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ARTICLE

Gut content metabarcoding reveals potential importance of fisheries discards consumption in marine fauna

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Abstract: Fisheries discards have become a source of concern for the perennation of marine resources. To reduce discards, the European Union adopted a Landing Obligation under the reform of its Common Fisheries Policy. However, food web consequences of reducing discards remain uncertain since their degree and pathway of reintegration are understudied. We used multi-marker DNA metabarcoding of gut contents and an ecological network approach to quantify marine fauna reliance on discarded fish and functional importance of discard consumers in coastal fishing grounds. We show that potential discard consumption is widespread across fish and invertebrates, but particularly important for decapods, which were also pinpointed as functionally important. Potential discard consumption may represent up to 66% of all interactions involving fish prey in the reconstructed network. We highlight that discard reliance may be more important than previously assessed in some fishing areas and support functionally important taxa. While reducing discarding remains a conservation priority, it is crucial to understand discards reintegration in marine food webs to anticipate changes in the context of an ecosystem approach to fisheries management.

Résumé : Les rejets de pêche sont devenus une source de préoccupation pour la pérennité des ressources marines. Dans l'optique de réduire les rejets, l'Union européenne a adopté une obligation de débarquement dans le cadre de la réforme de sa politique commune de la pêche. Les conséquences trophiques de la réduction des rejets demeurent toutefois incertaines puisque l'ampleur et les voies de leur réintégration sont sous-étudiées. Nous avons utilisé le métacodage à barres d'ADN de contenus stomacaux et une approche par réseau écologique pour quantifier la dépendance par les animaux marins aux poissons rejetés et l'importance fonctionnelle des consommateurs de rejets dans les zones de pêche côtières. Nous démontrons que la consommation potentielle de rejets est répandue chez les poissons et invertébrés, mais qu'elle est particulièrement importante pour les décapodes, dont l'importance fonctionnelle est également relevée. La consommation potentielle de rejets pourrait représenter jusqu'à 66 % de toutes les interactions impliquant des poissons en tant que proies dans le réseau reconstitué. Nous soulignons le fait que le recours aux rejets pourrait être plus important que ce qui avait été estimé dans certaines zones de pêche et pourrait soutenir des taxons qui jouent un important rôle fonctionnel. Si la réduction des rejets demeure une priorité de conservation, il est crucial de comprendre la réintégration des rejets dans les réseaux trophiques marins afin d'anticiper les changements dans le contexte d'une approche écosystémique de gestion des pêches. [Traduit par la Rédaction]

Introduction

Fishing activity is considered one of the most important threats to marine ecosystems worldwide (Jackson et al. 2001; Guillen et al. 2018). The impact of fishing activity on marine ecosystems has been mainly studied with respect to the removal of species from their environment and its direct and indirect effects, which can have complex cascading effects on food web structure and stability, ultimately affecting ecosystem functioning (Bascompte et al. 2005; Scheffer et al. 2005). Yet, another aspect of fisheries activities that could significantly impact food web structure and ecosystem functioning is fisheries discards (i.e., animals caught, but returned to the sea, dead or alive). Discarding arises from economic or legislative imperatives (low commercial value, over quota restriction, below the minimum allowable size, damaged catches; Kelleher 2005; Zeller et al. 2018). Discards are estimated to represent between 7 and 10 million metric tonnes (t) per year at a global scale, representing almost 10% of global catches (Kelleher 2005; Zeller et al. 2018). In Europe, the Northeast Atlantic ocean has been identified as a "discard hotspot" (Guillen et al. 2018), with fishing activities that can reach up to 50% discard rates, as in the case of *Nephrops* fishery in the Bay of Biscay (Kelleher 2005). Because of the considerable discard rates of some fishing practices, they are now also being recognized as an important factor of biomass dispersion and have become a cause of concern for the perennation of marine resources in many countries (Zeller et al. 2018). The European Union adopted a discard ban in the form of a Landing Obligation (LO) under the reform of its Common Fisheries Policy, with a gradual implementation over the years 2015–2019 (European Union 2013). This reform applies a restriction on the discarding of commercial species under total allowable catches (TAC) and quota, have a minimum landing size, as well as an obligation to return prohibited species to the sea. However, the short- and long-term environmental effects of reducing fishing discards remain uncertain since their degree and pathway of reintegration into the marine food web are understudied,

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albeit being topics of growing interest (Jenkins et al. 2004; Kopp et al. 2016; Sherley et al. 2020). Understanding the importance of fisheries discards reintegration in marine food webs is crucial in the context of an ecosystem approach to fisheries management, both to understand how fishing affects the functioning of marine ecosystems and to predict the impact of a reduction in fisheries discards on marine communities.

Scavenging behaviors and interactions with fisheries have been well documented in seabirds worldwide (Karris et al. 2018; Votier et al. 2013), with evidence that discards may support a large community of scavenger seabirds and be an important determinant of population dynamics (Sherley et al. 2020). However, a substantial part of discards is not directly consumed by seabirds (Garthe et al. 1996) and sinks through the water column, ending up on the seabed (Hill and Wassenberg 2000). Multiple studies have demonstrated that some marine species have developed scavenging feeding strategies targeting dead or damaged fauna originating from fishing activities, including fisheries discards (Groenewold and Fonds 2000; Jenkins et al. 2004; Ramsay et al. 1997). Results from these studies suggest that constant input of organic matter in the form of fisheries discards might play an important role in shaping marine communities and food webs. Such scavenging behaviors could potentially impact marine food webs in diverse ways, by resupplying a part of the biomass extracted by fisheries to the scavenger community and potentially other nontypically scavenger species, and enhancing secondary production (Bluhm and Bechtel 2003). It could also disproportionately affect some species compared to others depending on their level of specialization on fisheries discards, as is suspected in seabirds (Tyson et al. 2015). Yet, to what extent such scavenging behaviors may be common and determine the diet of marine fauna, including that of nontypical scavengers (Olaso et al. 2002), remains largely unknown. These questions are timely since depending on the existence and strength of trophic links with fisheries discards, current efforts to limit discarding practices, including the LO, may directly affect species feeding in fishing grounds, community stability and potentially propagate changes through the marine food web (Kopp et al. 2016).

