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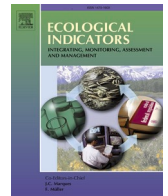
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Original Articles

Environmental DNA as a method to reconstruct food webs and assess ecosystem health

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ABSTRACT

Food web indicators have been developed to describe ecosystem structure and dynamics. Their potential to inform ecosystem management and conservation is undeniable (e.g., Marine Strategy Framework Directive / Descriptor 4). Here, a method was developed to obtain a set of indicators of ecosystem structure and functioning (e.g., mean trophic level, connectivity, redundancy) based on food webs reconstructed using environmental DNA (eDNA). Indeed, eDNA metabarcoding datasets were used to identify the trophic groups and their predator–prey interactions were assessed from the literature. Traditional methods generally require collecting extensive data on biomasses and diets. Such methods are robust but time-consuming, expensive, subject to bias and they require lethal sampling. eDNA-based food web reconstructions are more exhaustive (due to the high detectability of species using eDNA), rapid and non-invasive. In 2023, a study was conducted in Normandy (France) to compare this innovative approach to a more commonly used method (Ecopath with Ecosim), based on the same set of indicators. Correlations were observed, especially on response patterns. With eDNA, ‘complete’ snapshots of food webs can be obtained to monitor the effects of natural and anthropogenic changes (e.g. development projects) as well as the effects of conservation or restoration actions on ecosystem properties.

1. Introduction

To better consider the complexity of natural processes, marine conservation and management have gradually shifted from single-species to ecosystem-based approaches by considering trophic relationships (Jennings, 2004). It is now recognized that food web studies can lead to better-informed ecosystem management (McDonald-Madden et al., 2016). Food webs describe the links between resources and consumers, from primary producers to top predators, and are a common representation of species interactions in an ecosystem. They are considered as a useful approach towards a global understanding of ecosystem structure and dynamics for many decades (Elton, 1927). Researchers have long been studying how food web properties can affect the stability of an ecosystem (Pimm and Lawton, 1977). Food web indicators have been developed to describe different ecological properties that can be interpreted as a response to an environmental or anthropogenic change. In Europe, the Marine Strategy Framework Directive (MSFD) requires that

European Union member states achieve “Good Environmental Status” in respect of 11 descriptors of the marine environment, among which Descriptor 4 addresses marine food webs (D4 Commission Decision 2017/848 of May 2017).

Among the different approaches available to study food webs, Ecological Network Analysis (ENA) is a method that analyzes ecosystem interactions (i.e., all carbon flows and energy transfers among the various components of a food web) to identify global properties (Fath et al., 2007; Niquil et al., 2014). Indeed, species interactions can be interpreted as energy flows (Lindeman, 1942). Applications of ENA have been used to characterize ecosystem functioning (e.g., Ulanowicz & Puccia 1990), to identify key components of the ecosystem (e.g., Borrett 2013), to conduct before–after analyses of sudden events (Le Guen et al., 2019) and to define indicators of ecosystem health status (Fath et al., 2019; Safi et al., 2019). Most ENA indicators (e.g., System Omnivory Index (SOI), Connectance Index, Relative Redundancy, Mean Trophic Level (MTL)) have been proposed as management tools in the OSPAR

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Convention context (Safi et al., 2019). The set of ENA indicators can be obtained with trophic modelling tools that are mainly based on biomasses (for all compartments) and diets (which establish interactions between predators and prey), two essential types of data to build trophic networks. Among the existing modelling tools, Ecopath with Ecosim (EwE) has been intensively used over the last decades and was applied on hundreds of aquatic ecosystems worldwide (Polovina, 1984; Christensen et al., 2005). A diet matrix can be built based on stomach contents or/and stable isotope analyses. To gain a better understanding of the strength of the relationships occurring within a food web, a measure of the amounts of carbon (or energy) being transferred from one compartment (species or group of species) to another is calculated in EwE. ENA indicators can then be calculated in EwE based on these estimated carbon flows.

The approach described above using ENA enables the reconstruction of food webs based on in-situ observational data (for species identification and biomasses) and on stomach contents or isotopic analyses. Although this approach is very powerful to describe an ecosystem, it requires heavy logistic means, time, and expert knowledge. It is also lethal for the sampled individuals and subject to bias (observer and fishing gear during data collection, settings for parameters in EwE). In the present study, we used environmental DNA (eDNA) to get a species inventory, essential to build food webs and calculate ENA indicators. Environmental DNA refers to DNA that organisms leave in their environment via skin cells, hair, scales, eggs, feces, etc. and that can be extracted from environmental samples such as water, air or soil (see Taberlet et al., 2012a; Taberlet et al., 2018). After extracting DNA from samples, the DNA strands can be amplified and sequenced to identify the species who released these DNA fragments. Recent advances in the field of eDNA are having a major influence on biodiversity research as it is a non-invasive, efficient and inexpensive ecosystem surveying method (Bakker et al., 2019; Berry et al., 2019; Seymour et al., 2020; Banerjee et al., 2022; Blackman et al., 2024). For example, eDNA can be used to detect single species (e.g., endangered or rare species), to track the unwanted spread of invasive species or to conduct a full species inventory (see Taberlet et al., 2018). Indeed, eDNA metabarcoding techniques exist to identify the list of species (animals, plants or microorganisms) that are present in a sample (e.g. air or water) (Bourlat et al., 2013), creating a snapshot of biodiversity in the studied area. Such techniques are known to be very powerful compared to traditional techniques for species inventories. For instance, Boussarie et al., 2018 investigated the potential of eDNA metabarcoding by comparing the shark diversity across the New Caledonian archipelago obtained with eDNA, underwater visual census and baited videos. Authors found that despite a lower sampling effort with eDNA (two orders of magnitude), eDNA enabled the detection of 44 % more shark species than each of the two other methods. Indeed, with only 22 eDNA samples, authors were able to prove the presence of 13 shark species (out of 16 all methods combined), compared to 9 with 2758 visual census and 9 with 385 baited videos.

Such high-quality species inventories can be used to build the nodes of a food web. Recently, D'Alessandro & Mariani (2021) showed it is possible to reconstruct and analyze marine trophic networks using eDNA metabarcoding and data on predator-prey interactions from the literature. The metrics they calculated were indicators of the food web structure such as link density (i.e., the number of connections per node), average and maximum chain length. Authors concluded that despite the simplified representation of food webs with eDNA, they are realistic networks which meet our needs (D'Alessandro & Mariani, 2021). They emphasize that food web reconstructions based on eDNA data are affordable, rapid and easy to implement, allowing researchers to replicate this process for an efficient monitoring of the same ecosystem or to apply this concept over larger areas. Later, Blackman et al. (2022) used eDNA to study the food web properties of a freshwater ecosystem (river network of 700 km²). Authors built a metaweb based on co-occurrence of genera (nodes) and they calculated indicators of food web structure as

well as omnivory and functional redundancy (number of taxa within a trophic compartment). Because they studied a large area over several seasons, they identified the main strengths of eDNA to study food webs: the suitability of the method to monitor large areas, its replicability and its non invasivity (Blackman et al., 2022).

