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## 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.

**Keywords :** Diet, Discard ban, DNA metabarcoding, Fishing impact, Landing Obligation, Marine food web, Molecular network analysis, Scavengers, Trophic ecology

34 **Introduction**

35 Fishing activity is considered one of the most important threats to marine ecosystems  
36 worldwide (Jackson *et al.* 2001; Guillen *et al.* 2018). The impact of fishing activity on marine  
37 ecosystems has been mainly studied with respect to the removal of species from their  
38 environment, and its direct and indirect effects, which can have complex cascading effects on  
39 food web structure and stability, ultimately affecting ecosystem functioning (Bascompte *et al.*,

40 2005; Scheffer et al., 2005). Yet, another aspect of fisheries activities that could significantly  
41 impact food web structure and ecosystem functioning is fisheries discards (i.e. animals caught,  
42 but returned to the sea, dead or alive). Discarding arises from economic or legislative  
43 imperatives (low commercial value, over quota restriction, below the minimum allowable size,  
44 damaged catches; Kelleher, 2005; Zeller et al., 2018). Discards are estimated to represent  
45 between 7 and 10 million tons per year at a global scale, representing almost 10% of global  
46 catches (Kelleher 2005; Zeller *et al.* 2018). In Europe, the North-East Atlantic ocean has been  
47 identified as a 'discard hotspot' (Guillen *et al.* 2018), with fishing activities that can reach up  
48 to 50% discard rates, as in the case of *Nephrops* fishery in the Bay of Biscay (Kelleher 2005).  
49 Because of the considerable discard rates of some fishing practices, they are now also being  
50 recognized as an important factor of biomass dispersion and have become a cause of concern  
51 for the perennation of marine resources in many countries (Zeller *et al.* 2018). The European  
52 Union adopted a discard ban in the form of a Landing Obligation (LO) under the reform of its  
53 Common Fisheries Policy, with a gradual implementation over the years 2015-2019 (European  
54 Union 2013). This reform applies a restriction on the discarding of commercial species under  
55 total allowable catches (TAC) and quota, have a minimum landing size, as well as an obligation  
56 to return prohibited species to the sea. However, the short- and long-term environmental effects  
57 of reducing fishing discards remain uncertain since their degree and pathway of reintegration  
58 into the marine food web are understudied, albeit being topics of growing interest (Jenkins et  
59 al., 2004; Kopp et al., 2016; Sherley et al., 2019). Understanding the importance of fisheries  
60 discards reintegration in marine food webs is crucial in the context of an ecosystem approach  
61 to fisheries management, both to understand how fishing affects the functioning of marine  
62 ecosystems and to predict the impact of a reduction in fisheries discards on marine  
63 communities.

64 Scavenging behaviors and interactions with fisheries have been well documented in  
65 seabirds worldwide (Karris et al., 2018; Votier et al., 2013), with evidence that discards may  
66 support a large community of scavenger seabirds and be an important determinant of population  
67 dynamics (Sherley *et al.* 2019). However, a substantial part of discards is not directly consumed  
68 by seabirds (Garthe et al., 1996), and sinks through the water column, ending up on the seabed  
69 (Hill & Wassenberg 2000). Multiple studies have demonstrated that some marine species have  
70 developed scavenging feeding strategies targeting dead or damaged fauna originating from  
71 fishing activities, including fisheries discards (Groenewold & Fonds, 2000; Jenkins et al., 2004;  
72 Ramsay et al., 1997). Results from these studies suggest that constant input of organic matter  
73 in the form of fisheries discards might play an important role in shaping marine communities  
74 and food webs. Such scavenging behaviors could potentially impact marine food webs in  
75 diverse ways, by resupplying a part of the biomass extracted by fisheries to the scavenger  
76 community and potentially other non-typically scavenger species, and enhancing secondary  
77 production (Bluhm & Bechtel 2003). It could also disproportionately affect some species  
78 compared to others depending on their level of specialization on fisheries discards, as is  
79 suspected in seabirds (Tyson et al., 2015). Yet, to what extent such scavenging behaviors may  
80 be common and determine the diet of marine fauna, including that of non-typical scavengers  
81 (Olaso et al., 2002) remains largely unknown. These questions are timely since depending on  
82 the existence and strength of trophic links with fisheries discards, current efforts to limit  
83 discarding practices, including the LO, may directly affect species feeding in fishing grounds,  
84 community stability and potentially propagate changes through the marine food web (Kopp *et*  
85 *al.* 2016).

86 Estimation of discard consumption by marine fauna is hindered by inherent limitations  
87 of traditionally used diet assessment techniques. On the one hand, studies on discard  
88 consumption have relied mainly on baited remote underwater video systems (e.g. Bluhm &

89 Bechtel, 2003; Hill & Wassenberg, 2000; Ramsay et al., 1997), yielding results that do not  
90 allow one to ponder the importance of fisheries discards consumption relative to other items in  
91 the diet at a population level. On the other hand, traditional gut content and stable isotope  
92 analyses which constitute the main techniques used to assess discard contribution to the diet of  
93 marine fauna often only allow broad trophic assignments of consumers. DNA metabarcoding  
94 of gut contents, a molecular approach to diet assessment which combines next generation  
95 sequencing (NGS) with DNA barcoding to identify food items (Nielsen *et al.* 2017), may be  
96 useful to address some of the shortfalls of these techniques to study the fate of fisheries discards  
97 (McInnes *et al.* 2017). It is a well-established technique that can yield unparalleled resolution  
98 in taxonomic identification and is being increasingly used to document trophic interactions in  
99 marine ecosystems (Casey *et al.* 2019; Takahashi *et al.* 2020). Finally, discards identity and  
100 rates may vary considerably across time and geographic location, making it impossible to  
101 generally classify some prey as being always ingested as discards and others as originating from  
102 'natural' predation (Zeller *et al.* 2018). Coupling knowledge of local discard identity and rates  
103 with prior knowledge of predator-prey interaction of the studied consumers may allow to  
104 classify ingested prey according to their probability of originating from fisheries discards,  
105 providing that the time frame of discard frequencies and the trophic tracer are similar.

106 In this study, we first aim to quantify the potential consumption of fish originating from  
107 fisheries discards in marine fauna feeding in coastal fishing grounds, by using multi-marker  
108 DNA metabarcoding of gut contents. We developed an original approach to evaluate the  
109 probability of prey items as originating from fisheries discards based on prior knowledge of  
110 predator-prey interactions, consumers trophic guilds and a record of local discarding activity  
111 based on data collected during the study period, the whole year and the year before. Second,  
112 we aim to identify whether discarded taxa or taxa relying on fisheries discards may constitute  
113 functionally important nodes within the local network of trophic interactions by using a

114 molecular ecological network approach and therefore identify the potential pathways through  
115 which the LO may propagate changes through the food web. We hypothesized that (1) all  
116 sampled consumers within the fishing ground community would have ingested fish likely  
117 originating from discarding practices (i.e. even non-typically scavenging, non-piscivorous and  
118 low trophic level species), but that this resource would be more important for some species than  
119 others and particularly for benthic invertebrate macrofauna; and (2) potential discard  
120 consumption would represent a significant portion of all trophic interactions within the  
121 reconstructed network and support functionally important taxa such as decapods, which may  
122 therefore trigger cascading effects potentially affecting food web and community structure  
123 following discards reduction.