Estimation of discard consumption by marine fauna is hindered by inherent limitations of traditionally used diet assessment techniques. On the one hand, studies on discard consumption have relied mainly on baited remote underwater video systems (e.g., Bluhm and Bechtel 2003; Hill and Wassenberg 2000; Ramsay et al. 1997), yielding results that do not allow one to ponder the importance of fisheries discards consumption relative to other items in the diet at a population level. On the other hand, traditional gut content and stable isotope analyses that constitute the main techniques used to assess discard contribution to the diet of marine fauna often only allow broad trophic assignments of consumers. DNA metabarcoding of gut contents, a molecular approach to diet assessment that combines next generation sequencing (NGS) with DNA barcoding to identify food items (Nielsen et al. 2018), may be useful to address some of the shortfalls of these techniques to study the fate of fisheries discards (McInnes et al. 2017). It is a well-established technique that can yield unparalleled resolution in taxonomic identification and is being increasingly used to document trophic interactions in marine ecosystems (Casey et al. 2019; Takahashi et al. 2020). Finally, discards identity and rates may vary considerably across time and geographic location, making it impossible to generally classify some prey as being always ingested as discards and others as originating from "natural" predation (Zeller et al. 2018). Coupling knowledge of local discard identity and rates with prior knowledge of predator–prey interaction of the studied consumers may allow to classify ingested prey according to their probability of originating from fisheries discards, providing that the time frame of discard frequencies and the trophic tracer are similar.

In this study, we first aim to quantify the potential consumption of fish originating from fisheries discards in marine fauna feeding in coastal fishing grounds, by using multi-marker DNA metabarcoding of gut contents. We developed an original approach to evaluate the probability of prey items as originating from fisheries discards based on prior knowledge of predator–prey interactions, consumers trophic guilds and a record of local discarding activity based on data collected during the study period, the whole year and the year before. Second, we aim to identify whether discarded taxa or taxa relying on fisheries discards may constitute functionally important nodes within the local network of trophic interactions by using a molecular ecological network approach and therefore identify the potential pathways through which the LO may propagate changes through the food web. We hypothesized that (1) all sampled consumers within the fishing ground community would have ingested fish likely originating from discarding practices (i.e., even nontypically scavenging, nonpiscivorous and low trophic level species), but that this resource would be more important for some species than others and particularly for benthic invertebrate macrofauna; and (2) potential discard consumption would represent a significant portion of all trophic interactions within the reconstructed network and support functionally important taxa such as decapods, which may therefore trigger cascading effects potentially affecting food web and community structure following discards reduction.

Materials and methods

Gut content and local discarding data collection

Data were collected in April 2019 in the Bay of Bourgneuf (Bay of Biscay, France, Northeast Atlantic). Despite being shallow (from 0 to 34 m depth) and covering a relatively small area (320 km^2), this bay is diverse, both in terms of substrate type (i.e., composed of a variety of patchy rocky, sandy and muddy bottoms) and species occurrence (Gauduchon et al. 2020). Both sampling for gut content analysis and recording of local discarding activity at the time of sampling (identity and importance of discards) were carried out onboard of the same fishing boat, a 10.95 m long commercial trawler rigged with a single bottom trawl used to target multispecies fish assemblages (20 m headline and 70 mm diamond mesh codend) and are based on 12 trawl hauls over a 3-day period during normal operation of this boat. This trawler is representative of the commercial trawling fleet of the Bay of Bourgneuf, which are <12 m long (Ifremer. Système d'Informations Halieutiques 2020). Among fishing activities in the ICES 8a and 8b divisions where our sampling took place, bottom trawling is the main source of discards (i.e., 5000 $t \cdot year^{-1}$; Kopp et al. 2016). Coupling sampling and discarding observation was done to obtain information on the identity and importance of discarded species at a timescale as close as possible to that of diet information provided by gut content metabarcoding and at the exact same location. Our own in situ observations of discarding at the time of sampling were further compared and complemented using data from the OBSMER database (Cornou et al. 2021). OBSMER is a sampling program that separately collects catch and discard data (species identity, tonnage and sizes) of commercial fisheries on all French maritime facades. Data are collected by at-sea observers on board of professional fishing vessels. They monitor a complete fishing trip and randomly sample one-third to half of fishing operations of the vessel during each trip. Data request from OBSMER concerned years 2018 and 2019, métier OTB_DEF and OTB_CEP, and ICES subdivision 23E7BB. These data were used to calculate a "discard importance score" for each prey item as follows: 0 = no discarding observed in any dataset, 1 = low discard importance (i.e., species was discarded and met the following criteria: (*a*) discarded in less than 25% of the hauls and with less than 10 individuals per haul according to our own observational data OR (*b*) discarded in mean quantities

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(weight) per haul lower than the mean of all discarded fish per haul according to OBSMER data) and 2 = high discard frequency (all discarded species above the aforementioned thresholds in at least one dataset; see online Supplementary Table S1[1] for details).

Individuals captured for gut content analysis were directly frozen to be further dissected in the lab, except for *Conger conger*, *Raja undulata* and *Scyliorhinus canicula*, for which only the digestive tract was frozen due to their large body size (dissected on board). In total, metabarcoding was performed on 369 individuals belonging to 22 consumer taxa spanning 7 different Classes, which are representative of marine fauna and size classes captured by commercial trawling in the bay. All data were collected and analysed in accordance with the authorizations and ethics approval delivered by the Ministère de la transition écologique et solidaire (NOR: TREL1902817S/168).

Gut content acquisition

Dissections were conducted in sterile conditions (i.e., equipment and surfaces were disinfected and sterilized between dissections by bleaching and flame sterilization). Frozen individuals were thawed at ambient temperature and subsequently dissected to extract the digestive tract. Digestive content was scraped along the entire length of the digestive tract, homogenized and finally subsampled to a maximum of 15 g that was preserved dried in silica gel.

Production of metabarcoding data

Samples preserved in silica gel were sent to Argaly SA (Sainte-Hélène-du-Lac, France; https://www.argaly.com) for metabarcoding. Each sample was ground and homogenized using a mortar. DNA extractions were carried out using the NucleoSpin Soil kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's instructions. The final elution was performed with 100 μL of SE buffer and diluted 5 times prior to PCR (final volume = 0.5 mL). A combination of two complementary primer pairs was used for diet assessment: "Euka02" (TTTGTCTGSTTAATTSCG and CACAGACCTGTTATTGC; Guardiola et al. 2015), targeting the 18S rDNA region, was used as a generalist marker to obtain a full picture of the animal fraction of the diet, and "Tele02" (AAACTCGTGCCAGCCACC and GGGTATCTA ATCCCAGTTTG; Taberlet et al. 2018), targeting the 12S mitochondrial rDNA, was used to characterize more precisely the ingested fish (Actinopterygii and Chondrichthyes). Together, they allow to globally estimate the fraction of the ingested fish within the animal fraction of the diet of each consumer. Amplification of each DNA extract was carried out in four independent PCR replicates, for each marker. Bioinformatic discrimination of PCR replicates after sequencing was allowed by adding tags to the 5′ end of both the forward and reverse primers so that each PCR replicate was represented by a unique combination of forward and reverse tags. Tags were constituted of an eight-nucleotide sequence, with at least five nucleotide differences among them (Coissac 2012) to which two to four random nucleotides were added (on the 5′ end), to ensure sufficient sequence diversity and efficient detection of clusters during sequencing. PCR amplifications were performed in a 20-μL volume containing 2 μL of DNA extract, 0.4 μmol·L^{-1} of each primer, 10 μL of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, California, USA) and 0.16 μL of 20 mg·mL^{-1} bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland). Thermocycling conditions included an initial DNA polymerase activation step of 10 min at 95 °C, 45 cycles with a 30 s denaturation at 95 °C, a 30 s annealing at 45 °C for Euka02 and 54 °C for Tele02, and a 60 s elongation at 72 °C, followed by a final extension step of 7 min at 72 °C. Purification of PCR products (combined in equal volumes), were conducted using the MinElute PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Gel electrophoresis (E-Gel Power Snap; Invitrogen, Carlsbad, California, USA) was used to check for amplification and purification success. Library preparation and sequencing were outsourced to Fasteris SA (Geneva, Switzerland; https://www.fasteris.com/dna/). Two sequencing libraries (one per marker) were prepared following the PCR-free MetaFast protocol (Taberlet et al. 2018), which limits the formation of chimeras. All amplicons were sequenced on a NextSeq 500 sequencing platform (Illumina, San Diego, California, USA), using the rapid run mode generating 250 bp paired-end sequences. Extraction and PCR negatives (20 at each step; i.e., one by extraction batch and PCR plate), as well as unused tag combinations (240 in total; i.e., 12 by PCR plate) were included to control for potential contaminations and false positives caused by tag jumps (Schnell et al. 2015).