Despite the numerous and undeniable advantages that eDNA brings to the analysis of food webs, D'Alessandro & Mariani 2021 mentioned that further research is needed to validate the approach. They recommended comparing eDNA-based food webs with traditional methods and investigating the influence of the use of binary data for diet reconstructions. The present study compares the eDNA-based food web reconstruction and analysis with a more traditional approach: EwE. EwE requires biomasses for the different trophic groups as well as diets to assess ecosystem flows. This is not a kind of information that is easily accessible with eDNA (see Blackman et al., 2024). Therefore, a code was developed in R (hereafter named TROPHII script) to calculate trophic indicators based on a food web reconstructed without biomasses or carbon flows. The same set of indicators (ENA indicators) was calculated for both methods to characterize the same ecosystem. The objective of the present study is to compare different models to assess the influence of nodes and associated biomasses as well as precise diets inferred by stomach contents on ENA indicators. Based on these results, the relevance of eDNA to study food web structure and functioning can be assessed.

2. Materials and methods

2.1. Study site

The Bay of Seine covers an area of approximately 5,000 km² and has a roughly quadrilateral shape, measuring about 50 km latitudinally and 100 km longitudinally. It is predominantly open to the north, leading into the Eastern Basin of the English Channel. Its depth is 30 m on average and does not exceed 50 m. Tidal currents can have speeds of up to 3 knots. These tidal currents, along with the natural water drift, play an important role in the distribution of sediment and benthic communities. This dynamic process creates a gradient within the bay. Offshore areas are mainly made up of pebbles, gravel, and coarse sand, while coastal regions are mainly composed of fine sand and silty/muddy fine sands.

The sampling site was located at the SMILE (System of Measurement Integrated for Littoral and Environment) buoy, with coordinates 49°21'14.00"N and 0°19'41.00"W. It is an instrumented buoy with advanced sensors to analyze seawater for another project (Claquin et al., 2018). The resulting dataset does not feed the present study. The study site is positioned 2 nautical miles north of the town of Luc-sur-mer, within the Central English Channel.

2.2. Data collection and modelling for EwE approach

Fieldwork was conducted on February 25-26th 2022 and March 15th 2023 to acquire data on biomasses for the different trophic compartments (phytoplankton, zooplankton, fish, benthic communities etc.).

2.2.1. General approach

The Ecopath with Ecosim (EwE) software, created by Polovina in 1984 and later improved by Christensen and Walters (2004) and Christensen et al. (2008), was used to model the food web at the sampling site. The approach in EwE is to consider all trophic groups of the system simultaneously with their interactions, enhancing our understanding of the structure and dynamics of ecosystems. Within the EwE software, the Ecopath module provides an algorithm to estimate a snapshot of the food web. Ecopath operates on a mass-balance principle, focusing on the equilibrium of flows without considering year-to-year variations. It is a model with a single solution that calculates fluxes between defined trophic compartments, where each of them represents

either a species or a group of species that share similar diets and metabolic rates.

The Ecopath model is calibrated using dry-weight biomasses (B , gC m^{-2}), production-to-biomass ratios (P/B , $year^{-1}$), consumption-to-biomass ratios (Q/B , $year^{-1}$), and a diet matrix (DC) that details the feeding interactions between all predators and prey. The aim of the parameterization of a model in Ecopath is to comply with two equations. The first equation (Eq. (1)) defines the production of each prey group (i) in the system based on the consumption over biomass ratio (Q/B) of its predators (j), the fishing mortality (Y_i , in grams of carbon per square meter), net migration (E_i ; emigration-immigration, $year^{-1}$), biomass accumulation (BA_i , $year^{-1}$), and natural mortality ($1-EE_i$). In this context, EE represents the Ecotrophic Efficiency, which reflects the part of the biomass that is consumed for each compartment.

$$B\left(\frac{P}{B}\right)_i = \sum_j B_j \left(\frac{Q}{B}\right)_j DC_{ji} + Y_i + E_i + BA_i + B_i \left(\frac{P}{B}\right)_i (1 - EE_i) \quad (1)$$

The second equation (Eq. (2)) describes the energy balance within a trophic group, ensuring that the consumption of the compartment (Q_i) corresponds to the sum of its production (P_i), respiration (R_i), and excretion of unassimilated food (U_i). In the model, all flows are expressed in gC $m^{-2} y^{-1}$.

$$Q_i = P_i + R_i + U_i \quad (2)$$

To realize a mass-balanced model, the input variables (i.e., B , P/B , Q/B , EE , and diets) were manually adjusted to ensure that the EE constraint remained below 1. Additionally, verifications were made for physiological rates to ensure they adhere to predefined limits for each group: (1) P/Q between 0.1 and 0.3 for consumers, and (2) R/B ranging from 1 to 10 for fish groups.

The functional groups were determined based on biological and ecological characteristics of species. 15 groups were selected for the first Ecopath model called "M1 2022" (Fish piscivorous, Fish benthos feeders, Flatfish, Fish planktivorous, Benthic invertebrates (predators, scavengers/omnivorous, selective deposit feeders, non-selective deposit feeders, filter feeders), suprabenthos, meiofauna, zooplankton, bacteria, phytoplankton and detritus). The second Ecopath model called "M1 2023" had 14 groups due to the disappearance of the functional group 'Fish piscivorous'.

2.2.2. Fish compartments

Data on the abundance and biomass of demersal fish were collected using a 3 m beam trawl. Five hauls were performed against the current for a sampling duration of 20 min at a speed of 1.5 knots. Fish were identified, measured, weighed, and then preserved in a 10 % formaldehyde solution. Stomach content analyses were carried out to evaluate the contribution of benthic prey to the diet of demersal fish species. A conversion factor of 0.35 was used to turn wet weights into dry weights and then dry weight into carbon content (Boët et al., 1999). For each group, the average biomass was calculated. Q/B and P/B ratios were obtained from Mackinson and Daskalov (2007). The diet matrix was constructed based on the analyses of stomach contents performed as part of the present study.

2.2.3. Benthic invertebrates

Benthic macrofauna and meiofauna were sampled with five replicates. Benthic invertebrates were collected using a 0.1 m^2 Van Veen grab, and the samples were sieved onboard through a circular 1 mm mesh. The specimens were preserved in 10 % formalin until further analysis in the laboratory, where the organisms were identified to the species level when possible. The biomass of each species was calculated and reported in grams of Ash-Free Dry Weight (AFDW) per m^2 , which represents the weight loss of dry organisms after being heated for 5 h at 500 °C.

