu-DNA water protocol. Using this hybrid protocol allowed for efficient lysis of a more viscous sample than water (containing many cells and organismal fluids), while also removing PCR inhibitors associated with seawater (McKee et al. 2015). For each slush sample, four DNA extractions were performed and extracts were pooled two by two in identical volumes to maximize DNA retrieval (Fig. 1C).

We amplified (1) an ~167 bp fragment of the mitochondrial 12S gene using the Teleo02 primers specifically targeting fishes, with an estimated (in silico) species detection >98% for teleosts (Taberlet et al. 2018); (2) a ~313 bp fragment of the COI gene using the universal metazoan Leray-XT primers (Wangensteen et al. 2018). To uniquely distinguish each sample and aid in the detection of PCR/sequencing cross-contamination, each primer pair carried unique eight-base-pair tags, the same tag for both forward and reverse primers. DNA extracts were PCR amplified three times independently to minimize stochasticity. Each reaction (25 μL) contained 16 μL Ampliqaq Gold Master Mix (Applied Biosystems, Foster City, California, USA), 0.16 μL of Bovine Serum Albumin, 5.84 μL of water, 2 μL of purified DNA extract, and 1 μL of each forward and reverse primer. The cycling profile for 12S primers included polymerase activation at 95°C for 10 minutes, followed by 35 cycles of denaturation and amplification (95°C for 30 s, 54°C for 45 s, 72°C for 30 s), and a final elongation of 72°C for 5 minutes. The cycling profile for COI primers included polymerase activation at 95°C for 10 minutes, followed by 35 cycles of denaturation and amplification (94°C for 1 minute, 45°C for 1 minute, 72°C for 1 minute), and a final elongation of 72°C for 5 minutes. PCR replicates were then pooled prior to sequencing. Overall, we had six replicates per site (a combination of both field and technical replicates; Fig. 1C). Note that both field and laboratory controls (i.e., six seawater blanks, two extraction blanks, and a negative PCR control for each marker) were amplified to ensure quality of procedures and to assess contaminations at each step. Seawater blanks were processed identically to slush samples.

Amplicons were pooled and then purified with 1 \times paramagnetic beads (MagBio Genomics, Gaithersburg, Maryland, USA). For 12S, we prepared a PCR-free, single-indexed library with the KAPA Hyper Prep Kit (Roche, Basel, Switzerland), following the manufacturer's

instructions. The COI library was prepared using UDI (unique dual index) adapters as it was pooled with other libraries from other projects. Libraries were cleaned of adapters and quantified using qPCR. The 12S library was loaded onto an Illumina (San Diego, California, USA) MiSeq platform, at 8 pMol concentration, for 2 \times 150 paired-end sequencing. The COI library was loaded on a larger Illumina HiSeq 2,500 run, alongside samples for unrelated projects, at Macrogen (Seoul, South Korea) for 2 \times 250 paired-end sequencing.

Data pre-processing

Bioinformatic procedures for quality check and data pre-processing consisted of the following steps implemented in OBITools packages (Boyer et al. 2016). First, we merged paired ends reads (minimum score = 40) and de-multiplexed samples based on their individual tag, allowing for a single base mismatch error in each tag. Second, we discarded sequences with low base-call accuracy (i.e., average quality < 30 Phred score), sequences out of the expected length range (i.e., 12S = 129–209 bp; COI = 303–323 bp), and singletons. Third, identical sequences were collapsed (de-replication) before taxonomic assignment.

Reference database

A reference database was created for 12S and COI, separately (Fig. 1C): the former included 12S sequences of Mediterranean fish species, while the latter contained COI sequences of Mediterranean taxa targeted by bottom trawl fishery (i.e., teleosts, elasmobranchs, cephalopods, and decapods). Sequences were downloaded from the NCBI nucleotide database on 9 January 2020: 3,513 and 33,655 accessions were gathered for 12S and COI, respectively. Note that the list of Mediterranean fish species ($N = 755$) is accessible in FishBase (*available online*).² The list of cephalopods and decapods ($N = 384$) was obtained combining lists from SeaLifeBase and the Italian Society of Marine Biology checklists (*database available online*).^{3,4}