Sequences analyses, curation and conversion to occurrence data

The OBITools package (Boyer et al. 2016) was used to analyse raw reads. First, paired-end reads were assembled with illuminapairedend function, only retaining sequences with an alignment score higher than 40. Second, ngsfilter function was used to assign aligned sequences to the corresponding sample by allowing two and zero mismatches on primers and tags, respectively. Third, obiuniq function was used for sequence dereplication. Sequences whose length fell outside the expected size interval (shorter than 36 bp for Euka02, or 129 bp for Tele02), bad-quality sequences (i.e., containing "N"), and those never observed at least ten times in at least one PCR replicate were filtered out. Finally, ecotag function was used to perform taxonomic assignment of each molecular operational taxonomic unit (mOTU), for each marker, by comparing the mOTU sequence to a database of full-length reference metabarcodes. This reference database was constructed combining local reference sequences (Supplementary Table S2[1]), and sequences obtained from the GenBank public database (release 135) using ecoPCR (Ficetola et al. 2010). More specifically, ecoPCR carried out an in silico PCR on GenBank with the primer pair used for the experiment and allowing three mismatches per primer. The obtained reference sequences were further curated by keeping only sequences assigned at least at the family level. For Euka02, when a mOTU was 100% identical with a sequence from the local reference database, this taxonomic assignment was favored to avoid any potential dilution of the local taxonomic information with that, probably less relevant, available in GenBank.

ROBITools package (https://git.metabarcoding.org/obitools/ROBITools) in R was used for further data filtering, removing spurious or contaminant sequences that can bias ecological conclusions drawn from DNA metabarcoding data (Calderón-Sanou et al. 2020). We discarded from our dataset (1) potential chimeras (i.e., mOTUs with a best identity <95% with the local reference database or reference sequences from GenBank release 135); (2) contaminants (i.e., mOTUs that were more numerous in the negative control replicates than in true PCR replicates); (3) mOTUs assigned to taxa other than marine organisms for Euka02 and mOTUs assigned to taxa other than marine fish for Tele02, because Tele02 can also amplify taxa belonging to other classes (mammals, birds); and (4) PCR replicates with a low sequencing coverage. In total, 40 211 815 clusters were sequenced for Tele02 and 58 843 304 for Euka02. Total numbers of sequences after data filtering were 15 874 979 and 25 948 409 for Tele02 and Euka02 datasets, respectively.

After this, we removed "self-hits" (sequences identified as the studied species within its own gut samples) from both datasets, as these sequences can come from naturally degrading cells from the guts of the studied species and cannot be distinguished from potential consumption of conspecifics, which is considered as an

[1]Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2021-0267.

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intrinsic limitation of gut content metabarcoding (Taberlet et al. 2018; Casey et al. 2019). Only three species depicted high rates of self-hits in the Euka02 dataset (*Aphrodita aculeata*, *Buccinum undatum* and *Echinus esculentus*), which were the taxa with the lowest sample sizes. High rates of self-hits for a given species were defined as when self-hits were the highest taxonomic assignment across the majority of individuals of that consumer species (Casey et al. 2019), often resulting in self-hit counts being on average higher than total read counts (Supplementary Table S3[1]). High rates of self-hits were expected in fish species with Tele02 dataset because many individuals that did not consume other fish (e.g., from species not belonging to the piscivore trophic guild; Thompson et al. 2020) would likely only amplify DNA from their own degrading cells with this marker. This was the case for *Callionymus lyra*, *Chelidonichtys lucerna*, *Conger conger*, *Pleuronectes platessa* and *Trisopterus luscus*. Total number of sequences after self-hits removal were 9 065 600 and 15 191 780 for Tele02 and Euka02, respectively. Count data (number of sequences) were transformed to occurrence data (presence–absence). To avoid rare-item inflation that can bias diet estimation, normalize detection across samples and further limit the risk of potential contaminations to remain in the dataset after data filtering steps, we set a threshold of minimum sequence abundance to determine if a mOTU should be considered as an occurrence. We used a threshold of ≥1% of the total number of sequences amplified in the stomach content to define an occurrence (i.e., MOTUs representing <1% of the total number of sequences amplified in the stomach of each consumer were removed; Deagle et al. 2019). Finally, we built a consensus diet incorporating both molecular markers by merging the two datasets (Euka02 and Tele02) into a single one following the multi-marker merging procedure detailed in da Silva et al. (2019). Essentially, this merging procedure assumes that within the same gut content, a given food item recovered at higher taxonomic ranking (e.g., order or family) by the most generalist marker (Euka02) is the same as items of the same taxonomic group recovered at lower taxonomic ranking (e.g., genus or species) by the specialist marker (Tele02). Merged dataset contained a total of 154 identified mOTUs across all samples, with 40 belonging to Actinopterygii and Chondrichthyes.