For both Ecopath models (2022 and 2023), benthic invertebrates

were categorized into five functional groups (predators, scavengers/omnivores, selective deposit feeders, non-selective deposit feeders and filter feeders). AFDW biomasses were converted to carbon content using a conversion factor of 0.518 (Salonen et al., 1976). P/B , Q/B , and diets were derived from another study conducted in the Bay of Seine (Garcia, 2010).

2.2.4. Suprabenthos

The suprabenthos refers to organisms that live immediately above the seabed. Suprabenthic samples were taken using a suprabenthic sled (500 μm net) to sample the overlying water layer (0.10 - 0.40 m) above the seafloor. Two 10-minute suprabenthic sled tows were conducted against the current at a speed of 1.5 knots. All sampled specimens were cleaned, preserved in a 10 % neutralized formaldehyde solution, and subsequently placed in a 70 % ethanol solution. Individuals were identified and counted at the species level under a dissecting microscope. The biomass of each species was assessed and reported in gAFDW per m^2 . The same conversion factor as for benthic invertebrates was applied.

2.2.5. Zooplankton

Zooplankton was sampled vertically (from surface to bottom) using a WP2 plankton net with an opening of 0.25 m^2 . The net has a mesh size of 200 μm and is equipped with a flow meter to measure the volume of water that is filtered. All collected organisms were rinsed and passed through a 100-mm mesh for further processing. Then, organisms were identified and sorted. Biomasses were calculated in gAFDW per m^2 , a conversion factor of 0.4 was applied to turn them into carbon content (Feller and Warwick, 1988) and the production-to-biomass (P/B) ratio is based on the research conducted by Garcia in 2010.

2.2.6. Primary producers, bacteria, and detritus

The biomass and associated parameters for the three groups (primary producers, bacteria, and detritus) were not assessed in this study. Therefore, they were obtained from Garcia (2010).

2.2.7. Network analysis

Ecological network analysis (ENA) was conducted directly in EwE, using the network analysis plugin. ENA indices computed in the present study include: the Connectance, which measures the level of interactions in the system (Dunne et al., 2002); the Average Path Length (APL, Finn, 1980); the Mean trophic Level (Pauly et al., 1998); the System Omnivory Index (SOI) which informs on the level of trophic specialization of predators (Libralato, 2008); and the redundancy which highlights parallel pathways in the system (Ulanowicz, 1997). In addition, the Keystone Index was calculated for each group to identify which low biomass compartment that has a significant overall influence on others (Libralato et al., 2006).

2.3. Data collection and modelling for the eDNA-based approach

2.3.1. eDNA sampling

Fieldwork was conducted on March 15th, 2023. Seawater samples were collected at two stations around the Smile buoy, at the surface (~2 m deep), in the middle of the water column (~7.5 m deep), and just above the seabed (~15 m deep), with three replicates for each depth. One control sample (blank) was collected at each station using mineral water to account for potential field contamination. Seawater was collected using a 10L ©Niskin bottle and was transferred into a 10L bucket. For each sample, a total of 25L of seawater was filtered using a pump and a ©Waterra capsule containing a filter with a pore diameter of 0.45 μm immersed in the bucket. After filtration, the 20 capsules were emptied and filled again with 50 mL of preservation buffer to avoid DNA degradation. Capsules were agitated using a mechanical shaker directly in the field and sent to the ARGALY lab for analysis. Six marine sediments samples were also collected (three replicates at each station) using a Van Veen grab (0.1 m^2). For each, a subsample of 15 g was put

into a jar with silica gel, to keep it dry until it was sent to the ARGALY lab.

2.3.2. DNA extraction, amplification, sequencing and sequence analysis

DNA extractions from water and sediment samples were performed in two separate laboratories dedicated to these two types of environmental samples. DNA from the 20 seawater filtrations was extracted using the NucleoSpin eDNA Water protocol (Macherey Nagel) with a few modifications. The resulting DNA extracts were eluted in a final volume of 100 μ L. Sediment samples were extracted using the Phosphate buffer method (Taberlet et al., 2012b). The resulting DNA extracts were eluted in a final volume of 100 μ L.

Four different markers, targeting respectively bacterial (Bact01), metazoan (COI), eukaryotic (Euka03) and fish (Fish16S) communities were selected to amplify eDNA (Supplementary Material 1). Each DNA was amplified in eight replicates with each primer pair. Each PCR replicate was uniquely identified by a combination of two eight-base tags on the 5' end of each PCR primer. These tags are used to assign sequences to the corresponding replicate during bioinformatics analysis. After amplification, all samples were combined and purified with the MinElute purification kit (Qiagen GmbH). The success of amplifications and purifications was verified by capillary electrophoresis (E-gel electrophoresis system, ThermoFisher®). The construction of the sequencing libraries (one per marker) and the sequencing itself were performed by FASTERIS (Geneva, Switzerland). The sequencing libraries were prepared according to the Metafast protocol (<https://www.fasteris.com/en-us/NGS/DNA-sequencing/Amplicons-sequencing/Short-Amplicon-sequencing>). The sequencing libraries were then sequenced on Illumina MiSeq runs with paired-end reads of 2×150bp for the Euka03 and Fish16S markers and 2×300bp for the Bact01 and COI markers.

Different controls (extraction, negative PCR, positive PCR and bioinformatics) were performed to detect potential contaminations and to allow a better interpretation of the results.

For all markers, bioinformatic analysis was performed using the OBITools (Boyer et al., 2016), a suite of programs specially dedicated to the analysis of metabarcoding data for assemblage and filtering. The clustering was performed using the sumacust program (Mercier et al., 2013) with a 97 % clustering threshold value, to obtain Molecular Operational Taxonomic Units (MOTUs). The most abundant sequence within a cluster was chosen as representative of the cluster, and only MOTUs observed at least ten times in at least one PCR replicate were retained for the remainder of the analyses.

The taxonomic assignment of the remaining MOTUs was performed using the ecotag program of the OBITools, which compared each MOTU to a reference database of full-length metabarcode sequences constructed from GenBank v249 with the ecoPCR program (Ficetola et al., 2010).

After taxonomic assignment, several data filtering steps were thus conducted in R (R Core Team 2020) with the *metabar* package (Zinger et al., 2021), to remove three types of sequences: (1) probable chimeric MOTUs, i.e. MOTUs with a low sequence homology with the reference database (best identity parameter < 85 % for Bact01 and Euka03, < 75 % for COI and < 95 % for Fish16S); (2) contaminant MOTUs, defined as MOTUs more abundant in negative control replicates than in true PCR replicates, and (3) tag jumps, i.e. MOTUs with a very low abundance within a PCR replicate compared to its abundance overall.