124

## 125 **Material and methods**

### 126 ***Gut content and local discarding data collection***

127 Data were collected in April 2019 in the Bay of Bourgneuf (Bay of Biscay, France, northeast  
128 Atlantic). Despite being shallow (from 0 to 34 m depth) and covering a relatively small area  
129 (320 km<sup>2</sup>), this bay is diverse, both in terms of substrate type (i.e. composed of a variety of  
130 patchy rocky, sandy and muddy bottoms) and species occurrence (Gauduchon *et al.* 2020). Both  
131 sampling for gut content analysis and recording of local discarding activity at the time of  
132 sampling (identity and importance of discards) were carried out onboard of the same fishing  
133 boat, a 10.95 m long commercial trawler rigged with a single bottom trawl used to target  
134 multispecies fish assemblages (20 m headline and 70 mm diamond mesh codend) and are based  
135 on 12 trawl hauls over a 3 days period during normal operation of this boat. This trawler is  
136 representative of the commercial trawling fleet of the Bay of Bourgneuf, which are < 12 m long  
137 (Ifremer. Système d'Informations Halieutiques 2020). Among fishing activities in the ICES 8a  
138 and b divisions where our sampling took place, bottom trawling is the main source of discards

139 (i.e. 5000 t/year; Kopp et al., 2016). Coupling sampling and discarding observation was done  
140 to obtain information on the identity and importance of discarded species at a timescale as close  
141 as possible to that of diet information provided by gut content metabarcoding and at the exact  
142 same location. Our own *in situ* observations of discarding at the time of sampling were further  
143 compared and complemented using data from the OBSMER database (Cornou *et al.* 2021).  
144 OBSMER is a sampling program that separately collects catch and discard data (species  
145 identity, tonnage and sizes) of commercial fisheries on all French maritime facades. Data are  
146 collected by at-sea observers on board of professional fishing vessels. They monitor a complete  
147 fishing trip and randomly sample one-third to half of fishing operations of the vessel during  
148 each trip. Data request from OBSMER concerned years 2018 and 2019, métier OTB\_DEF and  
149 OTB\_CEP, and ICES sub-division 23E7BB. These data were used to calculate a 'discard  
150 importance score' for each prey item as follows: 0 = No discarding observed in any dataset, 1  
151 = low discard importance (i.e. species was discarded and met the following criteria: (a)  
152 discarded in less than 25% of the hauls and with less than 10 individuals per haul according to  
153 our own observational data OR (b) discarded in mean quantities (weight) per haul lower than  
154 the mean of all discarded fish per haul according to OBSMER data) and 2 = high discard  
155 frequency (all discarded species above the aforementioned thresholds in at least one dataset;  
156 see Table S1 for details).

157 Individuals captured for gut content analysis were directly frozen to be further dissected in  
158 the lab, except for *Conger conger*, *Raja undulata* and *Scyliorhinus canicula* for which only the  
159 digestive tract was frozen due to their large body size (dissected on board). In total,  
160 metabarcoding was performed on 369 individuals belonging to 22 consumer taxa spanning 7  
161 different Classes which are representative of marine fauna and size classes captured by  
162 commercial trawling in the bay. All data were collected and analyzed in accordance with the

163 authorizations and ethics approval delivered by the Ministère de la transition écologique et  
164 solidaire (NOR: TREL1902817S / 168).

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### 166 ***Gut content acquisition***

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

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### 173 ***Production of metabarcoding data***

174 Samples preserved in silica gel were sent to Argaly SA (Sainte-Hélène-du-Lac, France;  
175 <https://www.argaly.com>) for metabarcoding. Each sample was ground and homogenized using  
176 a mortar. DNA extractions were carried out using the NucleoSpin Soil kit (Macherey–Nagel,  
177 Düren, Germany) according to the manufacturer’s instructions. The final elution was performed  
178 with 100 µL of SE buffer and diluted 5 times prior to PCR (final volume = 0.5 mL). A  
179 combination of two complementary primer pairs was used for diet assessment: ‘Euka02’  
180 (TTTGTCTGSTTAATTSCG and CACAGACCTGTTATTGC; Guardiola et al., 2015),  
181 targeting the 18S rDNA region was used as a generalist marker to obtain a full picture of the  
182 animal fraction of the diet, and ‘Tele02’ (AAACTCGTGCCAGCCACC and  
183 GGGTATCTAATCCCAGTTTG; Taberlet et al., 2018) targeting the 12S mitochondrial rDNA  
184 was used to characterize more precisely the ingested fish (Actinopterygii and Chondrichthyes).  
185 Together, they allow to globally estimate the fraction of the ingested fish within the animal  
186 fraction of the diet of each consumer. Amplification of each DNA extract was carried out in  
187 four independent PCR replicates, for each marker. Bioinformatic discrimination of PCR



188 replicates after sequencing was allowed by adding tags to the 5' end of both the forward and  
189 reverse primers so that each PCR replicate was represented by a unique combination of forward  
190 and reverse tags. Tags were constituted of an eight-nucleotide sequence, with at least five  
191 nucleotide differences among them (Coissac 2012) to which two to four random nucleotides  
192 were added (on the 5' end), to ensure sufficient sequence diversity and efficient detection of  
193 clusters during sequencing. PCR amplifications were performed in a 20- $\mu$ L volume containing  
194 2  $\mu$ L of DNA extract, 0.4  $\mu$ M of each primer, 10  $\mu$ L of AmpliTaq Gold 360 Master Mix  
195 (Applied Biosystems, Foster City, CA, USA) and 0.16  $\mu$ L of 20 mg.ml<sup>-1</sup> bovine serum albumin  
196 (BSA; Roche Diagnostics, Basel, Switzerland). Thermocycling conditions included an initial  
197 DNA polymerase activation step of 10 min at 95°C, 45 cycles with a 30s denaturation at 95°C,  
198 a 30s annealing at 45°C for Euka02 and 54°C for Tele02, and a 60s elongation at 72°C, followed  
199 by a final extension step of 7 min at 72°C. Purification of PCR products (combined in equal  
200 volumes), were conducted using the MinElute PCR purification kit (Qiagen, Hilden, Germany)  
201 following the manufacturer's instructions. Gel electrophoresis (E-Gel Power Snap; Invitrogen,  
202 Carlsbad, CA, USA) was used to check for amplification and purification success. Library  
203 preparation and sequencing were outsourced to Fasteris SA (Geneva, Switzerland;  
204 <https://www.fasteris.com/dna/>). Two sequencing libraries (one per marker) were prepared  
205 following the PCR-free MetaFast protocol (Taberlet *et al.* 2018), which limits the formation of  
206 chimeras. All amplicons were sequenced on a NextSeq 500 sequencing platform (Illumina, San  
207 Diego, CA, USA), using the rapid run mode generating 250 bp paired-end sequences.  
208 Extraction and PCR negatives (20 at each step, i.e. one by extraction batch and PCR plate), as  
209 well as unused tag combinations (240 in total, i.e. 12 by PCR plate) were included to control  
210 for potential contaminations and false positives caused by tag jumps (Schnell *et al.*, 2015).