Calculation of a score of "probability of consumption as fisheries discard"

All fish prey were classified according to their probability of being ingested as fisheries discard by calculating a discard probability score (DPS), ranging from 0 to 3, as follows: 0 = very low, 1 = low, 2 = medium, 3 = high probability of being ingested as fisheries discard (details on DPS calculation for each predator–prey interaction are provided in Supplementary Table S4[1]). The score was calculated by compiling information on the discarding importance of the prey (i.e., "discard importance score" detailed above) and known diet and trophic guild affiliation of the consumer (i.e., "trophic link score"). The "trophic link score", set either as 0 or 1 was assigned to each consumer–prey combination appearing in the metabarcoding results based on a literature review as follows: 0 = natural predation is documented in the specific literature of the consumer or possible based on trophic guild affiliation of the consumer (i.e., predation on the family of the prey was observed in other species belonging to the consumer's trophic guild), 1 = natural predation is not documented either in the specific literature of the consumer or in other species belonging to the consumer's trophic guild. Trophic guild affiliations of fish were derived from Thompson et al. (2020), which source stomach content records from ICES (1997) and DAPSTOM (Pinnegar 2014) to establish trophic guilds. Specific diet of invertebrate taxa were complemented with the trophic traits "feeding type", "feeding habit" and "trophic level" available from Sealifebase (https://www.sealifebase.ca; Palomares and Pauly 2021) and the Biological Traits Information Catalogue (www.marlin.ac.uk/biotic; MarLIN 2006). The DPS was calculated in an additive way as DPS = "discard importance score" + "trophic link score". Using trophic guilds and traits to determine "the trophic link score" allows to limit biases and overestimating of discard consumption due to the unequal state of knowledge on the specific diet of the different taxa. Additionally, we adopted a conservative approach to avoid overamplifying the DPS by assigning a DPS = 0 to all prey items whose "discard importance score" = 0. This means that even if predation on a given prey is undocumented to our knowledge and according to the larger spectrum of the trophic guild ("trophic link score" = 1), but the prey was not observed being discarded at the time of sampling, the resulting DPS = 0. Following the same conservative approach, whenever a prey mOTU was identified to the genus level or higher, we assigned the lowest "discard importance score" of all species included within its taxonomic group.

Diet analysis

Information on the occurrence of each mOTU in the gut content of each species was summarized using frequency of occurrence (FOO), which is calculated for each consumer species as the number of occurrences of a given mOTU divided by the total number of gut contents samples of that consumer species. The weighted percentage of occurrence (wPOO) was obtained following Deagle et al. (2019); occurrences of food items are weighted (standardized) across the entire diet at the individual level, then these weighted occurrences are summed at the species level and divided by species sample size. Diet richness was assessed at the population and individual levels using raw richness of mOTUs.

Molecular ecological network analysis

We built a network of trophic interactions among consumers sampled in the Bay of Bourgneuf fishing grounds (i.e., species and sizes targeted by commercial trawling) and their prey using gut content metabarcoding data of the 22 consumer species to quantify the importance of trophic links potentially involving discard consumption at the scale of the modelled network and whether discards may be fueling structurally important species (Meyer et al. 2020). The network was directed from consumers to prey and interactions were weighted using the wPOO values. We used Gephi (version 0.9.2; Bastian et al. 2009) to visualize and calculate all network metrics. First, we mapped the network of trophic interactions using Forced Atlas 2 layout, a force-directed algorithm used to depict the modular aspect of network structure (Jacomy et al. 2014). Second, to obtain a view of the importance of trophic links involving discard consumption at the scale of the modeled network, we quantified the proportion of trophic links involving fish prey within the network and among those, the proportion of links likely involving discarded fish (links involving prey with DPS ≥ 2). Third, we measured the relative functional importance of each taxon within the modeled network by calculating (*i*) weighted in-degree centrality (i.e., the number of links directed to the node, weighted according to wPOO values), which indicates the degree to which a taxon is an important food source for other taxa in the network, identifying central taxa whose loss may trigger cascading effects, indirectly impacting the community (Elhesha et al. 2017), and (*ii*) Eigenvector centrality, which measures the node's importance within the network while accounting for the importance of its neighbors, further helping to identify taxa that potentially strongly influence the abundance of others (Allesina and Pascual 2009).

Results

Diet composition across the marine community

We sampled 22 consumers for a total of 369 individuals (Table 1). Individual sample size ranged between 4 for *Aphrodita aculeata* (Polychaeta) and 27 for *Sepia officinalis* (Cephalopoda), except for *Echinus esculentus* (Echinoidea) and *Buccinum undatum* (Gastropoda), which included only one individual. Sample sizes of *A. aculeata*, *E. esculentus* and *B. undatum* were judged too low to allow for any generalization of

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Table 1. Diet richness in molecular operational taxonomic units (mOTUs), expressed for the whole diet and the fish fraction, both at the population (total) and individual level (mean ± SE), and network centrality metrics (weighted in-degree and eigenvector centrality) for each consumer.

			Total richness			Fish richness			Centrality metrics	
Class	Species	*N*	Total	Mean	SE	Total	Mean	SE	Weighted in-degree	Eigenvector
Actinopterygii	*Callionymus lyra*	10	26	5.1	0.7	4	0.9	0.3	31.3	0.49
	Chelidonichthys lucerna	23	24	2.9	0.5	12	1.0	0.3	43.4	0.45
	Conger conger	19	25	4.1	0.3	14	1.6	0.3	29.0	0.63
	Merlangius merlangus	20	23	3.5	0.5	11	2.3	0.3	26.1	0.60
	Merluccius merluccius	19	16	2.5	0.3	9	1.7	0.2	120	0.78
	Pleuronectes platessa	20	24	5.5	0.5	8	1.7	0.3	25.8	0.38
	Pollachius pollachius	17	36	5.4	0.7	13	2.4	0.5	20.6	0.34
	Trachurus trachurus	25	34	7.4	0.5	15	4.6	0.3	93.8	0.71
	Trisopterus luscus	20	33	4.2	0.4	11	1.4	0.2	84.2	0.84
Cephalopoda	*Sepia officinalis*	27	38	4.6	0.4	24	3.3	0.5	1.7	0.10
Chondrichthyes	*Raja undulata*	21	21	4.3	0.4	13	2.2	0.2	19.6	0.56
	Scyliorhinus canicula	19	39	7.0	0.8	16	2.4	0.5	11.6	0.46
Echinoidea	*Echinus esculentus*	1	16	16.0	NA	9	9.0	NA	0 (7.9*)	0 (0.23*)
	Psammechinus miliaris	19	57	6.4	0.9	18	2.3	0.5	0 (7.9*)	0 (0.23*)
Gastropoda	*Buccinum undatum*	1	4	4.0	NA	1	1.0	NA	4.7	0.13
Malacostraca	*Atelecyclus undecimdentatus*	20	26	4.1	0.6	18	2.2	0.6	170.5	0.59
	Cancer pagurus	17	25	3.5	0.5	16	1.9	0.5	0	0
	Crangon sp.	13	25	7.9	1.4	19	6.3	1.3	147.0	0.50
	Maja brachydactyla	23	18	2.1	0.4	8	0.6	0.2	0	0
	Necora puber	16	25	4.2	0.7	16	2.2	0.5	94.6	0.82
	Paguroidea	15	22	3.8	0.5	11	1.1	0.3	8.9	0.27
Polychaeta	*Aphrodita aculeata*	4	6	2.3	0.6	2	1.0	0.4	0	0