Molecular taxonomic identification

We performed the taxonomic assignment of sequences that passed the previous filtering steps: unique sequences were compared to custom-made reference databases using the megaBLAST alignment algorithm implemented in the R-package rBLAST. For each query, the best match was chosen according to the maximum (bit) score. The use of such customized database allowed the automatic removal of non-target taxa (e.g., DNA of

² https://www.fishbase.se/trophiceco/FishEcoList.php?ve_code=13

³ https://www.sealifebase.ca/speciesgroup/index.php?group=mollusks&c_code=380&action=list

⁴ https://www.sealifebase.ca/speciesgroup/index.php?group=crustaceans&c_code=380&action=list

humans, chicken, cow), which are frequently found in such experiments due to contamination (Taberlet et al. 2018), and mitigated erroneous taxonomic assignments (e.g., assignment to non-Mediterranean species) that may occur when barcode sequences of a Mediterranean species are missing or shared with a non-Mediterranean species, or the barcode diversity is underrepresented compared to that of closely related exotic species. A manual inspection was performed to validate the taxonomic assignment of the more common sequences: when a sequence was shared between multiple species, we conservatively chose the one observed in catches. Only sequences showing >98% identity match combined with a >90% alignment coverage were unambiguously assigned to a species. We filtered out residual artefacts, likely originated by tag jumping (Schnell and Bohmann 2015) and/or cross-contamination, taking advantage of negative controls (i.e., six seawater blanks, two extraction blanks and a PCR negative). Specifically, the maximum frequency of reads per species observed in negative controls (i.e., 0.11% and 0.04% for 12S and COI, respectively) was assumed as the contamination threshold. Thus, species within a sample occurring with a relative abundance below such threshold were discarded. The above-mentioned procedures are summarized in the “bioinformatics” box in Fig. 1C.

Qualitative comparison between catch and metabarcoding

To effectively compare between overall compositions of slush (data from 12S and COI were qualitatively combined together) and catch data, we drew Venn diagrams for each taxon (teleosts, elasmobranchs, cephalopods, and decapods) at both species and family level. Secondly, we used nonmetric multidimensional scaling (NMDS) based on Jaccard distances to assess and visualize qualitative differences in species assemblages among sampling sites and sources (i.e., visual catch examination vs. slush metabarcoding) simultaneously. NMDS was implemented in the R package *vegan* (Oksanen et al. 2018) and performed twice including (1) all detected species or (2) only species shared between slush and catch. In both cases, we combined COI and 12S data and replicates were treated separately.

Finally, we formally tested differences between samples, considering their source (slush or catch) as a factor of the analysis together with sampling sites, with PERMANOVA (1,000 permutations) using the *vegan* function *adonis*.

Quantitative relationship between catch composition and number of DNA reads

We summed read counts from six replicates per species and site, for 12S and COI separately. Summed reads were used to investigate the quantitative relationship with catch composition, along with environmental and “taxonomic” variables, by fitting the following model:

$$\log(\text{Reads}_{s,i} + 1) \sim \log(N_{s,i} + 1) + \log(W_{s,i} + 1) + \text{Bathymetry}_i + \text{Family}_s$$

where $\text{Reads}_{s,i}$ is the number of reads for the species s at site i , $N_{s,i}$ is the number of individuals of species s at site i (from catch data), $W_{s,i}$ is the weight of species s at site i (from catch data), Bathymetry_i is the sea bottom depth at site i , and Family_s is the taxonomic family of species s . *Bathymetry* and *Family* variables were included a priori, as they are both likely to affect the DNA amount in the slush: fishes caught in deeper sites may release more fluids than those caught in shallow water (e.g., because swim bladder often collapses during hauling procedure); while taxonomic groups may shed DNA differently, due to their physical features (e.g., crustaceans; Tréguier et al. 2014). Given that the dependent variable ($\text{Reads}_{s,i}$) was clearly zero inflated (Martin et al. 2005), we fitted a zero-inflated regression model for count data via maximum likelihood, using the function *zeroinfl* of the R package *pscl* (Jackman et al. 2020). This kind of modelling approach returns a two-component mixture model combining a binary outcome model (i.e., Bernoulli), devised to model the inflation of zero in the observed values, and a truncated count model (e.g., Poisson or negative binomial). Using this approach, it is possible to estimate the probability that a species is present and then, given it is present, estimate the relative mean number of individuals (Martin et al. 2005). Coefficients for the zero and count components, respectively, are returned.