2.3.3. Food web reconstruction using eDNA

Species identified with eDNA metabarcoding analyses were used as the nodes of the food web. Links between these nodes were identified from the literature (DORIS website <https://doris.ffessm.fr/>, Fishbase website <https://www.fishbase.org.au/v4>, species factsheets from Ifremer database <https://peche.ifremer.fr/Fiches-par-espece>, and Morin et al., 2015). These links only represent a presence of a predatory relationship, without any proportion of the prey in the diet of the predator.

A pairwise predator–prey interaction matrix was established for all species considered in the present study (from all methods combined). For each model tested, this binary diet matrix was adjusted. If a node was not present, all associated links were removed from the diet matrix. Data was imported into R and the *cheddar* package (Hudson et al., 2013) was used to visualize food webs and measure their basic structural properties (number of nodes, number of links, number of chains, chain length). All the other indicators were manually developed and calculated in the TROPHII script (Supplementary Material 2). Unlike for EwE, there are no biomasses or carbon flows in TROPHII.

2.4. Food web indicators

The set of indicators chosen to assess ecosystem health in this study is described in Table 1.

2.5. Summary of models and statistical analyses

The different models assessed are listed in Table 2. The model M1 refers to the traditional approach with EwE, in which nodes are identified from fishing surveys after aggregating species into broader compartments (e.g., planktivorous fish or invertebrates filter feeders) and links are obtained from stomach contents. This is the reference model in the present study. The model M2 is based on the same data as M1 for the nodes and the links (without diet proportions) but the indicators are calculated with the TROPHII script rather than EwE. The comparison between M1 and M2 will inform us on the way to calculate indicators rather than on the way to characterize the nodes. The model M3 was run with the TROPHII script but nodes were identified with eDNA and links were obtained from the literature. This is the model of interest to demonstrate the potential of eDNA-based food webs. Two additional models were tested to investigate the optimal way to characterize the nodes of a food web. The model M4 is based on nodes identified from the combination of eDNA and fishing results but species were not grouped. This is the optimal model in the present study but it requires a high sampling effort. Finally, the model M5 is based on nodes from fishing only but species were not grouped either.

Shapiro-Wilk tests were conducted on the different datasets for normality checks. For normally distributed datasets, Student t tests were used to compare the different food web indicators between models when sample variances were equal (F test for equality of variances) and Welch tests were used in case of non-equal variances. When data were not normally distributed, Wilcoxon tests were conducted. Finally, the equality of two proportions obtained from independent samples was tested using the Pearson chi-square test. The alpha level for all significance tests was set at 0.05. Results are generally presented as mean \pm standard errors (SE).

3. Results

3.1. Identification of nodes

In 2022, 15 nodes were identified based on fishing data (Supplementary material 3). In 2023, all species detected were gathered into 84 different nodes based on their feeding ecology (literature-based assessment). Some groups contained only one species. Among these 84 nodes, 46 (55 %) were detected using traditional methods (fishing gears), 61 (73 %) were detected using eDNA, and there were 23 shared between methods. More details are provided in Table 3.

3.2. Temporal changes in food web structure and dynamics using traditional approach

The number of links in the food web slightly decreased between 2022 and 2023 as a direct result of a decrease in the number of nodes, due to the loss of “piscivorous fish”, which was represented by only one species:

Table 1
Definition and ecological interpretation of seven commonly used food web indicators.

ENA indicator	Definition and interpretation
System Omnivory Index (SOI) <i>ENA indicator suggested in Safi et al. (2019)</i>	Omnivory is a feeding strategy which consists in feeding on several trophic levels. The System Omnivory Index (SOI) informs on the average variability (i.e., the variance) of trophic levels of prey for all predators in the food web (Libralato 2008). Low SOI values are observed in food webs with a chain-like structure (i.e., with fewer omnivorous pathways between the different trophic groups) (Dimitrios et al., 2018). On the opposite, high SOI values are associated with networks that are more flexible, resilient and better fitted to face disturbances (Libralato 2008). When omnivorous links are more numerous in a food web, the distance between a compartment and basal nodes is reduced, which in turn reduces the vulnerability of this compartment to potential trophic cascades (Kruess & Tschardtke, 1994). In the TROPHII script, the formula of SOI was adjusted to account for the fact that diet proportions and biomasses are not available with eDNA.
Redundancy (R) <i>ENA indicator suggested in Safi et al. (2019)</i>	The concept of redundancy was introduced by Ulanowicz (1997) to reveal the fact that energy can circulate between trophic groups through multiple parallel trophic itineraries. Alternative pathways enhance ecosystem resilience as any disfunction in a redundant route can be offset by an increase in energy transfer of other routes (Ulanowicz 2018). Another way to describe redundancy, based on links rather than chains, has been suggested by Allesina et al. (2009). Authors oppose redundant links to functional links and explain that not all links contribute to the robustness of the food web. Indeed, if a redundant link is removed from the system, the robustness of the food web is not altered. According to Allesina et al. (2009), “a connection is redundant if it connects a given prey, say x, with a predator that also preys upon species that are direct or indirect prey of x itself”. With both definitions, redundancy enhances the resilience of ecosystems, making more robust to face perturbations like species extinction (Sanders et al., 2018). When several species perform similar functions, if something catastrophic happens to one of them, functions can still be maintained overall. Hereafter, we distinguish the Chain Redundancy (CR) from the Link Redundancy (LR). Connectance is calculated as the number of realized links divided by the total possible number of links in the system and informs on food web complexity. A low CI is related to few pathways between compartments and can occur under stressing conditions such as fishing (Eddy et al., 2017). On the opposite, higher CI values bring stability and resilience to the ecosystem.
Connectance Index (CI) <i>ENA indicator suggested in Safi et al. (2019)</i>	
Neighbour Connectivity (NC)	The Neighbour Connectivity (NC) corresponds to the average number of links (i.e., neighbours) per node and is a measure of the complexity of interactions across a food web (Maslov & Sneppen, 2002; D’Alessandro & Mariani, 2021).
Mean Trophic Level (MTL)	The MTL is widely used in the context of fisheries as it gives information on the structural changes resulting from fishing. The removal of large fish, with high trophic levels, induces a decrease in MTL values (Pauly et al., 1998). In the TROPHII script, this indicator was adjusted to account for the fact that biomasses are not available with eDNA.
Keystone Species (KS)	A keystone species refers to a species whose disappearance precipitates other species’ declines due to cascading effects in the ecosystem (Mills et al., 1993). The definition described in Libralato et al. (2006) is slightly different, with the idea that a keystone species has a significant overall impact on other groups despite having a relatively low biomass. In the TROPHII script, this indicator was calculated based on the definition of Mills et al., 1993 as biomasses are not available with eDNA.