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### 212 ***Sequences analyses, curation and conversion to occurrence data***

213 The OBITools package (Boyer *et al.* 2015) was used to analyse raw reads. First, paired-end  
214 reads were assembled with *illuminapairedend* function, only retaining sequences with an  
215 alignment score higher than 40. Second, *ngsfilter* function was used to assign aligned sequences  
216 to the corresponding sample by allowing two and zero mismatches on primers and tags,  
217 respectively. Third, *obiuniq* function was used for sequence dereplication. Sequences whose  
218 length fell outside the expected size interval (shorter than 36 bp for Euka02, or 129 bp for  
219 Tele02), bad-quality sequences (i.e. containing “N”) and those never observed at least ten times  
220 in at least one PCR replicate were filtered out. Finally, *ecotag* function was used to perform  
221 taxonomic assignment of each molecular Operational Taxonomic Unit (mOTU), for each  
222 marker, by comparing the mOTU sequence to a database of full-length reference metabarcodes.  
223 This reference database was constructed combining local reference sequences (Table S2), and  
224 sequences obtained from the GenBank public database (release 135) using *ecoPCR* (Ficetola *et*  
225 *al.*, 2010). More specifically, *ecoPCR* carried out an *in silico* PCR on GenBank with the primer  
226 pair used for the experiment and allowing three mismatches per primer. The obtained reference  
227 sequences were further curated by keeping only sequences assigned at least at the family level.  
228 For Euka02, when a mOTU was 100% identical with a sequence from the local reference  
229 database, this taxonomic assignment was favored in order to avoid any potential dilution of the  
230 local taxonomic information with that, probably less relevant, available in GenBank.

231 ROBITools package (<https://git.metabarcoding.org/obitools/ROBITools>) in R was used  
232 for further data filtering, removing spurious or contaminant sequences that can bias ecological  
233 conclusions drawn from DNA metabarcoding data (Calderón-Sanou *et al.*, 2019). We discarded  
234 from our dataset (1) potential chimeras (i.e. mOTUs with a best identity < 95% with the local  
235 reference database or reference sequences from GenBank release 135); (2) contaminants (i.e.  
236 mOTUs that were more numerous in the negative control replicates than in true PCR replicates);  
237 (3) mOTUs assigned to taxa other than marine organisms for Euka02 and mOTUs assigned to

238 taxa other than marine fish for Tele02, because Tele02 can also amplify taxa belonging to other  
239 classes (mammals, birds); and (4) PCR replicates with a low sequencing coverage. In total,  
240 40,211,815 clusters were sequenced for Tele02 and 58,843,304 for Euka02. Total numbers of  
241 sequences after data filtering were 15,874,979 and 25,948,409 for Tele02 and Euka02 datasets.

242 After this, we removed 'self-hits' (sequences identified as the studied species within its  
243 own gut samples) from both datasets, as these sequences can come from naturally degrading  
244 cells from the guts of the studied species and cannot be distinguished from potential  
245 consumption of conspecifics, which is considered as an intrinsic limitation of gut content  
246 metabarcoding (Taberlet *et al.* 2018; Casey *et al.* 2019). Only three species depicted high rates  
247 of self-hits in the Euka02 dataset (*Aphrodita aculeata*, *Buccinum undatum* and *Echinus*  
248 *esculentus*), which were the taxa with the lowest sample sizes. High rates of self-hits for a given  
249 species were defined as when self-hits were the highest taxonomic assignment across the  
250 majority of individuals of that consumer species (Casey *et al.* 2019), often resulting in self-hit  
251 counts being on average higher than total read counts (Table S3). High rates of self-hits were  
252 expected in fish species with Tele02 dataset because many individuals which did not consume  
253 other fish (e.g. from species not belonging to the piscivore trophic guild; Thompson *et al.* 2020),  
254 would likely only amplify DNA from their own degrading cells with this marker. This was the  
255 case for *Callionymus lyra*, *Chelidonichthys lucerna*, *Conger conger*, *Pleuronectes platessa* and  
256 *Trisopterus luscus*. Total number of sequences after self-hits removal were 9,065,600 and  
257 15,191,780 for Tele02 and Euka02, respectively. Count data (number of sequences) were  
258 transformed to occurrence data (presence-absence). To avoid rare-item inflation that can bias  
259 diet estimation, normalize detection across samples and further limit the risk of potential  
260 contaminations to remain in the dataset after data filtering steps, we set a threshold of minimum  
261 sequence abundance to determine if a mOTU should be considered as an occurrence. We used  
262 a threshold of  $\geq 1\%$  of the total number of sequences amplified in the stomach content to define

263 an occurrence (i.e. MOTUs representing < 1% of the total number of sequences amplified in  
264 the stomach of each consumer were removed) (Deagle *et al.* 2019). Finally, we built a consensus  
265 diet incorporating both molecular markers by merging the two datasets (Euka02 and Tele02)  
266 into a single one following the multi-marker merging procedure detailed in da Silva *et al.*  
267 (2019). Essentially, this merging procedure assumes that within the same gut content, a given  
268 food item recovered at higher taxonomic ranking (e.g. order or family) by the most generalist  
269 marker (Euka02) is the same as items of the same taxonomic group recovered at lower  
270 taxonomic ranking (e.g. genus or species) by the specialist marker (Tele02). Merged dataset  
271 contained a total of 154 identified mOTUs across all samples, with 40 belonging to  
272 Actinopterygii and Chondrichthyes.

#### 274 ***Calculation of a score of ‘probability of consumption as fisheries discard’***

275 All fish prey were classified according to their probability of being ingested as fisheries discard  
276 by calculating a discard probability score (DPS), ranging from 0 to 3, following: 0 = very low,  
277 1 = low, 2 = medium, 3 = high probability of being ingested as fisheries discard (details on DPS  
278 calculation for each predator-prey interaction are provided in Table S4). The score was  
279 calculated by compiling information on the discarding importance of the prey (i.e. ‘discard  
280 importance score’ detailed above) and known diet and trophic guild affiliation of the consumer  
281 (i.e. ‘trophic link score’). The ‘trophic link score’, set either as 0 or 1 was assigned to each  
282 consumer-prey combination appearing in the metabarcoding results based on a literature review  
283 as follows: 0 = natural predation is documented in the specific literature of the consumer or  
284 possible based on trophic guild affiliation of the consumer (i.e. predation on the family of the  
285 prey was observed in other species belonging to the consumer’s trophic guild), 1 = natural  
286 predation is not documented either in the specific literature of the consumer or in other species  
287 belonging to the consumer’s trophic guild. Trophic guild affiliations of fish were derived from