These analyses were carried out considering only species detectable by both methods (i.e., metabarcoding and catches) to avoid that technical issues may affect the relationship (e.g., the incompleteness of reference database for taxonomic assignment). Furthermore, we removed co-absences (i.e., species missing in both slush and catches in a certain site), which would artificially improve the robustness of models. The results of this model were represented as scatterplots overlapping a representation of the confusion matrixes generated by the comparison between expected (from catch) and observed (in the slush) number of reads per species.

RESULTS

After bioinformatic analysis, 12S PCR products yielded 5,433,845 reads and allowed detection of 32 species of teleosts and 8 elasmobranchs. From COI PCR products, we obtained 716,091 reads, returning 49 species of teleosts, 9 elasmobranchs, 14 cephalopods, and 5 crustaceans. Twenty-three teleosts and 4 elasmobranchs were shared between 12S and COI PCR products (Appendix S1: Table S2). Fifty-eight species were shared across slush and catch sampling methods (Fig. 2A). Over 30% of reads included the most important target species for Mediterranean demersal catches (Russo et al. 2019): the European hake (*Merluccius merluccius*), the two red mullets (*Mullus barbatus* and *Mullus*

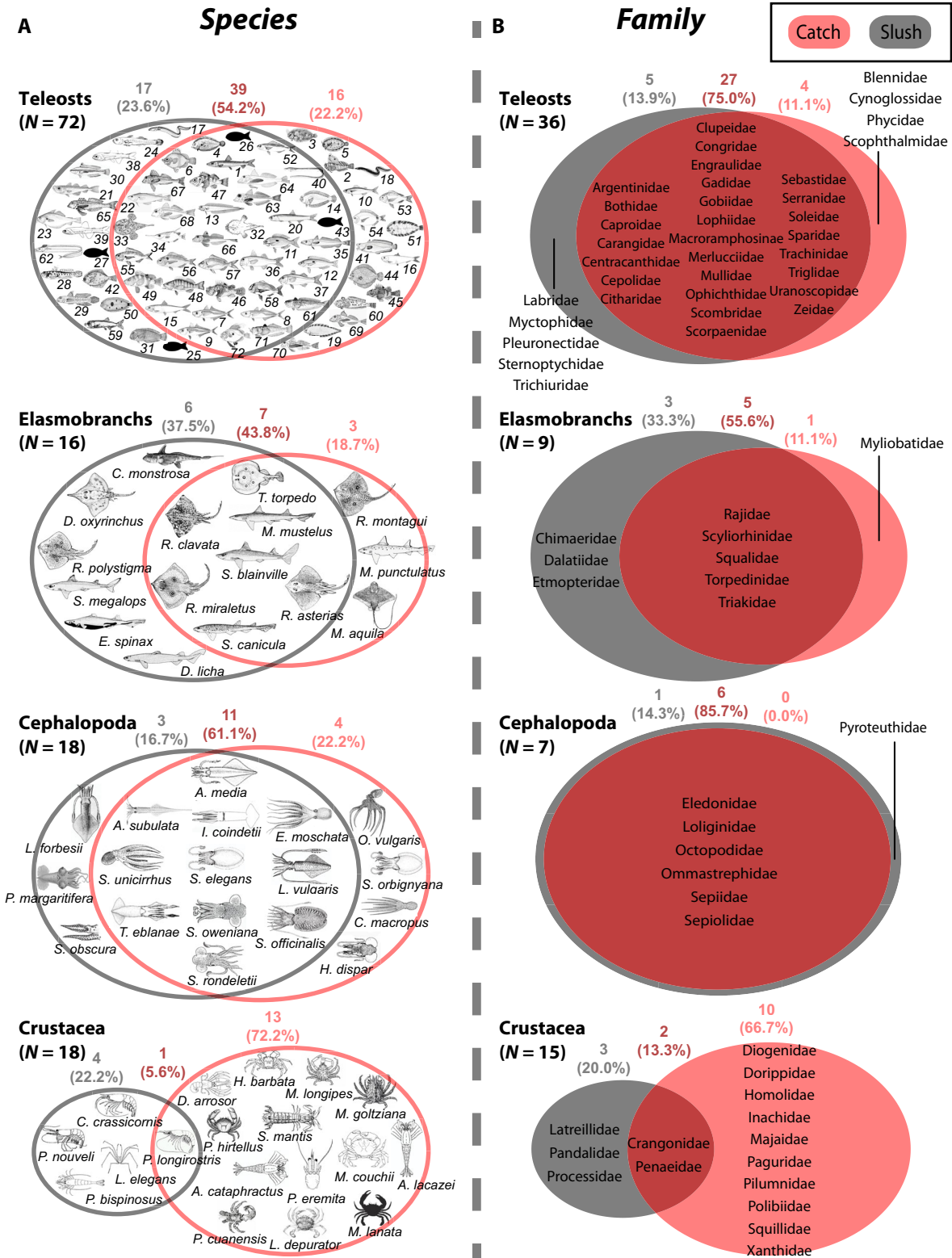


FIG. 2. Venn diagram of (A) species and (B) families detected via metabarcoding of DNA in the slush and catch. Taxa identified using the 12S marker and COI markers are combined. Diagram areas are proportional to the number of taxa. Refer to Appendix S1: Table S2 for the names of teleost species denoted by numbers, and genera names for Elasmobranchs, Cephalopoda and Crustacea. Drawings were reproduced with permission of FAO Copyright Office (<http://www.fao.org/3/a-i7429e.pdf>), with some exceptions (see Appendix S1: Table S5).

surmuletus), three species of sea bream (*Pagellus acarne*, *Pagellus bogaraveo*, and *Pagellus erythrinus*), and the deep-water rose shrimp (*Parapenaeus longirostris*). However, 30 species were detected only in the slush and 36 only in the catch (Fig. 2A). Fig. 2B shows the comparison between sampling methods at the family level (further details on detection overlaps can be found in Appendix S1: Table S3).