Table 2
Table containing the sources for nodes and links and the tool used to run analyses for different models assessed in 2022 and 2023.

Year	Model	Source for nodes	Source for links	Tool
2022	M1	Fishing (broad functional groups)	Diets with proportions (from stomach contents)	EwE
	M2	Fishing (broad functional groups)	Presence/Absence from M1	TROPHII
2023	M1	Fishing (broad functional groups)	Diets with proportions (from stomach contents)	EwE
	M2	Fishing (broad functional groups)	Presence/Absence from M1	TROPHII
	M3	eDNA only	Presence/Absence from literature	TROPHII
	M4	eDNA + fishing (species not grouped)	Presence/Absence from literature	TROPHII
	M5	Fishing (species not grouped)	Presence/Absence from literature	TROPHII

Table 3
Number of nodes identified by each method in 2023. Functional groups are in rows while models are in columns. Values in parentheses express the percentage of nodes within a group that are detected with the method out of the total number of identified nodes within the taxonomic group all methods combined.

	Number of nodes from fishing data (Model M5)	Number of nodes from eDNA data (from water and sediment samples) (Model M3)	Shared nodes between M5 and M3	Total number of nodes (eDNA and fishing data combined) (Model M4)
Elasmobranch	2 (100 %)	0 (0 %)	0	2
Benthic/ benthopelagic fish	12 (43 %)	25 (89 %)	9	28
Pelagic fish	1 (9 %)	10 (91 %)	0	11
Mollusk bivalve	8 (100 %)	2 (25 %)	2	8
Mollusk gasteropod	3 (60 %)	2 (40 %)	0	5
Mollusk cephalopod	0 (0 %)	0 (0 %)	0	0
Mollusk other	1 (100 %)	0 (0 %)	0	1
Crustacean decapoda	6 (86 %)	2 (29 %)	1	7
Crustacean other	3 (60 %)	3 (60 %)	1	5
Echinoderm	1 (50 %)	2 (100 %)	1	2
Cnidaria	0 (0 %)	2 (100 %)	0	2
Worms	2 (67 %)	3 (100 %)	2	3
Rotifera	0 (0 %)	1 (100 %)	0	1
Other nodes (bacteria, fungi, phytoplankton...)	7	9	7	9
All groups combined	46 (55 %)	61 (73 %)	23	84

Merlangius merlangius (Supplementary materials 3 and 4). In turn, there was a decreasing number of chains, from 548 to 516 (Table 4). This shows the importance of having a good representation of the ecosystem from the beginning, with a relatively exhaustive list of species present.

Using the traditional approach (EwE), there was no significant difference between the two years (models M1 2022 and M1 2023) for the following indicators: Neighbour Connectivity (NC) calculated with import (Student test: $t = -0.021827$, $df = 25.958$, $p\text{-value} = 0.9828$), Mean Trophic Level (MTL) calculated with biomasses (Student test: $t = 0.19747$, $df = 26.997$, $p\text{-value} = 0.8449$), System Omnivory Index (SOI) calculated with biomasses (Student test: $t = -0.30326$, $df = 21.471$, $p\text{-value} = 0.7646$) and Connectance Index (CI) calculated with import: (Khi^2 : $\chi^2 = 0.3037$, $df = 1$, $p\text{-value} = 0.5816$) (Table 4; Fig. 1). However, a significant difference was observed for the Average Chain Length (ACL) (Wilcoxon test: $W = 175275$, $p\text{-value} = 2.531.10^{-12}$). No statistical analysis could be performed for Chain Redundancy (CR) due to the

Table 4

Results from models M1 and M2 for 2022 and 2023 with significance levels (ns = not significant; * = p-value < 0.05; ** = p-value < 0.01 and *** = p-value < 0.001). The significance is between years and not between approaches. Up and down triangles respectively reflect an increase and a decrease in indicators between years.

	M1 (2022) Nodes: Fishing (groups) Links: Gut content Tool: EwE	M1 (2023) Nodes: Fishing (groups) Links: Gut content Tool: EwE	M2 (2022) Nodes: M1 Links: M1 Tool: TROPHII	M2 (2023) Nodes: M1 Links: M1 Tool: TROPHII
Number of nodes (N)	15	14 ▼	15	14 ▼
Number of links (L)	69	65 ▼	69	65 ▼
Number of trophic chains (TC)	548	516 ▼	548	516 ▼
Connectance index (CI)	0.36(with import)	0.39 ▲ ^{ns} (with import)	0.33(without import)	0.36 ▲ ^{ns} (without import)
Neighbour Connectivity (NC)	9.3 ± 0.69(with import)	9.3 ± 0.54 ▲ ^{ns} (with import)	9.2 ± 0.81(without import)	9.3 ± 0.68 ▲ ^{ns} (without import)
Average chain length (ACL)	4.7 ± 0.05	4.2 ± 0.05 ▼*	4.7 ± 0.05	4.2 ± 0.05 ▼*
Redundancy (R)	50.94(chains)	47 ▼(chains)	67 %(links)	66 ▼ ^{ns} (links)
Mean Trophic Level (MTL)	2.60 ± 0.22(with biomass)	2.40 ± 0.21 ▼ ^{ns} (with biomass)	2.81 ± 0.26(without biomass)	2.78 ± 0.26 ▼ ^{ns} (without biomass)
System Omnivory (SOI)	0.14 ± 0.03(with biomass)	0.15 ± 0.02 ▲ ^{ns} (with biomass)	0.31 ± 0.05(without biomass)	0.35 ± 0.05 ▲ ^{ns} (without biomass)
Keystone species	Benthic Invertebrates omnivorous &detritivorous	Fish Benthic feeders	Piscivorous fish	Benthic Invertebrates omnivorous &detritivorous