288 Thompson *et al.* (2020) which source stomach content records from ICES (1997) and  
289 DAPSTOM (Pinnegar 2014) to establish trophic guilds. Specific diet of invertebrate taxa were  
290 complemented with the trophic traits ‘feeding type’, ‘feeding habit’ and ‘trophic level’ available  
291 from Sealifebase (<https://www.sealifebase.ca>; Palomares & Pauly 2021) and the Biological  
292 Traits Information Catalogue ([www.marlin.ac.uk/biotic](http://www.marlin.ac.uk/biotic); MarLIN 2006). The discard  
293 probability score, was calculated in an additive way as  $DPS = \text{‘discard importance score’} +$   
294  $\text{‘trophic link score’}$ . Using trophic guilds and traits to determine ‘the trophic link score’ allows  
295 to limit biases and overestimating of discard consumption due to the unequal state of knowledge  
296 on the specific diet of the different taxa. Additionally, we adopted a conservative approach to  
297 avoid overamplifying the DPS by assigning a  $DPS = 0$  to all prey items whose ‘discard  
298 importance score’ = 0. This means that even if predation on a given prey is undocumented to  
299 our knowledge and according to the larger spectrum of the trophic guild (‘trophic link score’ =  
300 1), if the prey was not observed being discarded at the time of sampling, the resulting  $DPS = 0$ .  
301 Following the same conservative approach, whenever a prey mOTU was identified to the genus  
302 level or higher, we assigned the lowest ‘discard importance score’ of all species included within  
303 its taxonomic group.

304

### 305 ***Diet analysis***

306 Information on the occurrence of each mOTU in the gut content of each species was  
307 summarized using Frequency of Occurrence (FOO), which is calculated for each consumer  
308 species as the number of occurrences of a given mOTU divided by the total number of gut  
309 contents samples of that consumer species. The weighted Percentage of Occurrence (wPOO)  
310 was obtained following Deagle *et al.* (2018): occurrences of food items are weighted  
311 (standardized) across the entire diet at the individual level, then these weighted occurrences are

312 summed at the species level and divided by species sample size. Diet richness was assessed at  
313 the population and individual levels using raw richness of mOTUs.

314

### 315 ***Molecular Ecological Network Analysis***

316 We built a network of trophic interactions among consumers sampled in the Bay of Bourgneuf  
317 fishing grounds (i.e. species and sizes targeted by commercial trawling) and their prey using  
318 gut content metabarcoding data of the 22 consumer species to quantify the importance of  
319 trophic links potentially involving discard consumption at the scale of the modelled network,  
320 and whether discards may be fueling structurally important species (Meyer et al., 2020). The  
321 network was directed from consumers to prey and interactions were weighted using the wPOO  
322 values. We used Gephi (version 0.9.2; Bastian et al., 2009) to visualize and calculate all network  
323 metrics. First, we mapped the network of trophic interactions using Forced Atlas 2 layout, a  
324 force-directed algorithm used to depict the modular aspect of network structure (Jacomy et al.,  
325 2014). Second, to obtain a view of the importance of trophic links involving discard  
326 consumption at the scale of the modeled network, we quantified the proportion of trophic links  
327 involving fish prey within the network and among those, the proportion of links likely involving  
328 discarded fish (links involving prey with  $DPS \geq 2$ ). Third, we measured the relative functional  
329 importance of each taxon within the modeled network by calculating: (i) Weighted in-degree  
330 centrality (i.e. the number of links directed to the node, weighted according to wPOO values),  
331 which indicates the degree to which a taxon is an important food source for other taxa in the  
332 network, identifying central taxa whose loss may trigger cascading effects, indirectly impacting  
333 the community (Elhesha et al., 2017); (ii) Eigenvector centrality, which measures the node's  
334 importance within the network while accounting for the importance of its neighbors, further  
335 helping to identify taxa that potentially strongly influence the abundance of others (Allesina &  
336 Pascual 2009).

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## 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 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; Fig. S1). 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).

360 **Table 1:** Diet richness in mOTUs, expressed for the whole diet and the fish fraction, both at  
 361 the population (total) and individual level (Mean  $\pm$  SE), and network centrality metrics  
 362 (weighted in-degree and eigenvector centrality) for each consumer. \*Centrality values of both  
 363 echinoids are those of the mOTU 'Echinoidea' since they could not be differentiated during  
 364 metabarcoding analysis.

Class	Species	N	Total richness			Fish richness			Centrality metrics	
			Total	Mean	SE	Total	Mean	SE	Weighted in-degree	Eigen vector
Actinopterygii	<i>Callionymus lyra</i>	10	26	5.1	0.7	4	0.9	0.3	31.3	0.49
	<i>Chelidonichthys lucerna</i>	23	24	2.9	0.5	12	1.0	0.3	43.4	0.45
	<i>Conger conger</i>	19	25	4.1	0.3	14	1.6	0.3	29	0.63
	<i>Merlangius merlangus</i>	20	23	3.5	0.5	11	2.3	0.3	26.1	0.60
	<i>Merluccius merluccius</i>	19	16	2.5	0.3	9	1.7	0.2	120	0.78
	<i>Pleuronectes platessa</i>	20	24	5.5	0.5	8	1.7	0.3	25.8	0.38
	<i>Pollachius pollachius</i>	17	36	5.4	0.7	13	2.4	0.5	20.6	0.34
	<i>Trachurus trachurus</i>	25	34	7.4	0.5	15	4.6	0.3	93.8	0.71
	<i>Trisopterus luscus</i>	20	33	4.2	0.4	11	1.4	0.2	84.2	0.84
Cephalopoda	<i>Sepia officinalis</i>	27	38	4.6	0.4	24	3.3	0.5	1.7	0.10
Chondrichthyes	<i>Raja undulata</i>	21	21	4.3	0.4	13	2.2	0.2	19.6	0.56
	<i>Scyliorhinus canicula</i>	19	39	7.0	0.8	16	2.4	0.5	11.6	0.46
Echinoidea	<i>Echinus esculentus</i>	1	16	16.0	NA	9	9.0	NA	0 (7.9*)	0 (0.23*)
	<i>Psammechinus miliaris</i>	19	57	6.4	0.9	18	2.3	0.5	0 (7.9*)	0 (0.23*)
Gastropoda	<i>Buccinum undatum</i>	1	4	4.0	NA	1	1.0	NA	4.7	0.13
Malacostraca	<i>Atelecyclus undecimdentatus</i>	20	26	4.1	0.6	18	2.2	0.6	170.5	0.59
	<i>Cancer pagurus</i>	17	25	3.5	0.5	16	1.9	0.5	0	0
	<i>Crangon</i> sp.	13	25	7.9	1.4	19	6.3	1.3	147	0.50
	<i>Maja brachydactyla</i>	23	18	2.1	0.4	8	0.6	0.2	0	0
	<i>Necora puber</i>	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
Polychaeta	<i>Aphrodita aculeata</i>	4	6	2.3	0.6	2	1.0	0.4	0	0