β -diversity reconstructed through different methods (DNA metabarcoding or visual inspection), showed a clear intra-sites affinity and a coherent distribution of samples according to the depth strata (Fig. 3A,B). Both NMDS performed on the whole data set (Fig. 3A) and on the subset of species shared across slush and catch (Fig. 3B) separated samples according to their spatial origin (habitat/biotope), with a clear depth gradient along the first dimension. Most notably, DNA data appear to convey very effectively the greater β -diversity of the mid-depth layer (stations “B”).

PERMANOVA on the whole data set detected a significant difference between samples, with most of the variance explained by stations (69%), and only 3% contribution of source (i.e., slush or catch). Fig. 3C,D show the zero-inflated model fits (see also Appendix S1: Table S4), in which observed values are plotted against predicted values. The zero-inflated models for 12S and COI data sets returned good fits for both zero and count components, with a clear linear relationship between predicted and observed values when both are greater than zero.

DISCUSSION

The idea behind this work is that fishing nets can concentrate shed material from captured species, increasing the amount of their DNA relative to the trace DNA from all the species present in the surrounding environment. Our results confirm that the water draining from trawling gear is indeed an effective source of concentrated DNA. Given that capturing sufficient quantities of DNA is one of the first critical steps in the application of eDNA-related techniques (Spens et al. 2017), and that the oceans remain largely under-surveyed, this finding opens the door to potentially important applications for fisheries and biodiversity monitoring.

Visual and DNA-based assessment methods (catch and slush) corroborate each other, indicating that water draining from net cod-end is a significantly good proxy for composition of commercial catch (Figs. 2A, 3A), and that important information on β -diversity of fishing grounds can be readily gleaned through DNA monitoring. The 30 species detected by DNA but not recovered via visual inspection reflect the power of metabarcoding approaches to identify rare and cryptic species (e.g., *R. polystigma* vs. *R. montagu*), and record taxa that are present in the environment but not catchable via trawling. The accuracy of taxa detection was further improved by the use of two barcode sequences, a