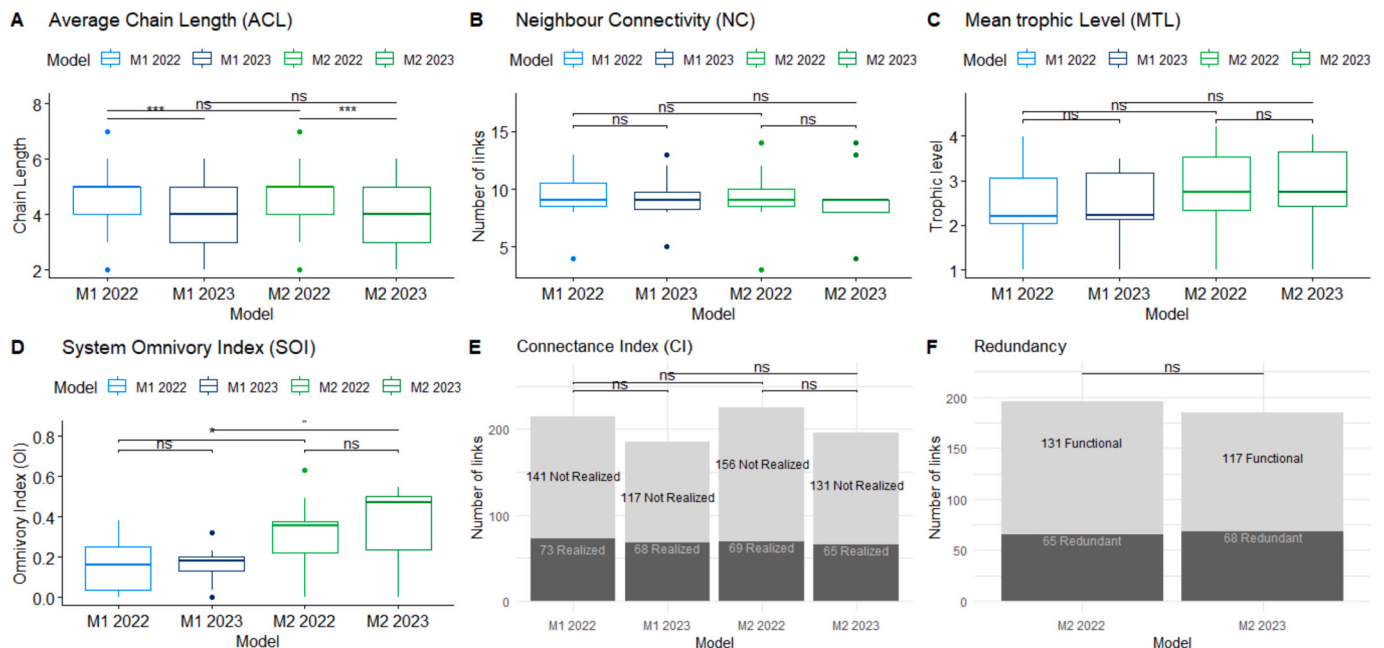


Fig. 1. Results for the models M1 and M2 and the years 2022 and 2023, presented as boxplots (for ACL, NC, MTL and SOI) and stacked histograms (for CI and redundancy). Results are given with significance levels (ns = not significant; * = p-value < 0.05; ** = p-value < 0.01 and *** = p-value < 0.001). For CI and redundancy, the equality of proportions between models was tested using the Pearson chi-square test. For the other indicators, comparisons of means were conducted using Student, Welch or Wilcoxon tests depending on the data distribution (more details can be found in section 2.5).

format of the EwE output of this indicator.

Using the TROPHII script, there was no significant difference between the two years (models M2 2022 and M2 2023) for the following indicators: Neighbour Connectivity (NC) calculated without import (Wilcoxon test: $W = 120$, p-value = 0.5155), Mean Trophic Level (MTL) calculated without biomasses (Student test: $t = 0.068249$, $df = 26.906$, p-value = 0.9461), System Omnivory Index (SOI) calculated without biomasses (Wilcoxon test: $W = 59$, p-value = 0.3127), Connectance index (CI) calculated without import ($\text{Khi}^2: \chi^2 = 0.30091$, $df = 1$, p-value = 0.5833), Link Redundancy (LR) ($\text{Khi}^2: \chi^2 = 0.0039463$, $df = 1$, p-value = 0.9499) (Table 4; Fig. 1). However, a significant difference was observed for the Average Chain Length (ACL) (Wilcoxon test: $W = 175275$, p-value = 2.531.10⁻¹²).

The models M1 2022 and M2 2022 were also compared to analyze the difference between modelling approaches. There was no significant

difference for the following indicators: Average Chain Length (ACL) (Wilcoxon: $W = 150152$, p-value = 1), Neighbour Connectivity (NC) (Wilcoxon: $W = 110.5$, p-value = 0.9495), Mean Trophic Level (MTL) (Student test: $t = 1.0243$, $df = 27.363$, p-value = 0.3147), Connectance Index (CI) ($\text{Khi}^2: \chi^2 = 0.595$, $df = 1$, p-value = 0.4405) (Table 4; Fig. 1). However, there was a significant difference for SOI (Student test: $t = 2.6477$, $df = 21.406$, p-value = 0.01491). No statistical analysis could be performed for Chain Redundancy (CR) due to the format of the EwE output of this indicator.

Similarly, the models M1 2023 and M2 2023 were compared. There was no significant difference for the following indicators: Average Chain Length (ACL) (Wilcoxon: $W = 133128$, p-value = 1), Neighbour Connectivity (NC) (Wilcoxon: $W = 93$, p-value = 0.8279), Mean Trophic Level (MTL) (Student test: $t = 1.1277$, $df = 24.999$, p-value = 0.2702), Connectance Index (CI) ($\text{Khi}^2: \chi^2 = 0.54086$, $df = 1$, p-value = 0.4621)

(Table 4; Fig. 1). However, there was a significant difference for SOI (Wilcoxon $W = 117$, p -value = 0.01005). No statistical analysis could be performed for Chain Redundancy (CR) due to the format of the EwE output of this indicator.

3.3. Comparative assessment: Traditional approach vs eDNA

Results for the different models are shown in Table 5 and Fig. 2. For the ACL, all models were significantly different. For NC, there was no significant difference between M2 and M3 and between M2 and M5. However, there was a significant difference for all other models. For the MTL, there was no significant difference between M2 and M5 and between M3 and M4. However, there was a significant difference for all other models. There was no significant difference for SOI between models (Kruskal-Wallis: $\chi^2 = 1.9509$, $df = 3$, p -value = 0.5827). For the CI, Khi^2 tests showed that all models were significantly different. For redundancy, results were not significant for M2 vs M3 and for M3 vs M4 but they were significant for the other models. More details on the statistical analyses are available in Supplementary Material 5.

4. Discussion

4.1. Differences between 2022 and 2023 to study the relevance of the TROPHII approach

Results for model M1 (run with EwE) show that there were 15 nodes in 2022 and 14 nodes in 2023. This is due to the disappearance of piscivorous fish in the system. It is important to note that this node was only represented by one species: *Merlangius merlangus*. The fact that this species was not encountered in 2023 led to the removal of the “piscivorous fish” node. As a mechanistic consequence of the removal of a top-level node, the Average Chain Length (ACL) was significantly lower in 2023. There was no significant difference for any of the other trophic indicators. To summarize, there was no major structural or functional difference between the two years. The slight variations observed are likely due to interannual variability. The same trends (between years) were observed for M1 and M2 for all food web indicators, which suggests that the TROPHII script seems valid to conduct such analyses. Precaution must be taken for the System Omnivory Index (SOI): values between models widely differ, suggesting that diet proportions are essential for

Table 5

Values of the different food web indicators for models M2, M3, M4 and M5 in 2023. Results are presented as mean \pm standard errors (SE).