*Centrality values of both echinoids are those of the mOTU "Echinoidea", since they could not be differentiated during metabarcoding analysis.

findings, therefore results concerning these three taxa are presented in tables but not extensively discussed. Total prey richness ranged between 4 and 57 mOTUs for *B. undatum* and *Psammechinus miliaris* (Echinoidea), respectively. All 22 consumer taxa ingested fish (i.e., mOTUs identified as Actinopterygii or Chondrichthyes; Fig. 1 and Supplementary Fig. S1[1]). At the population level, diversity of ingested fish mOTUs ranged between 4 and 24 for *C. lyra* and *S. officinalis*, respectively. It was notably high in the gut contents of *P. miliaris* (18 different fish mOTUs; Table 1), in the two Chondrichthyes *Raja undulata* and *Scyliorhinus canicula* (13 and 16 different fish mOTUs, respectively) and in Malacostraca, where it ranged between 8 for the spider crab *Maja brachydactyla* and 19 for the shrimp *Crangon* sp. In Actinopterygii, diversity of fish prey ranged between 4 for *Callionymus lyra* and 15 for *Trachurus trachurus*. At the individual level, richness of prey mOTUs were highest for *Crangon* sp., *S. officinalis* and *T. trachurus* (3.3–6.3 mean ingested fish mOTUs per individual) but generally low for all other taxa (0.9–2.4 mean ingested fish mOTUs per individual).

wPOO of fish mOTUs in the diet of the different consumers varied from a minimum of 15.9% in *M. brachydactyla* to a maximum of 77% in *Merluccius merluccius* (Supplementary Table S5[1]). Fish represented ≥50% of all ingested mOTUs in terms of wPOO in the fishes *Merlangius merlangus*, *M. merluccius*, *T. trachurus* and *R. undulata*, in the cuttlefish *S. officinalis*, but also in the echinoid *E. esculentus*, the shrimp *Crangon* sp. and the polychaete *A. aculeata* (Fig. 1 and Supplementary Fig. S1[1]).

Potential importance of discarded fish ingestion across consumer taxa

Prey with the highest DPS (= 3) were found in the gut contents of all consumers, except the three predatory fishes *M. merlangus*, *M. merluccius* and *T. trachurus*, and the polychaete *A. aculeata*. Fish prey that have a medium to high probability of having been consumed as fisheries discards (i.e., DPS ≥ 2) were found in the gut contents of all 22 consumers. We considered that the fish fraction of the diet of a species was dominated by potential discard ingestions when mOTUs with DPS ≥ 2 represented more than 50% of all ingested fish mOTUs in terms of wPOO. All consumer taxa had a fish fraction of the diet dominated by prey with DPS ≥ 2, except the five predatory fishes *Conger conger*, *M. merlangus*, *M. merluccius*, *T. trachurus* and *S. canicula*. In these five species, fish fraction of the diet was dominated by species belonging to the family Clupeidae, which were being discarded by local fisheries at the time of sampling according to our observations but are also known to be naturally predated upon by those consumers, hence their classification as DPS = 1 (Supplementary Tables S4 and S5[1]). However, in *T. trachurus*, fish prey with DPS = 2 still constituted "typical" prey (i.e., dominant items in terms of frequency of occurrence: FOO = 80%). Among fishes, the species that included the most prey with DPS ≥ 2 relative to other fish prey were *C. lyra*, *Pleuronectes platessa* and *R. undulata* (100%, 97.2% and 74.8% of their fish diet, respectively). All invertebrates excluding *S. officinalis* had a fish fraction of the diet almost entirely composed of prey with DPS ≥ 2 (73.9%–100% of fish mOTUs), which were also generally largely dominant in terms of FOO, indicative of a reliance on fish prey potentially mainly linked to scavenging on discards (Fig. 1; Supplementary Table S5[1]). Prey with DPS ≥ 2 represented 11.8% to 56.3% of the whole diet of these species. Fish fraction of the diet was entirely constituted of prey with DPS ≥ 2 in *E. esculentus*, Paguroidea and *A. aculeata*, constituting 56.3%, 27% and 50% of their whole diet, respectively. *Aphrodita aculeata*, Paguroidae, *M. brachydactyla* and *S. officinalis* had the lowest reliance on prey with DPS = 3 relative to their whole diet (0%–6.6% wPOO). Conversely, echinoids and all other decapods relied importantly on prey with DPS = 3, reaching 15.5% to 34.9% of their whole diet. The gastropod *B. undatum* relied exclusively on fish with DPS = 3, constituting 25% of its whole diet.

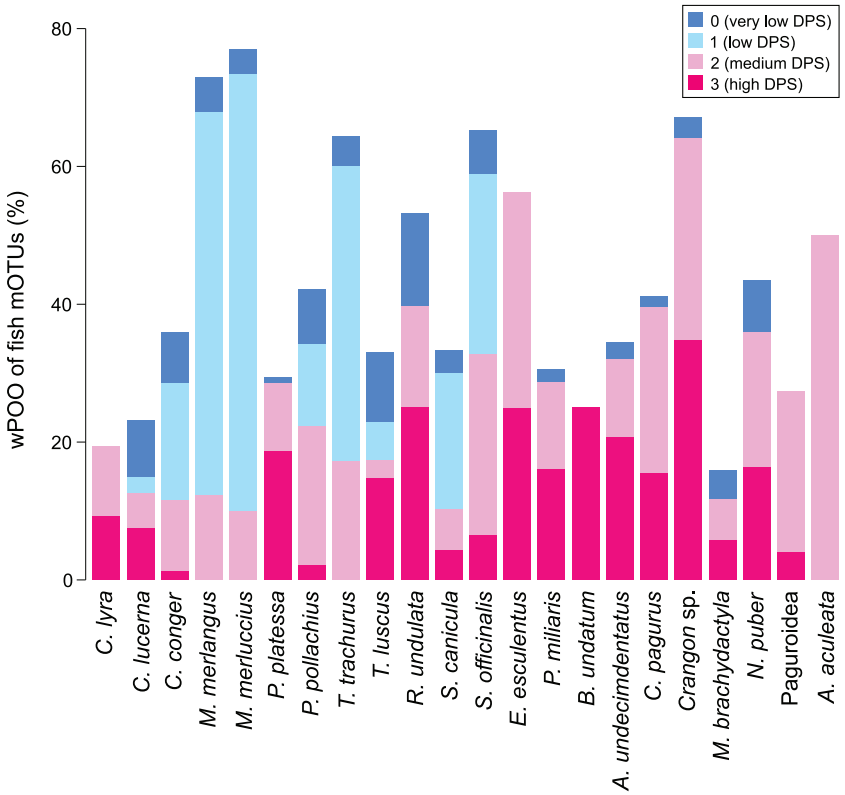
Molecular ecological network analysis

Diet information were used to model a network of trophic interactions among the 22 sampled consumers and their prey,