practice often advocated for high-biodiversity environments (McElroy et al. 2020). Although it may appear counterintuitive, positive detection of taxa not captured by the net can originate from a variety of processes: the physical action of the trawling gear on the substrate can suspend and retain biological material from organisms that may not be caught; this may include gametes, larvae and mucus and hence be visually undetectable in the haul; additional DNA may originate from feces and regurgitates from the fish that are caught, and damaged, in the net. Overall, ship deck and net contamination (i.e., from previous hauls) appear negligible based on the substantial among-site differences in species assemblages (Fig. 3A). On the other hand, the 36 species that were visually identified but not detected through DNA mostly reflect the incompleteness of sequence repositories and the lower taxonomic resolution of the 12S marker (Collins et al. 2019), which remains a significant challenge that must be met in the coming years in order to make DNA monitoring fully operational. This explanation is supported by the analysis at the family level, which shows a reduction of the mismatches between methods.

The DNA approach appears less efficient in the case of crustaceans, possibly due to the effect of the exoskeleton in reducing the amount of released extracellular DNA (Tréguier et al. 2014), compared to the various secretions typical of fish and cephalopods (Livia et al. 2006). Also note that we specifically extracted DNA from centrifuged pellets (i.e., excluding the supernatant), so this may have high-graded DNA through the concentration of fish and cephalopod mucus.

Despite the complexities discussed above, we were able to unveil a robust correlation between number of sequence reads and species abundance in the catch. Previous studies had also suggested that metabarcoding data could be used to infer abundance, at least to some extent, and at a higher taxonomic level than the species (Thomsen et al. 2016). Here we showed that by adding few sampling-associated predictor variables to a regression model, it is possible to explain up to 63% of the variance in reads abundance across samples. Undoubtedly, the unnatural biomass concentration achieved through trawling likely underlies the more pronounced quantitative nature of metabarcoding data compared to what is normally expected in “typical” eDNA studies based on seawater samples. This represents a promising feature of the “slush” approach, for instance, in the context of catch composition reconstruction, which may often differ from recorded landings as a consequence of by-catch discard, a widespread practice that interferes with fisheries management and has substantial consequences on some stocks and assemblages. Of course, further optimization will be required, particularly to identify biological and environmental factors substantially affecting the relationship between DNA amount in the water and actual abundances of taxa. The analysis of mock samples from controlled experimental conditions may provide an encouraging approach to address this issue.