	M2 (2023) Nodes: Fishing (gr) Links: Gut content Tool: TROPHII	M3 (2023) Nodes: eDNA Links: Literature Tool: TROPHII	M4 (2023) Nodes: eDNA + fishing (sp) Links: Literature Tool: TROPHII	M5 (2023) Nodes: fishing (sp) Links: Literature Tool: TROPHII
Nb of nodes	14	61	84	46
Nb of links	65	282	635	254
Nb of chains	516	68 168	550 934	5894
Connectance	0.36	0.08	0.09	0.12
Connectivity	9.3 \pm 0.68	9.3 \pm 0.81	15.1 \pm 0.96	10.9 \pm 0.80
Avg chain length	4.2 \pm 0.05	8.0 \pm 0.01	9.5 \pm 0.00	5.4 \pm 0.02
Redundancy	66 % (links)	52.8 % (links)	49.9 % (links)	31.1 % (links)
Keystone species	Benthic invertebrates – omnivorous/detritivorous	Carcinus maneas, brachyura, annelid, echinoderms	Echinoderm, carcinus maneas, brachyura, conger, annelid	Echinoderm, brachyura, maja, gobius, scylorhinus canicula
MTL	2.78 \pm 0.26	3.6 \pm 0.15	3.5 \pm 0.11	2.9 \pm 0.15
SOI	0.35 \pm 0.05	0.36 \pm 0.05	0.34 \pm 0.03	0.32 \pm 0.04

the calculation of this indicator. However, the fact that both SOI calculation methods seem correlated suggests that it is not a major issue.

4.2. The relevant use of eDNA to study food web properties

For 2023, four models ran with the TROPHII script were compared. The model M2 corresponds to the model built with the same nodes as M1 (from fishing data), in which species are aggregated into broad functional groups (14 nodes). The model M3 corresponds to the eDNA-based food web reconstruction (61 nodes). The model M4 corresponds to the model combining eDNA data and detailed fishing data (without aggregation of species). This is the model with the highest number of nodes in this study (84 nodes). Finally, the model M5 is built on detailed fishing data only (without aggregation) (46 nodes). It is interesting to note that there were 23 nodes in common between M3 and M5. The model M4, which can be seen as a combination of M3 and M5, shows that both approaches are somehow complementary. Indeed, eDNA was very powerful to detect benthic and pelagic fish, echinoderms and cnidaria species (Table 3). On the other side, fishing methods used to build M5 (beam trawl and Van Veen grab) were more efficient than eDNA to detect mollusk and crustacean species (Table 3). This is likely due to the selection of primers for eDNA analyses. Dedicated primers could be used to target the DNA of under-represented taxa.

The model M3 based on eDNA only showed the highest value of Mean Trophic Level (MTL = 3.6 \pm 0.15). This is due to the higher representation of fish species (with high trophic levels) in the ecosystem. Results for the Average Chain Length (ACL) also corroborate this effect. Indeed, the highest values of ACL were observed for models M4 and M3 involving eDNA (ACL = 9.5 \pm 0.00 and 8.0 \pm 0.01, respectively). Results for Neighbour Connectivity (NC) and Connectance Index (CI) show highest values for model M4. This is not surprising since M4 is a model with an overall complementarity between fish and invertebrates, allowing more trophic relationships for each node.

There is a significant difference in most indicators (CI, NC, ACL, MTL and Redundancy) between M4 and M5, showing that fishing data only are not sufficient to properly describe the ecosystem. In addition, values of most indicators for M3 (based on eDNA only) are closer to M4 values (based on eDNA and fishing data, i.e., optimal model) than to M2 values (based on aggregated fishing data like in EwE). As such, eDNA appears as a relevant and sufficient tool to build food webs (Fig. 3). In combination with other sampling techniques like fishing, the eDNA-based approach is even more powerful, but eDNA alone allows a rapid and non-invasive food web reconstruction. Because this approach is also cost-effective, it allows replicates of trophic web snapshots for the same ecosystem. Food web analyses based on opportunistic eDNA metabarcoding data could help researchers conduct more frequent or wider monitoring surveys with a limited cost (DiBattista et al., 2020).

4.3. Limits and perspectives

In future studies, it would be interesting to include additional primers to target species that were not optimally detected with eDNA and to make recommendations on the optimal set of primers to be used to build a food web and to calculate trophic indicators. It is possible that some species were not detected due to their intrinsic properties (e.g., crustacean may not release a lot of DNA fragments due to their exoskeleton) but additional markers could help us detect elasmobranch and cephalopods for instance.

The main limitation of the developed methodology is the fact that determining trophic links from literature is time consuming and not always very precise. Information on diet can be at the group level rather than at the species level for instance. Moreover, dietary data can be missing for some parts of the world. Therefore, it is likely that there are false positives and false negatives in the diet matrix. In the objective of comparing models for the same ecosystem, the most important thing is to have the same general diet matrix which can then be adapted to each