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Fig. 1. Weighted percentage of occurrence (wPOO, %) of the fish mOTUs (Actinopterygii and Chondrichthyes) identified via gut contents metabarcoding of the 22 consumers. Fish molecular operational taxonomic units (mOTUs) are categorized according to the "discard probability score" (DPS), from 0 (dark blue) = very low probability to 3 (dark pink) = high probability of being ingested as fisheries discard. [Colour online.]



totaling 563 weighted links (trophic interactions) between 159 nodes (sampled consumers and mOTUs; Fig. 2 and Supplementary Fig. S2[1]). Among the 563 trophic links, 48% (268 links) involved fish prey, that is 12% with DPS = 3, 19% with DPS = 2, 10% with DPS = 1 and 7% with DPS = 0. Among these 270 trophic links involving fish prey, 66% concerned fish prey that had a medium to high probability of having been consumed as fisheries discards (DPS $\geq$ 2); 40% with DPS = 2 and 26% with DPS = 3 relative to the consumer. Both centrality metrics provided complementary information identifying species whose fish fraction of the diet could be dominated by discards, or fish that were themselves importantly discarded, as functionally important nodes in the modeled interaction network (Table 1 and Supplementary Table S6[1]). Among taxa with the highest weighted in-degree centrality values, we found species whose fish fraction of the diet could be potentially largely dominated by discards (e.g., the decapods *Atelecyclus undecimdentatus*, *Crangon* sp. and *Necora puber*). The fish with the highest weighted in-degree centrality values were all part of species discarded by the commercial fleet in the area and at the time of sampling: *T. trachurus*, *Trisopterus luscus* and *C. lucerna*, which were observed among the most discarded species, as well as *M. merluccius* and multiple Clupeidae, which were observed to be discarded in lower quantities. A similar pattern was visible in Eigenvector centrality values, but with an increased importance of fish, mainly Clupeidae and *T. luscus*, and a slight decrease of decapods importance in the network (Table 1 and Supplementary Table S6[1]).

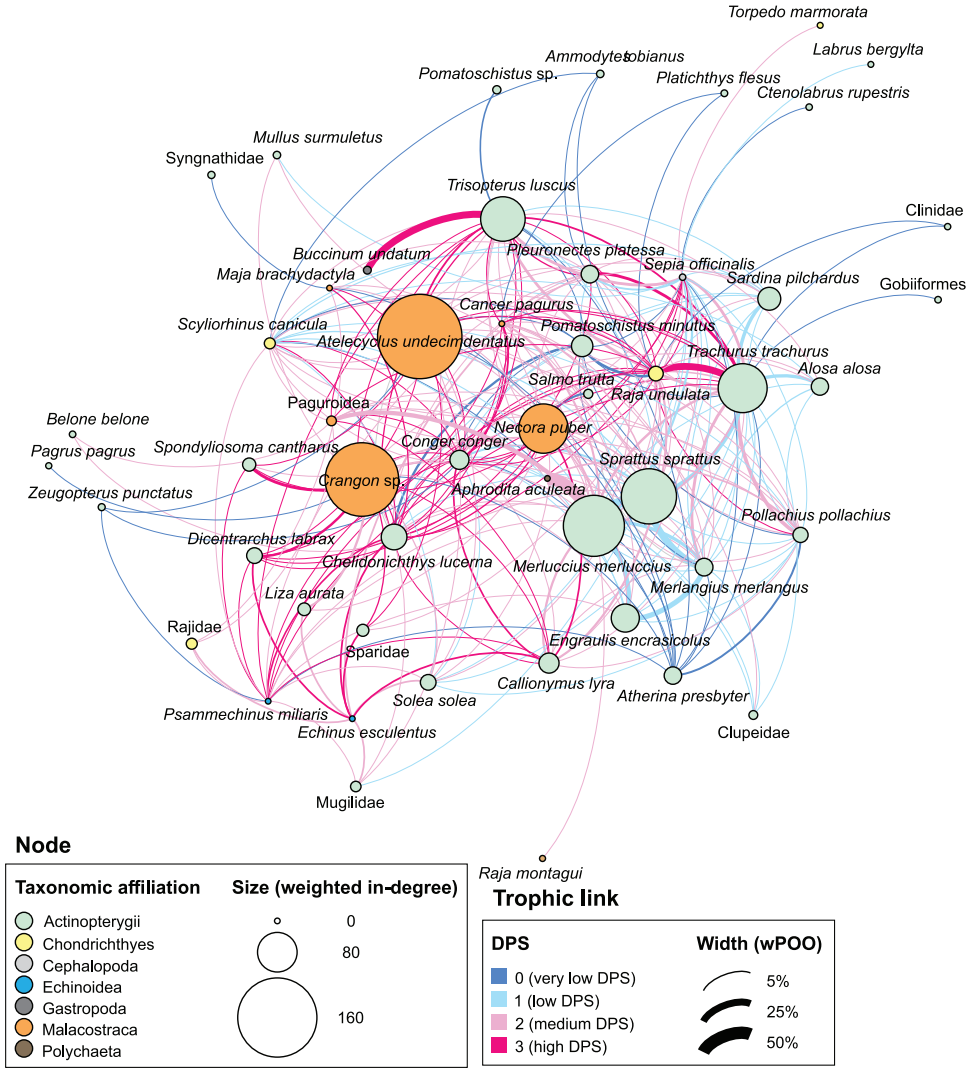
Discussion

Importance of discard consumption across marine fauna

With the introduction of the LO in European waters (European Union 2013), the amount of fisheries discards is expected to decline. However, it is not clear to which extent scavenging behaviors may be common and determine the diet of marine fauna (e.g., Bluhm and Bechtel 2003; Olaso et al. 2002; Ramsay et al. 1997), especially in fishing grounds subject to important discarding activity. Here, we showed that potential ingestion of discarded fish was generalized from low trophic level benthic invertebrates to predatory fishes in shallow coastal fishing grounds of the Bay of Bourgneuf, with a fish fraction of the diet likely dominated by potential discards in 17 of the 22 sampled taxa. Diet involving prey with DPS $\geq$ 2 generally corresponded to prey that may not be naturally predated upon. For example, gut content metabarcoding revealed that 30% of *Callionymus lyra*, a small coastal benthivore fish that feeds mainly on benthic invertebrates (Fricke 1986; Thompson et al. 2020), ingested *Conger conger* and *Merluccius merluccius*, which are larger and higher in the food chain (Arroyo et al. 2019). These ingestions accounted for 100% of the fish diet of this species. As both *C. conger* and *M. merluccius* are eviscerated on board, the ingestion of these two species by *C. lyra* likely corresponds to eating discarded viscera. Note that some fish mOTUs identified in the guts of some species might correspond to predation on eggs or early life stages, which could be underassessed in the diet literature, possibly introducing a bias in the interpretation of high DPS prey consumption. However, the trophic guild approach included in the calculation of the DPS (Thompson et al. 2020) is based on large databases of stomach content records that also report eggs and larvae consumption (i.e., the "Integrated Database and Portal for Fish Stomach Records" (DAPSTOM; Pinnegar 2014) and ICES "Year of the Stomach" database (ICES 1997)), which should limit such bias as much as possible.