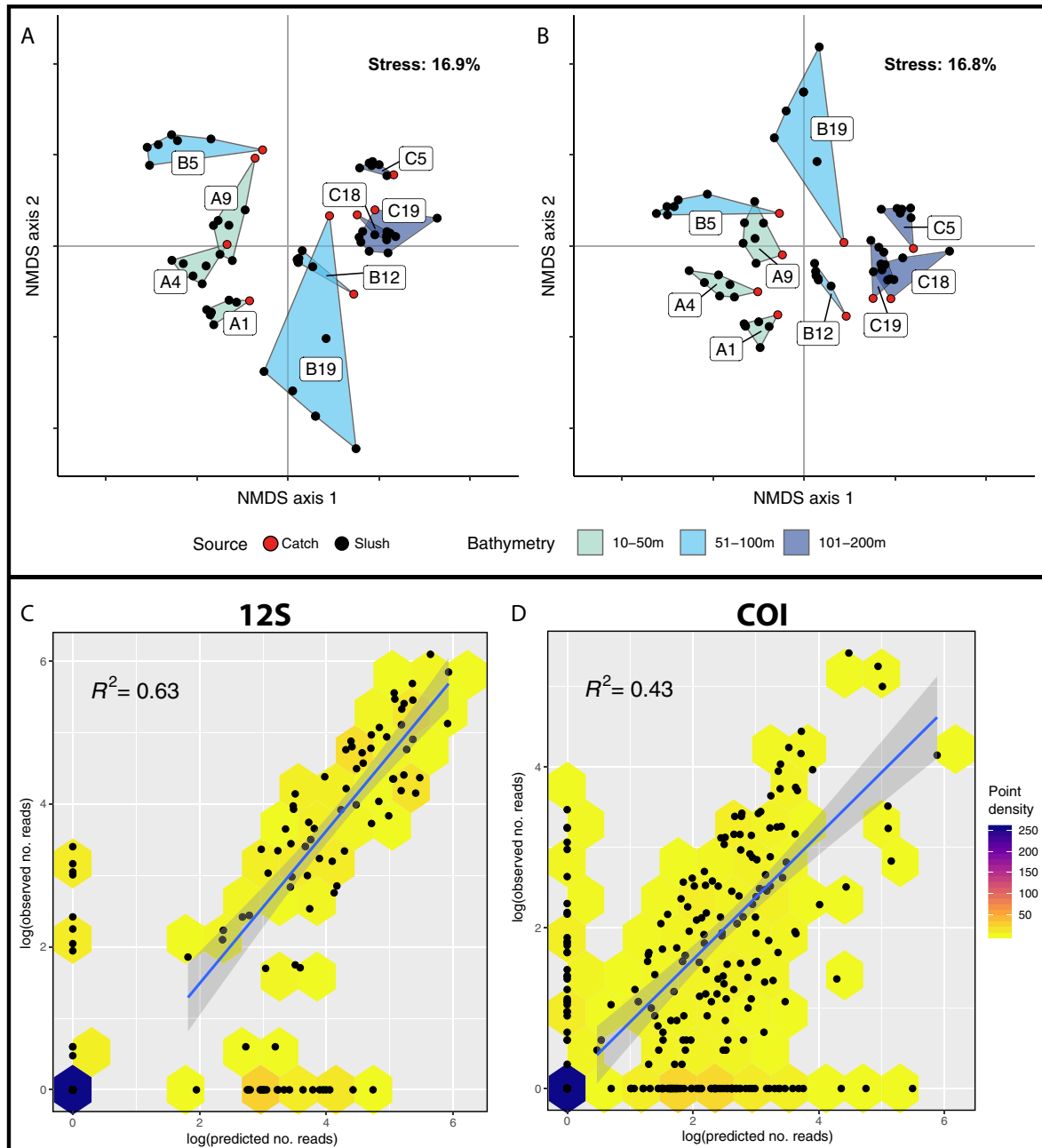


FIG. 3. (A–B) Pattern of species composition from visual sorting of survey catches and DNA sequences (12S and COI) retrieved from slush samples (six replicates per site), as returned by a nonmetric multidimensional scaling (NMDS) based on Jaccard's distance. Samples are grouped by sampling locations into convex hulls colored according to the corresponding depth layer and labelled according to the sampling station. (A) NMDS performed on the whole data set (125 species); (B) NMDS performed on restricted data set (58 species present in both slush and catch). (C–D) Scatterplot of observed (in the slush) and predicted (by the fitted zero-inflated models) \log_{10} -transformed number of reads by species and site, for (C) 12S and (D) COI data sets. Point density is represented by hexagons colored according to the scale placed to the right of the COI plot. Linear regressions (with standard errors and corresponding adjusted R^2 values), fitted on positive values, are also presented.

It should be stressed that scalability of DNA-based trawl assessments is only practically achievable in a context of low-tech, rapid, non-sterile sampling operations, as fishers and observers would not have the time to carry out sampling following strict eDNA protocols. For

instance, the presence of “carry-over” DNA traces from a previous haul may still be present on the deck and the fishing gear and potentially cause noise through false positive detections in the subsequent sample site. Nevertheless, our results demonstrate that this source of bias

is likely negligible: after filtering for basic contamination controls, we find that metabarcoding data mirror visual identifications, and the overall pattern of β -diversity reliably discriminates between hauls and depth strata (see also Appendix S1: Fig. S1 for further robustness testing).

The collection of slush samples is easy and quick (Fig. 1A), potentially providing a huge amount of data that could be collected by commercial fishing vessels operating across seas. Fishers are often the first to notice changes in marine assemblages (Bradley et al. 2019), so fishing vessels, combined with cutting-edge technology (including satellite-based tracking and electronic monitoring) are increasingly touted as potential scientific platforms not only for collecting necessary data for stock assessment but also for biodiversity data recording. The present study suggests that slush collection and storage would be a valuable low-effort task that can be carried out by most trawlers, across vast marine areas and in different seasons. This approach could be used to investigate species distribution across the oceans and, consequently, to assess species richness patterns, the spread of invasive species, and the loss of threatened and endangered species due to environmental change, ultimately providing a new tool to detect shifts in community composition (Jerde and Mahon 2015). If judiciously coordinated, fishing vessels could form an unparalleled fleet of sentinels for monitoring marine life and their changes in response to local and global perturbations, with the added bonus of potentially engendering a greater sense of marine stewardship among the fishers.