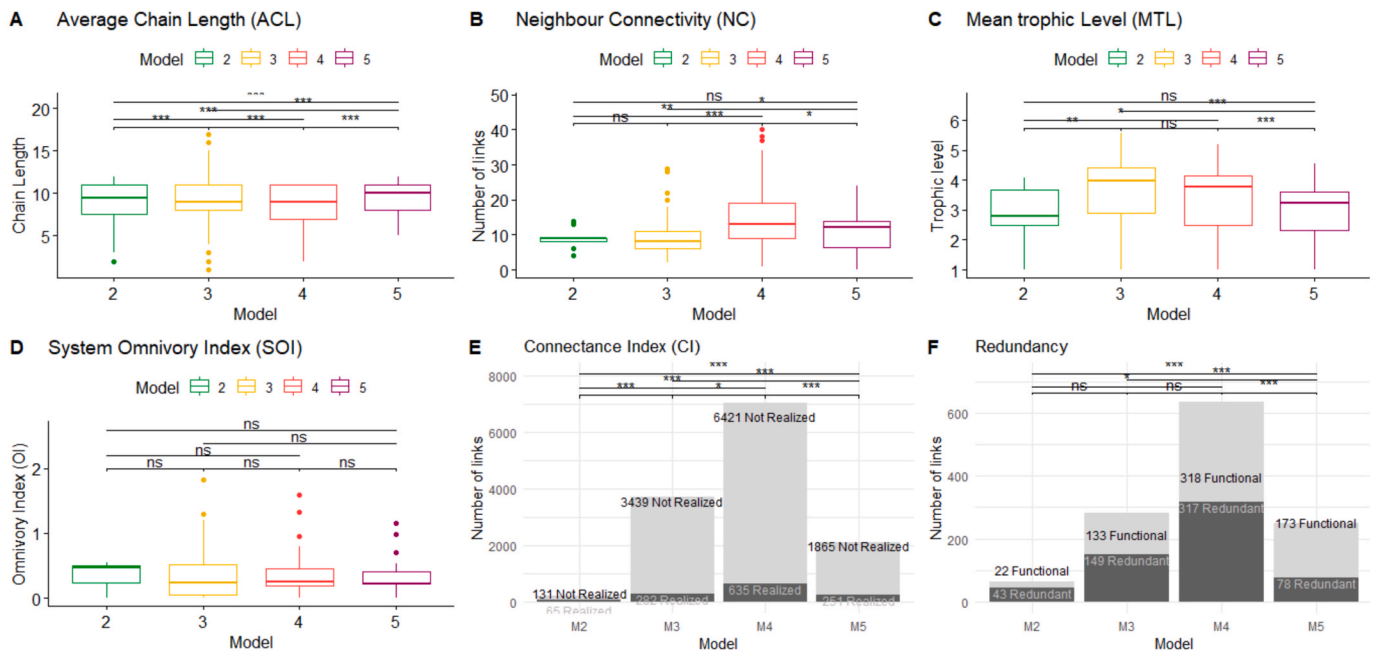


Fig. 2. Results for models M2, M3, M4 and M5 for 2023 presented as boxplots (for ACL, NC, MTL and SOI) and stacked histograms (for CI and redundancy). Plots were obtained using R and results are given with significance levels (ns = not significant; *= p -value < 0.05; **= p -value < 0.01 and ***= p -value < 0.001). For CI and redundancy, the equality of proportions between models was tested in R using the Pearson chi-square test. For the other indicators, comparisons of means were conducted in R using Student, Welch or Wilcoxon tests depending on the data distribution (more details can be found in section 2.5).

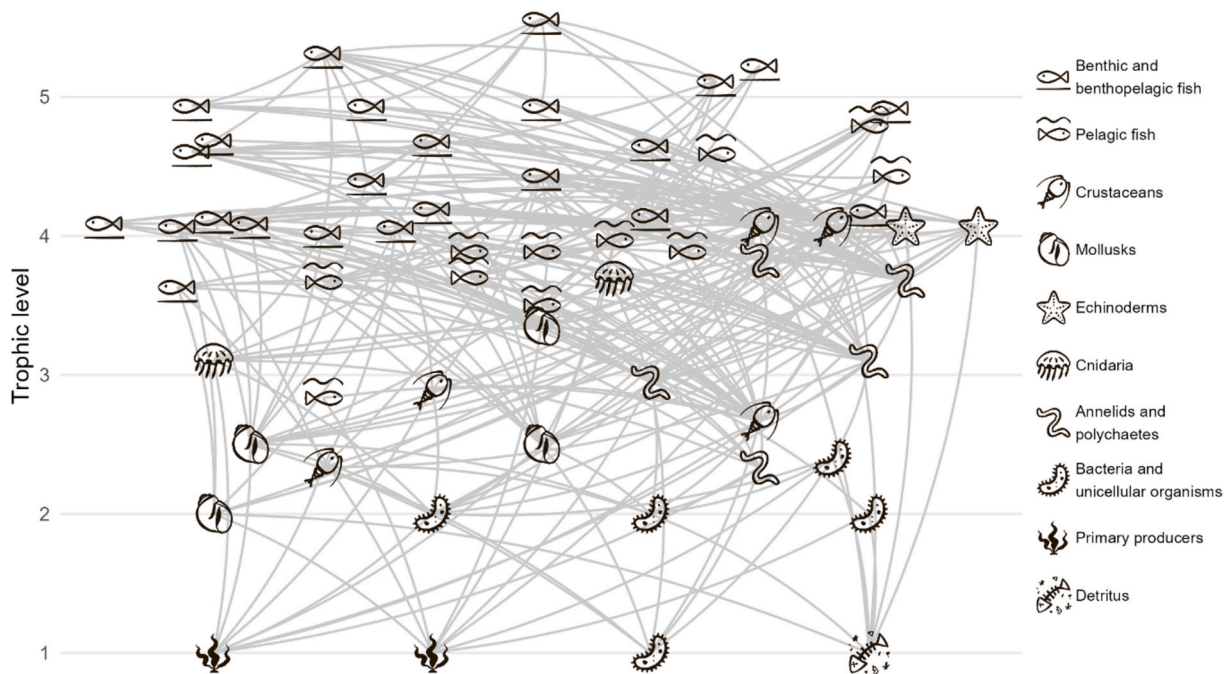


Fig. 3. Schematic representation of model M3 (eDNA-based) from 2023.

model. This limitation also applies to traditional approaches when diet data is not available (e.g. low trophic levels). The same conclusion applies to the fact that biomasses are not available with eDNA, leading to a simplified view of the ecosystem.

As already mentioned in D’Alessandro & Mariani (2021), another perspective would be to work on a standardization of the method regarding the selection of nodes (and the level of aggregation of species) and the identification of the trophic links from the literature. Harmonizing the different studies would lead to more robust comparisons.

Another limitation lies in the application of ENA metrics to the network topology reconstructed using qualitative data from the literature. ENA algorithms are versatile and can be applied to any network topology, including those where flows are not derived from linear inverse models. However, the accuracy and reliability of ENA outputs depend on the quality and resolution of the network topology, particularly the accuracy of trophic links. We recognize the potential limitations of applying ENA formulas to such networks. Future studies could explore integrating semi-quantitative or probabilistic approaches to

enhance the robustness of ENA metrics in cases where exact flow data are unavailable.

Despite these potential limitations, the promising results of the present study suggest that eDNA-based food webs are relevant for ecosystem-based management, especially since it can be used for different types of ecosystems (marine, freshwater, coastal, terrestrial, soil) (Bloor et al., 2021; Calderón-Sanou et al., 2024).

CRedit authorship contribution statement

Camille Mélanie Marie-Anne Le Guen: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aurore Raoux:** Writing – review & editing, Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Samuele Tecchio:** Writing – review & editing, Writing – original draft, Software, Methodology. **Nathan Chauvel:** Writing – review & editing, Formal analysis. **Thomas Merzi:** Writing – review & editing, Validation, Resources. **Eva Bellemain:** Writing – review & editing, Formal analysis. **Aurélié Bonin:** Writing – review & editing, Formal analysis. **Jean-Philippe Pezy:** Writing – review & editing, Formal analysis.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2025.113399>.

Data availability

Data will be made available on request.

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