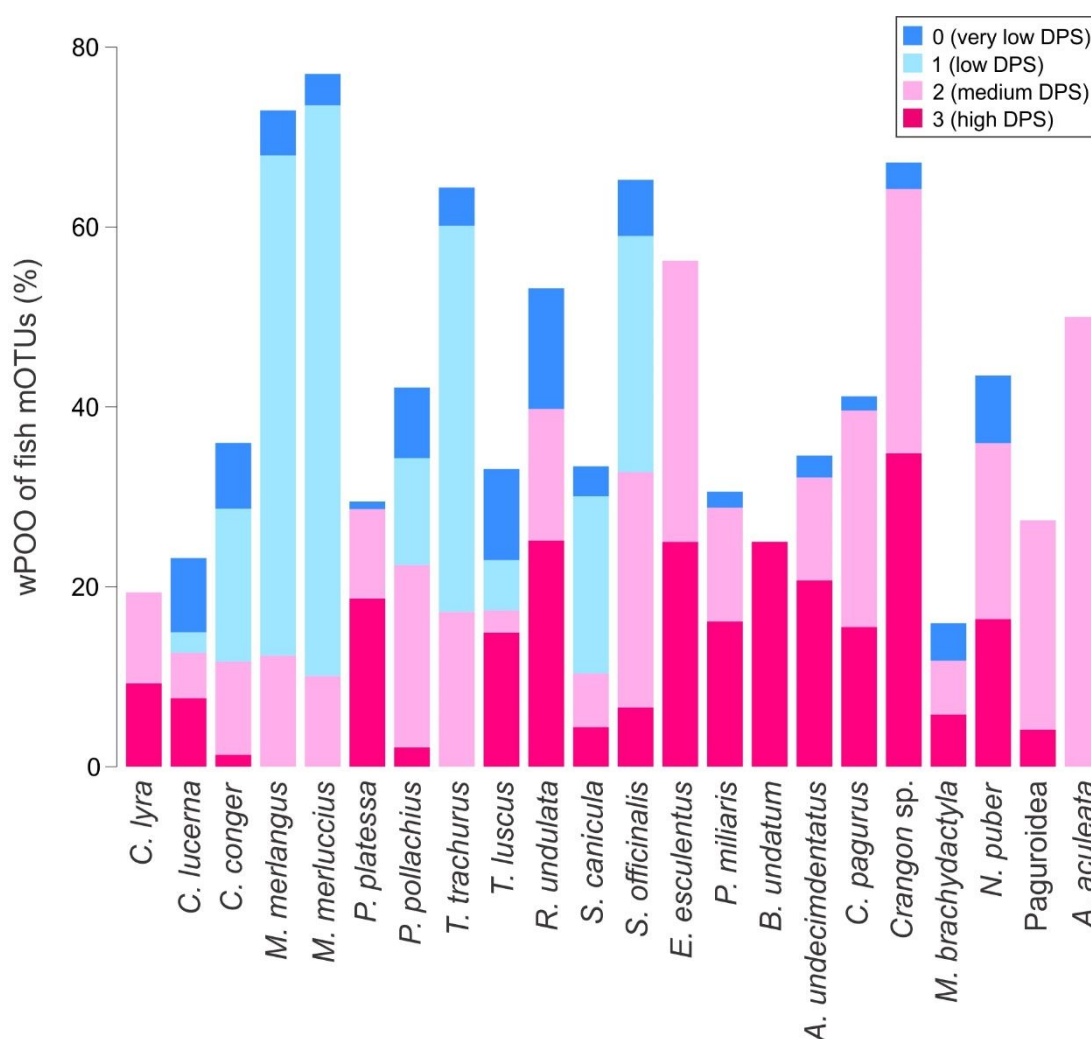
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367 Weighted percentage of occurrence (wPOO) of fish mOTUs in the diet of the different  
 368 consumers varied from a minimum of 15.9% in *M. brachydactyla* to a maximum of 77% in  
 369 *Merluccius merluccius* (Table S5). Fish represented  $\geq 50\%$  of all ingested mOTUs in terms of  
 370 wPOO in the fishes *Merlangius merlangus*, *M. merluccius*, *T. trachurus* and *R. undulata*, in the  
 371 cuttlefish *S. officinalis*, but also in the echinoid *E. esculentus*, the shrimp *Crangon* sp. and the  
 372 polychaete *A. aculeata* (Fig. 1; Fig. S1).

373



374

375 **Figure 1:** Weighted percentage of occurrence (wPOO [%]) of the fish mOTUs (Actinopterygii  
 376 and Chondrichthyes) identified via gut contents metabarcoding of the 22 consumers. Fish  
 377 mOTUs are categorized according to the 'discard probability score' (DPS), from 0 (dark blue)  
 378 = very low probability to 3 (dark pink) = high probability of being ingested as fisheries discard.

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### ***Potential importance of discarded fish ingestion across consumer taxa***

Prey with the highest discard probability score (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.  $DPS \geq 2$ ) 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  $DPS \geq 2$  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  $DPS \geq 2$ , 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  $DPS = 1$  (Table S4 and S4). 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  $DPS \geq 2$  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  $DPS \geq 2$  (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; Table S5). Prey with  $DPS \geq 2$  represented 11.8 to 56.3 % of the whole diet of these species. Fish fraction of the diet was entirely constituted of prey with  $DPS \geq 2$  in *E. esculentus*, Paguroidea and *A. aculeata* constituting 56.3%, 27% and 50% of their whole diet, respectively. *A. aculeata*, Paguroidea, *M. brachydactyla* and *S. officinalis* had the lowest