ACKNOWLEDGMENTS

T. Russo, S. Mariani, and S. Cataudella conceived and designed the study; F. Di Maio, F. Fiorentino, G. Garofalo, and D. Scannella contributed to the general idea, drafted the design of the sampling, and did fieldwork; F. Di Maio rinsed the samples; C. Baillie and G. Colosimo performed laboratory experiments; T. Russo, L. Talarico, G. Maiello, L. D'Andrea, and S. Franceschini analyzed data; T. Russo, S. Mariani, L. Talarico, and G. Maiello wrote the paper; all authors contributed to revising the manuscript.

LITERATURE CITED

- Bertrand, J. A., L. G. De Sola, C. Papaconstantinou, G. Relini, and A. Souplet. 2002. The general specifications of the MEDITS surveys. *Scientia Marina* 66:9–17.
- Boyer, F., C. Mercier, A. Bonin, Y. Le Bras, P. Taberlet, and E. Coissac. 2016. obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources* 16:176–182.
- Bradley, D., M. Merrifield, K. M. Miller, S. Lomonico, J. R. Wilson, and M. G. Gleason. 2019. Opportunities to improve fisheries management through innovative technology and advanced data systems. *Fish and Fisheries* 20:564–583.
- Collins, R. A., J. Bakker, O. S. Wangenstein, A. Z. Soto, L. Corrigan, D. W. Sims, M. J. Genner, and S. Mariani. 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution* 10:1985–2001.
- Dennis, D., É. Plagányi, I. Van Putten, T. Hutton, and S. Pascoe. 2015. Cost benefit of fishery-independent surveys: Are they worth the money? *Marine Policy* 58:108–115.
- Djurhuus, A., K. Pitz, N. A. Sawaya, J. Rojas-Márquez, B. Michaud, E. Montes, F. Muller-Karger, and M. Breitbart. 2018. Evaluation of marine zooplankton community structure through environmental DNA metabarcoding. *Limnology and Oceanography: Methods* 16:209–221.
- FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome, Italy. <https://doi.org/10.4060/ca9229en>
- Fonseca V. G., et al. 2010. Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature Communications* 1. <http://dx.doi.org/10.1038/ncomms1095>.
- Jackman, S., A. Tahk, A. Zeileis, C. Maimone, J. Fearon, and Z. Meers. 2020. pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University. Cran. <http://www.pscl.stanford.edu/>
- Jerde, C. L., and A. R. Mahon. 2015. Improving confidence in environmental DNA species detection. *Molecular Ecology Resources* 15:461–463.
- Kraan, M., S. Uhlmann, J. Steenbergen, A. T. M. Van Helmond, and L. Van Hoof. 2013. The optimal process of self-sampling in fisheries: Lessons learned in the Netherlands. *Journal of Fish Biology* 83:963–973.
- Lacoursière-Roussel, A., G. Côté, V. Leclerc, and L. Bernatchez. 2016. Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. *Journal of Applied Ecology* 53:1148–1157.
- Lamb, P. D., E. Hunter, J. K. Pinnegar, S. Creer, R. G. Davies, and M. I. Taylor. 2019. How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology* 28:420–430.
- Livia, L., P. Antonella, L. Hovirag, N. Mauro, and F. Panara. 2006. A nondestructive, rapid, reliable and inexpensive method to sample, store and extract high-quality DNA from fish body mucus and buccal cells. *Molecular Ecology Notes* 6:257–260.
- Martin, T. G., B. A. Wintle, J. R. Rhodes, P. M. Kuhnert, S. A. Field, S. J. Low-Choy, A. J. Tyre, and H. P. Possingham. 2005. Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. *Ecology Letters* 8:1235–1246.
- McElroy, M. E., et al. 2020. Calibrating environmental DNA metabarcoding to conventional surveys for measuring fish species richness. *Frontiers in Ecology and Evolution* 8:276.
- McInnes, J. C., et al. 2017. High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Molecular Ecology* 26:4831–4845.
- McKee, A. M., S. F. Spear, and T. W. Pierson. 2015. The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation* 183:70–76.
- Oksanen, J., et al. 2018. vegan: Community Ecology Package. Ordination methods, diversity analysis and other functions for community and vegetation ecologists. <https://CRAN.R-project.org/package=vegan>
- Pauly, D., and D. Zeller. 2016. Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nature Communications* 7:1–9.
- Plet-Hansen, K. S., H. Bergsson, and C. Ulrich. 2019. More data for the money: Improvements in design and cost efficiency of electronic monitoring in the Danish cod catch quota management trial. *Fisheries Research* 215:114–122.
- Russo, T., et al. 2019. Simulating the effects of alternative management measures of trawl fisheries in the central Mediterranean