Large-scale studies have provided contrasting results regarding the importance of discards for scavengers concluding to either influential (e.g., Catchpole et al. 2006) or limited effects (e.g., Depestele et al. 2019), but calling for more empirical studies in

Fig. 2. Subset of the molecular network constructed from gut content metabarcoding data, depicting trophic interactions among the 22 consumers and their prey; only the trophic links involving fish prey are presented (see Supplementary Fig. S2[1] for the complete network). Nodes are representing the consumers and prey mOTUs. Node colour depicts taxonomic affiliation (by class). Node size is proportional to its importance as a food source for other taxa (weighted in-degree centrality). Link width is proportional to wPOO of prey, and colour varies according to prey DPS. [Colour online.]



diverse environmental settings. In invertebrates, fish fraction of the diet was largely dominated by prey with DPS ≥ 2 (74%–100%), suggesting that all invertebrates sampled may rely mainly on discards concerning fish prey. This is consistent with the fact that most of these species, except *Sepia officinalis*, are unlikely to catch and feed upon live fish. Although some are known necrophageous, the proportion of fish ingestion likely originating from discarding was particularly high in the crabs *Cancer pagurus*, *Necora puber* and *Atelecyclus undecimdentatus*. Previous studies recorded discard consumption by *Buccinum undatum* (Evans et al. 1996), sea urchins (González-Irusta et al. 2014), crabs and spider crabs (Ramsay et al. 1997; Bozzano and Sardà 2002). While we judged that *Aphrodita aculeata*, *Echinus esculentus* and *Buccinum undatum* have too low sample sizes to allow for any generalization of findings, their reporting remain valuable regarding the paucity of information on the diet of these species. Overall, these results suggest that in coastal fishing grounds subject to important discarding activity, the reliance of local invertebrate macrofauna on fish likely ingested as discards may be important and reveal scavenging behaviors that appear to be under documented or whose strengths may be underappreciated.

In fish, potential discarded fish ingestions were more variable across taxa and less important in more predatory fishes compared to others. However, this may also be due to the fact that in predatory fishes for which piscivory is well established in the literature, such as *Conger conger*, *Merlangius merlangus*, *Merluccius merluccius*, *Trachurus trachurus* and *Scyliorhinus canicula* (see Supplementary Table S4[1] for references), most of the ingested fish mOTUs were known to be naturally predated upon, therefore lowering their DPS according to our methodology, even if they were importantly discarded in the area. A previous study by Olaso et al. (2002) acknowledged important consumption of discarded fish such as Clupeidae by *S. canicula*, while another study by Groenewold and Fonds (2000) evidenced that *M. merlangus* were attracted by fish carcasses. Fishes that should occupy lower trophic levels (*Callionymus lyra*, *Chelidonichthys lucerna*, *Pleuronectes platessa*, *Pollachius pollachius*, *Trisopterus luscus* and *Raja undulata*; Arroyo et al. 2019) showed unexpectedly important consumption of fish potentially linked to

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scavenging on discards (DPS ≥ 2). Such scavenging behaviors were already noted for Rajidae (Olaso et al. 2002), as well as for *P. platessa*, which were documented waiting and feeding at the chelae of crabs around discarded fish (Jenkins et al. 2004). *Callionymus lyra*, *C. lucerna*, *P. platessa* and *T. luscus* also showed attraction towards fish carcasses in the study by Groenewold and Fonds (2000). Our study suggests that *R. undulata*, *P. platessa*, *T. luscus* and *C. lyra* may be the main consumers of discards in the fish community.

Potential consequences of the Landing Obligation suggested by network analysis

Studying food web structure helps forecasting the impact of trophic changes on ecosystem functioning (Allesina and Pascual 2009; Meyer et al. 2020). Because of the lack of infrastructure on the ground, some areas benefitted from an extension of the application of the LO to 2020, as is the case of the Bay of Biscay (European Union 2019), explaining that species under TACs and quota were still being discarded at the time of study. This provided the opportunity to picture the structure of a network of trophic interactions among species feeding in coastal fishing grounds subject to important discarding and their prey. Network analysis revealed that in the studied fishing ground, 66% of the trophic links implying fish prey potentially involved discard consumption (DPS ≥ 2), including 26% with the highest DPS (= 3). This provides novel insights suggesting that discarded fish consumption might constitute a non-negligible factor affecting the structure of the local food web and community.

One may argue that discards might enhance secondary production in marine food webs (Bluhm and Bechtel 2003), in which case a reduction of discarding could lead to negative effects on populations most supported by them and to associated indirect effects. It is worth noting that not all discarded species are subject to the LO, implying that not all discarding will end with its full application in the area, but only those species under TAC and quota. Likewise, even if discarding is regulated, consumption of discarded viscera should still be possible since large catches such as *C. conger* or *M. merluccius* would continue to be eviscerated on board. However, species subject to the LO represented an important fraction of ingested fish in the sampled community (e.g., *Trachurus trachurus*, *Engraulis encrasicolus*, *Merluccius merluccius* or the different rays; see all species under TAC and quota among our samples in Supplementary Table S5[1]). Results show that decapods such as *Atelecyclus undecimdentatus*, *Crangon* sp. and *Necora puber*, or the fish *Trisopterus luscus*, which relied potentially importantly on discards, but also *Trachurus trachurus*, which was among the most discarded fish according to both our observations and OBSMER data, constitute some of the most important structuring nodes in the network. This implies that they may potentially strongly affect the local abundance of other taxa via bottom-up effects (Elhesha et al. 2017). This is consistent with studies describing the central role of decapods, including as food sources for diverse taxa (Boudreau and Worm 2012) and the fact that decapods seem to constitute the dominant invertebrate Order attracted by or consuming discards (Depestele et al. 2019). By reducing the amount of food supporting these central species or reducing their discarding, the LO may potentially trigger indirect effects, altering the local network of trophic interactions and community structure. Another consequence that is perhaps more complex to predict is the potential positive effect on fish populations that were previously strongly subject to discarding. On the one hand they could provide more food for species higher in the food chain, following natural predation. On the other hand they could increase predation pressure on species lower in the food chain and modify food web structure via top-down effects. For example, a discard ban on *Trachurus trachurus* may positively affect its population, increasing predation pressure on the variety of Clupeidae constituting its diet. Because Clupeidae are found among the most important structuring taxa, they may then also propagate changes to diverse taxa via bottom-up effects.