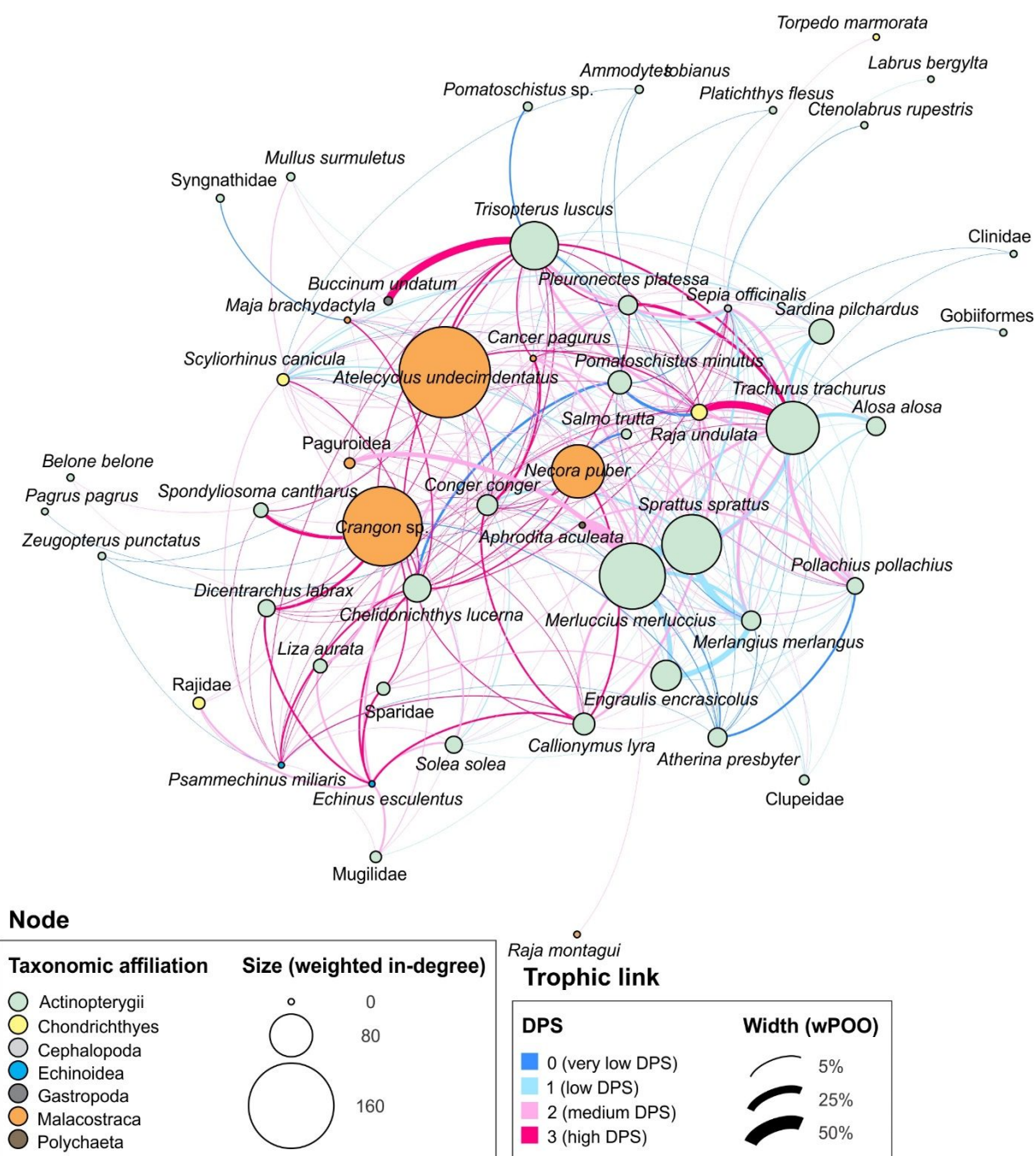
404 reliance on prey with DPS = 3 relative to their whole diet (0-6.6% wPOO). Conversely,  
405 echinoids and all other decapods relied importantly on prey with DPS = 3, reaching 15.5 to  
406 34.9% of their whole diet. The gastropod *B. undatum* relied exclusively on fish with DPS = 3,  
407 constituting 25% of its whole diet.

408

### 409 ***Molecular ecological network analysis***

410 Diet information were used to model a network of trophic interactions among the 22 sampled  
411 consumers and their prey, totaling 563 weighted links (trophic interactions) between 159 nodes  
412 (sampled consumers and mOTUs) (Fig. 2; Figure S2). Among the 563 trophic links, 48% (268  
413 links) involved fish prey, that is 12% with DPS = 3, 19% with DPS = 2, 10% with DPS = 1 and  
414 7% with DPS = 0. Among these 270 trophic links involving fish prey, 66% concerned fish prey  
415 that had a medium to high probability of having been consumed as fisheries discards (DPS  $\geq$   
416 2); 40% with DPS = 2 and 26% with DPS = 3 relative to the consumer. Both centrality metrics  
417 provided complementary information identifying species whose fish fraction of the diet could  
418 be dominated by discards, or fish that were themselves importantly discarded, as functionally  
419 important nodes in the modeled interaction network (Table 1; Table S6). Among taxa with the  
420 highest weighted in-degree centrality values, we found species whose fish fraction of the diet  
421 could be potentially largely dominated by discards (e.g. the decapods *Atelecyclus*  
422 *undecimdentatus*, *Crangon* sp. and *Necora puber*). The fish with the highest weighted in-degree  
423 centrality values were all part of species discarded by the commercial fleet in the area and at  
424 the time of sampling: *T. trachurus*, *Trisopterus luscus* and *C. lucerna* which were observed  
425 among the most discarded species, as well as *M. merluccius* and multiple Clupeidae which were  
426 observed to be discarded in lower quantities. A similar pattern was visible in Eigenvector  
427 centrality values, but with an increased importance of fish, mainly Clupeidae and *T. luscus*, and  
428 a slight decrease of decapods importance in the network (Table 1; Table S6).

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430

431 **Figure 2:** Subset of the molecular network constructed from gut content metabarcoding data,  
 432 depicting trophic interactions among the 22 consumers and their prey; only the trophic links  
 433 involving fish prey are presented, see Fig. S2 for the complete network. Nodes are representing  
 434 the consumers and prey mOTUs. Node color depicts taxonomic affiliation (by Class). Node  
 435 size is proportional to its importance as a food source for other taxa (weighted in-degree  
 436 centrality). Link width is proportional to wPOO of prey and color varies according to prey DPS.

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437

438 **Discussion**439 ***Importance of discard consumption across marine fauna***

440 With the introduction of the LO in European waters (European Union 2013), the amount of  
441 fisheries discards is expected to decline. However, it is not clear to which extent scavenging  
442 behaviors may be common and determine the diet of marine fauna (e.g. Bluhm & Bechtel, 2003;  
443 Olaso et al., 2002; Ramsay et al., 1997), especially in fishing grounds subject to important  
444 discarding activity. Here, we showed that potential ingestion of discarded fish was generalized  
445 from low trophic level benthic invertebrates to predatory fishes in shallow coastal fishing  
446 grounds of the Bay of Bourgneuf, with a fish fraction of the diet likely dominated by potential  
447 discards in 17 of the 22 sampled taxa. Diet involving prey with  $DPS \geq 2$  generally corresponded  
448 to prey that may not be naturally predated upon. For example, gut content metabarcoding  
449 revealed that 30% of *Callionymus lyra*, a small coastal benthivore fish that feeds mainly on  
450 benthic invertebrates (Fricke 1986; Thompson et al. 2020), ingested *Conger conger* and  
451 *Merluccius merluccius*, which are larger and higher in the food chain (Arroyo et al. 2019).  
452 These ingestions accounted for 100% of the fish diet of this species. As both *C. conger* and *M.*  
453 *merluccius* are eviscerated on board, the ingestion of these two species by *C. lyra* likely  
454 corresponds to eating discarded viscera. Note that some fish mOTUs identified in the guts of  
455 some species might correspond to predation on eggs or early life stages which could be  
456 underassessed in the diet literature, possibly introducing a bias in the interpretation of high DPS  
457 prey consumption. However, the trophic guild approach included in the calculation of the DPS  
458 (Thompson et al. 2020) is based on large databases of stomach content records which also  
459 report eggs and larvae consumption (i.e. the 'Integrated Database and Portal for Fish Stomach  
460 Records' [DAPSTOM; Pinnegar 2014]) and ICES 'Year of the Stomach' database [ICES  
461 1997]), which should limit such bias as much as possible.