- sea: application of a multi-species bio-economic modeling approach. *Frontiers in Marine Science* 6:1–23.
- Sampson, D. B. 2011. The accuracy of self-reported fisheries data: Oregon trawl logbook fishing locations and retained catches. *Fisheries Research* 112:59–76.
- Schnell, I. B., and K. Bohmann. 2015. Tag jumps illuminated—reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular ecology resources* 15:1289–1303.
- Seebens, H., N. Schwartz, P. J. Schupp, and B. Blasius. 2016. Predicting the spread of marine species introduced by global shipping. *Proceedings of the National Academy of Sciences USA* 113:5646–5651.
- Sellers, G. S., C. Di Muri, A. Gómez, and B. Hänfling. 2018. Mu-DNA: a modular universal DNA extraction method adaptable for a wide range of sample types. *Metabarcoding and Metagenomics* 2:e24556.
- Siegenthaler, A., O. S. Wangensteen, C. Benvenuto, J. Campos, and S. Mariani. 2019. DNA metabarcoding unveils multiscale trophic variation in a widespread coastal opportunist. *Molecular Ecology* 28:232–249.
- Spens, J., A. R. Evans, D. Halfmaerten, S. W. Knudsen, M. E. Sengupta, S. S. T. Mak, E. E. Sigsgaard, and M. Hellström. 2017. Comparison of capture and storage methods for aqueous microbial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods in Ecology and Evolution* 8:635–645.
- Stat, M., J. John, J. D. DiBattista, S. J. Newman, M. Bunce, and E. S. Harvey. 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology* 33:196–205.
- Taberlet, P., A. Bonin, E. Coissac, and L. Zinger. 2018. Environmental DNA: For Biodiversity Research and Monitoring. Oxford University Press, New York, New York, USA. <https://doi.org/10.1093/oso/9780198767220.001.0001>
- Talarico, L., et al. 2020. Data from: All is fish that comes to the net: metabarcoding for rapid fisheries catch assessment. Dryad, data set. <https://doi.org/10.5061/dryad.7m0cfxpsh>
- Thomsen, P. F., J. Kielgast, L. L. Iversen, P. R. Møller, M. Rasmussen, and E. Willerslev. 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* 7:e41732.
- Thomsen, P. F., P. R. Møller, E. E. Sigsgaard, S. W. Knudsen, O. A. Jørgensen, and E. Willerslev. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLoS ONE* 11: e0165252.
- Tréguier, A., J. M. Paillisson, T. Dejean, A. Valentini, M. A. Schlaepfer, and J. M. Roussel. 2014. Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. *Journal of Applied Ecology* 51:871–879.
- Vilas, C., L. T. Antelo, F. Martin-Rodriguez, X. Morales, R. I. Perez-Martin, A. A. Alonso, J. Valeiras, E. Abad, M. Quinzan, and M. Barral-Martinez. 2019. Use of computer vision onboard fishing vessels to quantify catches: The iObserver. *Marine Policy* 116:103714.
- Wangensteen, O. S., C. Palacín, M. Guardiola, and X. Turon. 2018. DNA metabarcoding of littoral hardbottom communities: High diversity and database gaps revealed by two molecular markers. *PeerJ* 2018:e4705.
- Worm, B., and T. A. Branch. 2012. The future of fish. *Trends in Ecology and Evolution* 27:594–599.

SUPPORTING INFORMATION

Additional supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/eap.2273/full>

DATA AVAILABILITY

Data are available from the Dryad Digital Repository (Talarico et al. 2020): <https://doi.org/10.5061/dryad.7m0cfxpsh>.