The "snapshot" character of trophic information obtained via gut content metabarcoding and the focus on a limited number of species corresponding to those targeted by the local commercial trawling fleet may not allow to fully apprehend the complex consequences of the LO on marine food web structure. This could be improved by expanding this study to a wider area, community and with repeated measures. The method of capture used in this study (bottom trawling) may introduce a bias in the representation of species against pelagic ones while focusing on the benthodemersal assemblage. However, this also reflects the specific environmental context in which the study took place, which is a shallow coastal fishing ground (4–30 m depth) representative of many coastal fisheries in the south of Europe. The presence of *T. trachurus* in our dataset shows that some pelagic species can nonetheless feed in such fishing grounds, underlying the increased benthopelagic coupling in shallow coastal areas (Kopp et al. 2015). More precise inference on the effects of discard reduction on the pelagic compartment of the food web could be drawn from conducting complementary studies using a larger variety of capture methods (e.g., including gill-nets, traps), as well as replicate our study design in deeper and open-water fisheries. Studies conducted in deep-sea offshore fisheries (200–800 m depth) dominated by trawling suggest a similar pattern, whereas important quantities of discards end up on the seabed and be consumed by various benthic fauna, including small crustaceans that are important prey of targeted commercial species (Castro et al. 2005). Offshore open-water fisheries may be dominated by other métiers (e.g., gill-nets, longlines) that often target single species, generate less discards (Cornou et al. 2021) and operate in a spottier fashion than in coastal fisheries that are more geographically comprehensively fished. In these contexts, the possibility of consumption by large pelagic predators before discards reach the bottom might reduce its integration into the benthic food web compared to our study system and encounter probability (i.e., the likelihood to come across fisheries discards) might be reduced as well for both pelagic and benthodemersal species (Depestele et al. 2019), possibly leading to a more diluted impact across the whole food web. Yet, we believe that more empirical studies are needed to clarify and quantify trophic links between marine fauna and discards in these structurally more complex systems, which would require a wider array of capture methods to be deployed to cover both pelagic and benthic species.

DNA metabarcoding to advance research on discards reintegration into marine food webs

Gut content metabarcoding promises to revolutionize food web studies by yielding unparalleled resolution of trophic interactions (Casey et al. 2019; Takahashi et al. 2020), but limitations should also be considered to advance research on discards reintegration into marine food webs. It was successfully used to identify species potentially originating from fisheries discards in the diet of albatrosses (McInnes et al. 2017) and quantify discard consumption in marine fauna in the present study. Here, gut content metabarcoding allowed to identify 154 mOTUs (considering a ≥1% relative read abundance threshold; Deagle et al. 2019), with 40 belonging to Actinopterygii and Chondrichthyes, which represents an unprecedented resolution in a demersal food web. Yet, taxonomic levels were sometimes dropped from species to genus, or further, whenever the similarity between primary and secondary reference sequences was equal or higher to the similarity between the primary reference and the query sequence during taxonomic assignment, resulting in sequence assignment to the most recent common ancestor. Such cases can occur because of incomplete reference databases. Improving the local reference database is therefore crucial to maximize the resolution of taxonomic identification (Casey et al. 2019). It is also worth

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noting that while the 18S rDNA region may provide an exceptional coverage across the prey items of interest in this study, it may provide poorly reliable taxonomic resolution. The use of complementary markers can improve taxonomic resolution within specific groups of interest (da Silva et al. 2019). Here, the complementary use of Tele02 (targeting 12S region) helped improve the resolution of fish mOTUs identification compared to Euka02 alone (targeting 18S region): Among the 40 fish mOTUs identified, 32 were assigned to species level, 1 to genus, 6 to family and 1 to order. Since taxonomic richness is dependent upon clustering level, taxonomic richness of the whole diet may be influenced by lower or variable resolution yielded by Euka02 for non-fish groups. Taxonomic richness of the fish fraction of the diet should be much less influenced by such problem. Specific markers for other taxonomic groups should similarly improve identification resolution. Using multiple markers could also help limit the intrinsic biases associated with primer choice and difference in amplification rate among sequences (Deagle et al. 2014).

One intrinsic limitation of applying metabarcoding to study diet is that one cannot decipher between conspecifics consumption and DNA coming from the consumer's own degrading cells, which is the reason for removing "self-hits" from diet data (i.e., DNA of the consumer within its own gut content; Taberlet et al. 2018; Casey et al. 2019). We found no evidence of an important role of cannibalism in the trophic ecology of the studied species based on gut content data from large databases used for DPS calculation. But beyond cannibalism of living conspecifics, one-way self-hits censoring might still impact the estimated diet is by making a part of discard consumption invisible and therefore unaccounted for, namely if a species is consuming discarded conspecifics. This might be more impactful for species that are importantly discarded in the area (e.g., *C. conger*, *T. trachurus*, *T. luscus*), as one would expect the probability of consuming discarded conspecifics to increase with discarding rate of that species. Important amount of consumer's DNA in a sample might impede the ability of PCR to reveal the true diversity of the diet because of a quick saturation of the reaction. But in our dataset self-hit rate was generally low, with only five fish species out of 22 depicting high self-hit rates with Tele02. High read counts of only their own DNA can be explained by the absence of fish consumption, which is confirmed by the absence of (other) fish mOTUs identified with Euka02. In this specific case, self-hits have little impact on diet estimates.

Finally, like traditional morphological identification, gut content metabarcoding only provides dietary information on a short time-scale. However, its sensitivity to identify highly degraded prey from intestinal content allows to access diet information that is often missed in traditional morphological identification (Takahashi et al. 2020). The use of long-term biomarkers such as stable isotopes or fatty acids as complementary tools may allow to decipher between opportunistic scavenging and specialized long-term strategies across the community, and better apprehend the potential consequences of the LO.

Conclusions

Ultimately, reducing bycatch and discarding remain conservation priorities, but it appears also crucial to understand and anticipate the potential consequences of reducing discards for the variety of marine species that may have become quite dependent upon them. Our results reveal that consumption of fish likely ingested as discards was important and generalized across marine fauna feeding in fishing grounds subject to important discarding and identified that discards may support multiple species potentially playing an important structuring role in the local food web, such as decapods. DNA metabarcoding of gut contents, together with reliant methodologies to establish the probability of prey to originate from discarding, offers the possibility to quickly assess dependence of a community on fisheries discards. These results could be further confirmed using stable isotopes or fatty acid tracers and repeated in various environmental settings to be implemented in ecosystem models such as Ecopath with ECOSIM in which trophic links with discards and corresponding energy fluxes may currently be underestimated.

Contributors' statement

DK, MAM and SM designed the study and methodology; DK and SM sampled and preserved gut contents; SM realized the observations on discard frequencies; BL designed the DPS calculation methodology, participated to sequence curation and analysed metabarcoding data; BL led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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