462 Large-scale studies have provided contrasting results regarding the importance of  
463 discards for scavengers concluding to either influential (e.g. Catchpole & Frid 2006) or limited  
464 effects (e.g. Depestele *et al.* 2019), but calling for more empirical studies in diverse  
465 environmental settings. In invertebrates, fish fraction of the diet was largely dominated by prey  
466 with  $DPS \geq 2$  (74-100%), suggesting that all invertebrates sampled may rely mainly on discards  
467 concerning fish prey. This is consistent with the fact that most of these species, except *Sepia*  
468 *officinalis*, are unlikely to catch and feed upon live fish. Although some are known  
469 necrophagous, the proportion of fish ingestion likely originating from discarding was  
470 particularly high in the crabs *Cancer pagurus*, *Necora puber* and *Atelecyclus undecimdentatus*.  
471 Previous studies recorded discard consumption by *Buccinum undatum* (Evans *et al.*, 1996), sea  
472 urchins (González-Irusta *et al.* 2014), crabs and spider crabs (Ramsay *et al.* 1997; Bozzano &  
473 Sardà 2002). While we judged that *Aphrodita aculeata*, *Echinus esculentus* and *Buccinum*  
474 *undatum* have too low sample sizes to allow for any generalization of findings, their reporting  
475 remain valuable regarding the paucity of information on the diet of these species. Overall, these  
476 results suggest that in coastal fishing grounds subject to important discarding activity, the  
477 reliance of local invertebrate macrofauna on fish likely ingested as discards may be important,  
478 and reveal scavenging behaviors that appear to be under documented or whose strengths may  
479 be underappreciated.

480 In fish, potential discarded fish ingestions were more variable across taxa and less  
481 important in more predatory fishes compared to others. However, this may also be due to the  
482 fact that in predatory fishes for which piscivory is well established in the literature, such as  
483 *Conger conger*, *Merlangius merlangus*, *Merluccius merluccius*, *Trachurus trachurus* and  
484 *Scyliorhinus canicula* (See Table S4 for references), most of the ingested fish mOTUs were  
485 known to be naturally predated upon, therefore lowering their DPS according to our  
486 methodology, even if they were importantly discarded in the area. A previous study by Olaso

487 et al. (2002) acknowledged important consumption of discarded fish such as Clupeidae by *S.*  
488 *canicula*, while another study by Groenewold and Fonds (2000) evidenced that *M. merlangus*  
489 were attracted by fish carcasses. Fishes that should occupy lower trophic levels (*Callionymus*  
490 *lyra*, *Chelidonichthys lucerna*, *Pleuronectes platessa*, *Pollachius pollachius*, *Trisopterus luscus*  
491 and *Raja undulata*; Arroyo et al., 2019) showed unexpectedly important consumption of fish  
492 potentially linked to scavenging on discards ( $DPS \geq 2$ ). Such scavenging behaviors were  
493 already noted for Rajidae (Olaso et al. 2002), as well as for *P. platessa* which were documented  
494 waiting and feeding at the chelae of crabs around discarded fish (Jenkins et al. 2004). *C. lyra*,  
495 *C. lucerna*, *P. platessa* and *T. luscus* also showed attraction towards fish carcasses in the study  
496 by Groenewold and Fonds (2000). Our study suggests that *R. undulata*, *P. platessa*, *T. luscus*  
497 and *C. lyra* may be the main consumers of discards in the fish community.

### 498

### 499 ***Potential consequences of the Landing Obligation suggested by network***

### 500 ***analysis***

501 Studying food web structure helps forecasting the impact of trophic changes on ecosystem  
502 functioning (Allesina & Pascual 2009; Meyer et al. 2020). Because of the lack of infrastructure  
503 on the ground, some areas benefitted from an extension of the application of the Landing  
504 Obligation (LO) to 2020, as is the case of the Bay of Biscay (European Union 2019), explaining  
505 that species under total allowable catches (TAC) and quota were still being discarded at the  
506 time of study. This provided the opportunity to picture the structure of a network of trophic  
507 interactions among species feeding in coastal fishing grounds subject to important discarding  
508 and their prey. Network analysis revealed that in the studied fishing ground, 66% of the trophic  
509 links implying fish prey potentially involved discard consumption ( $DPS \geq 2$ ), including 26%  
510 with the highest discard probability score ( $DPS = 3$ ). This provides novel insights suggesting

511 that discarded fish consumption might constitute a non-negligible factor affecting the structure  
512 of the local food web and community.

513 One may argue that discards might enhance secondary production in marine food webs  
514 (Bluhm & Bechtel 2003), in which case a reduction of discarding could lead to negative effects  
515 on populations most supported by them and to associated indirect effects. It is worth noting that  
516 not all discarded species are subject to the LO, implying that not all discarding will end with its  
517 full application in the area, but only those species under TAC and quota. Likewise, even if  
518 discarding is regulated, consumption of discarded viscera should still be possible since large  
519 catches such as *C. conger* or *M. merluccius* would continue to be eviscerated on board.  
520 However, species subject to the LO represented an important fraction of ingested fish in the  
521 sampled community (e.g. *Trachurus trachurus*, *Engraulis encrasicolus*, *Merluccius merluccius*  
522 or the different rays; see all species under TAC and quota among our samples in Table S5).  
523 Results show that decapods such as *Atelecyclus undecimdentatus*, *Crangon sp.* and *Necora*  
524 *puber*, or the fish *Trisopterus luscus* which relied potentially importantly on discards, but also  
525 *Trachurus trachurus* which was amongst the most discarded fish according to both our  
526 observations and OBSMER data, constitute some of the most important structuring nodes in  
527 the network. This implies that they may potentially strongly affect the local abundance of other  
528 taxa via bottom-up effects (Elhesha *et al.* 2017). This is consistent with studies describing the  
529 central role of decapods, including as food sources for diverse taxa (Boudreau & Worm 2012)  
530 and the fact that decapods seem to constitute the dominant invertebrate Order attracted by or  
531 consuming discards (Depestele *et al.* 2019). By reducing the amount of food supporting these  
532 central species or reducing their discarding, the LO may potentially trigger indirect effects,  
533 altering the local network of trophic interactions and community structure. Another  
534 consequence that is perhaps more complex to predict is the potential positive effect on fish  
535 populations which were previously strongly subject to discarding. On the one hand they could



536 provide more food for species higher in the food chain, following natural predation. On the  
537 other hand they could increase predation pressure on species lower in the food chain and modify  
538 food web structure via top-down effects. For example, a discard ban on *Trachurus trachurus*  
539 may positively affect its population, increasing predation pressure on the variety of Clupeidae  
540 constituting its diet. Because Clupeidae are found amongst the most important structuring taxa,  
541 they may then also propagate changes to diverse taxa via bottom-up effects.

542 The 'snapshot' character of trophic information obtained via gut content metabarcoding  
543 and the focus on a limited number of species corresponding to those targeted by the local  
544 commercial trawling fleet may not allow to fully apprehend the complex consequences of the  
545 LO on marine food web structure. This could be improved by expanding this study to a wider  
546 area, community and with repeated measures. The method of capture used in this study (bottom  
547 trawling) may introduce a bias in the representation of species against pelagic ones while  
548 focusing on the benthic-demersal assemblage. However, this also reflects the specific  
549 environmental context in which the study took place, which is a shallow coastal fishing ground  
550 (4-30m depth) representative of many coastal fisheries in the South of Europe. The presence of  
551 *T. trachurus* in our dataset shows that some pelagic species can nonetheless feed in such fishing  
552 grounds, underlying the increased benthic-pelagic coupling in shallow coastal areas (Kopp *et*  
553 *al.* 2015). More precise inference on the effects of discard reduction on the pelagic compartment  
554 of the food web could be drawn from conducting complementary studies using a larger variety  
555 of capture methods (e.g. including gill-nets, traps), as well as replicate our study design in  
556 deeper and open-water fisheries. Studies conducted in deep-sea offshore fisheries (200-800 m  
557 depth) dominated by trawling suggest a similar pattern whereas important quantities of discards  
558 end up on the seabed and be consumed by various benthic fauna, including small crustaceans  
559 which are important prey of targeted commercial species (Castro, Araújo & Monteiro 2015).  
560 Offshore open-water fisheries may be dominated by other métiers (e.g. gill-nets, longlines)

561 which often target single species, generate less discards (Cornou *et al.* 2021) and operate in a  
562 spottier fashion than in coastal fisheries which are more geographically comprehensively  
563 fished. In these contexts, the possibility of consumption by large pelagic predators before  
564 discards reach the bottom might reduce its integration into the benthic food web compared to  
565 our study system and encounter probability (i.e. the likelihood to come across fisheries discards)  
566 might be reduced as well for both pelagic and benthic-demersal species (Depestele *et al.* 2019),  
567 possibly leading to a more diluted impact across the whole food web. Yet, we believe that more  
568 empirical studies are needed to clarify and quantify trophic links between marine fauna and  
569 discards in these structurally more complex systems, which would require a wider array of  
570 capture methods to be deployed to cover both pelagic and benthic species.

571

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

574 Gut content metabarcoding promises to revolutionize food web studies by yielding  
575 unparalleled resolution of trophic interactions (Casey *et al.* 2019; Takahashi *et al.* 2020), but  
576 limitations should also be considered in order to advance research on discards reintegration into  
577 marine food webs. It was successfully used to identify species potentially originating from  
578 fisheries discards in the diet of Albatrosses (McInnes *et al.* 2017) and quantify discard  
579 consumption in marine fauna in the present study. Here, gut content metabarcoding allowed to  
580 identify 154 mOTUs (considering a  $\geq 1\%$  relative read abundance threshold; Deagle *et al.*  
581 2019), with 40 belonging to Actinopterygii and Chondrichthyes, which represents an  
582 unprecedented resolution in a demersal food web. Yet, taxonomic levels were sometimes  
583 dropped from species to genus, or further, whenever the similarity between primary and  
584 secondary reference sequences was equal or higher to the similarity between the primary  
585 reference and the query sequence during taxonomic assignment, resulting in sequence

586 assignment to the most recent common ancestor. Such cases can occur because of incomplete  
587 reference databases. Improving the local reference database is therefore crucial to maximize the  
588 resolution of taxonomic identification (Casey *et al.* 2019). It is also worth noting that while the  
589 18S rDNA region may provide an exceptional coverage across the prey items of interest in this  
590 study, it may provide poorly reliable taxonomic resolution. The use of complementary markers  
591 can improve taxonomic resolution within specific groups of interest (da Silva *et al.* 2019). Here,  
592 the complementary use of Tele02 (targeting 12S region) helped improve the resolution of fish  
593 mOTUs identification compared to Euka02 alone (targeting 18S region): Among the 40 fish  
594 mOTUs identified, 32 were assigned to species level, 1 to genus, 6 to family and 1 to order.  
595 Since taxonomic richness is dependent upon clustering level, taxonomic richness of the whole  
596 diet may be influenced by lower or variable resolution yielded by Euka02 for non-fish groups.  
597 Taxonomic richness of the fish fraction of the diet should be much less influenced by such  
598 problem. Specific markers for other taxonomic groups should similarly improve identification  
599 resolution. Using multiple markers could also help limit the intrinsic biases associated with  
600 primer choice and difference in amplification rate among sequences (Deagle *et al.*, 2014).

601 One intrinsic limitation of applying metabarcoding to study diet is that one cannot  
602 decipher between conspecifics consumption and DNA coming from the consumer's own  
603 degrading cells, which is the reason for removing 'self-hits' from diet data (i.e. DNA of the  
604 consumer within its own gut content) (Taberlet *et al.* 2018; Casey *et al.* 2019). We found no  
605 evidence of an important role of cannibalism in the trophic ecology of the studied species based  
606 on gut content data from large databases used for DPS calculation. But beyond cannibalism of  
607 living conspecifics, one way self-hits censoring might still impact the estimated diet is by  
608 making a part of discard consumption invisible and therefore unaccounted for, namely if a  
609 species is consuming discarded conspecifics. This might be more impactful for species that are  
610 importantly discarded in the area (e.g. *C. conger*, *T. trachurus*, *T. luscus*), as one would expect

611 the probability of consuming discarded conspecifics to increase with discarding rate of that  
612 species. Important amount of consumer's DNA in a sample might impede the ability of PCR to  
613 reveal the true diversity of the diet because of a quick saturation of the reaction. But in our  
614 dataset self-hit rate was generally low, with only five fish species out of twenty-two depicting  
615 high self-hit rates with Tele02. High read counts of only their own DNA can be explained by  
616 the absence of fish consumption, which is confirmed by the absence of (other) fish mOTUs  
617 identified with Euka02. In this specific case, self-hits have little impact on diet estimates.

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

625

## 626 **Conclusions**

627 Ultimately, reducing bycatch and discarding remain conservation priorities, but it appears also  
628 crucial to understand and anticipate the potential consequences of reducing discards for the  
629 variety of marine species that may have become quite dependent upon them. Our results reveal  
630 that consumption of fish likely ingested as discards was important and generalized across  
631 marine fauna feeding in fishing grounds subject to important discarding, and identified that  
632 discards may support multiple species potentially playing an important structuring role in the  
633 local food web, such as decapods. DNA metabarcoding of gut contents, together with reliant  
634 methodologies to establish the probability of prey to originate from discarding, offers the  
635 possibility to quickly assess dependence of a community on fisheries discards. These results

636 could be further confirmed using stable isotopes or fatty acid tracers and repeated in various  
637 environmental settings to be implemented in ecosystem models such as Ecopath with ECOSIM  
638 in which trophic links with discards and corresponding energy fluxes may currently be  
639 underestimated.

640

#### 641 **Authors' contributions**

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

646

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655

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