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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 km²), 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).

165

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.

172

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- μ L volume containing
194 2 μ L of DNA extract, 0.4 μ M of each primer, 10 μ L of AmpliTaq Gold 360 Master Mix
195 (Applied Biosystems, Foster City, CA, USA) and 0.16 μ L of 20 mg.ml⁻¹ 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).

211

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; 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.

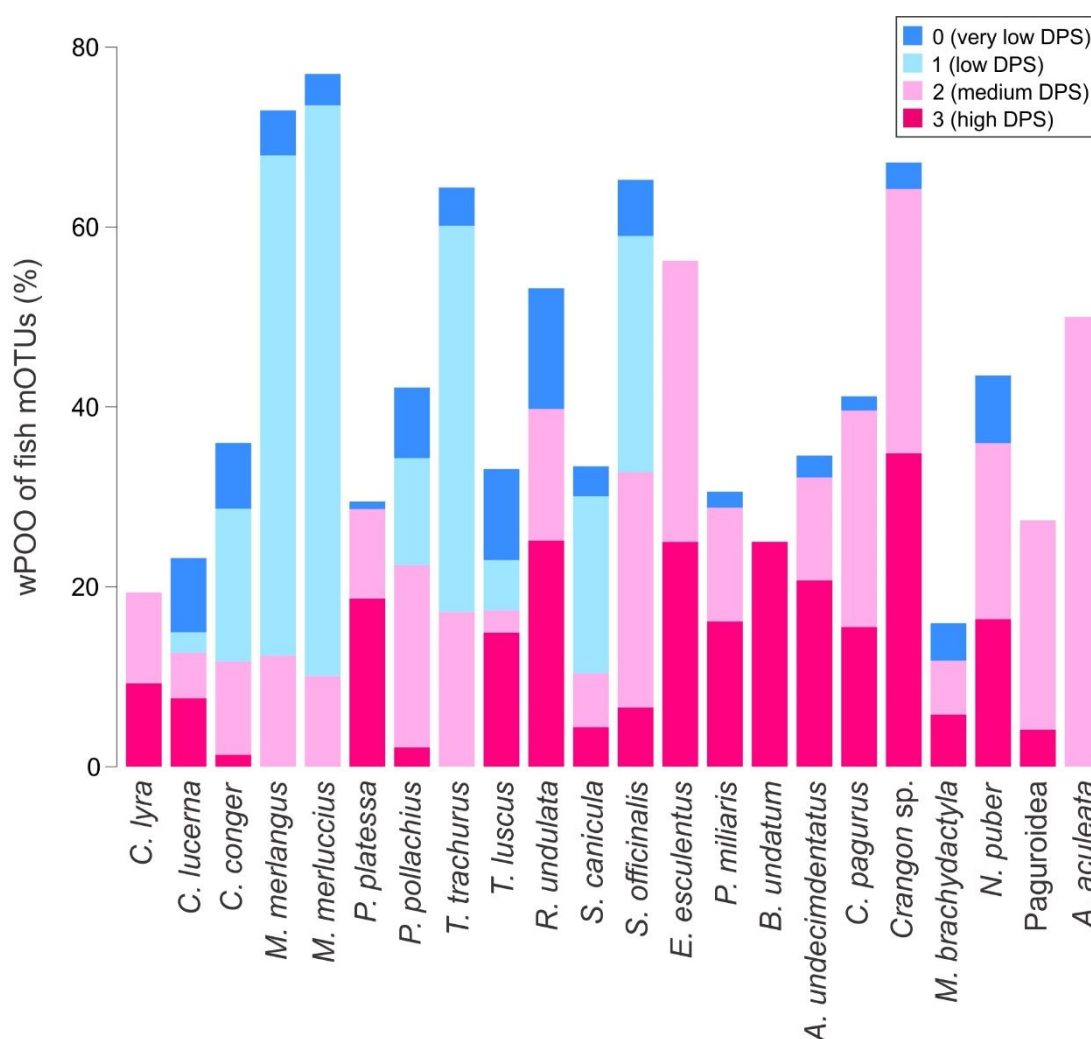
Class	Species	N	Total richness			Fish richness			Centrality metrics	
			Total	Mean	SE	Total	Mean	SE	Weighted in-degree	Eigen vector
Actinopterygii	<i>Callionymus lyra</i>	10	26	5.1	0.7	4	0.9	0.3	31.3	0.49
	<i>Chelidonichthys lucerna</i>	23	24	2.9	0.5	12	1.0	0.3	43.4	0.45
	<i>Conger conger</i>	19	25	4.1	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).

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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

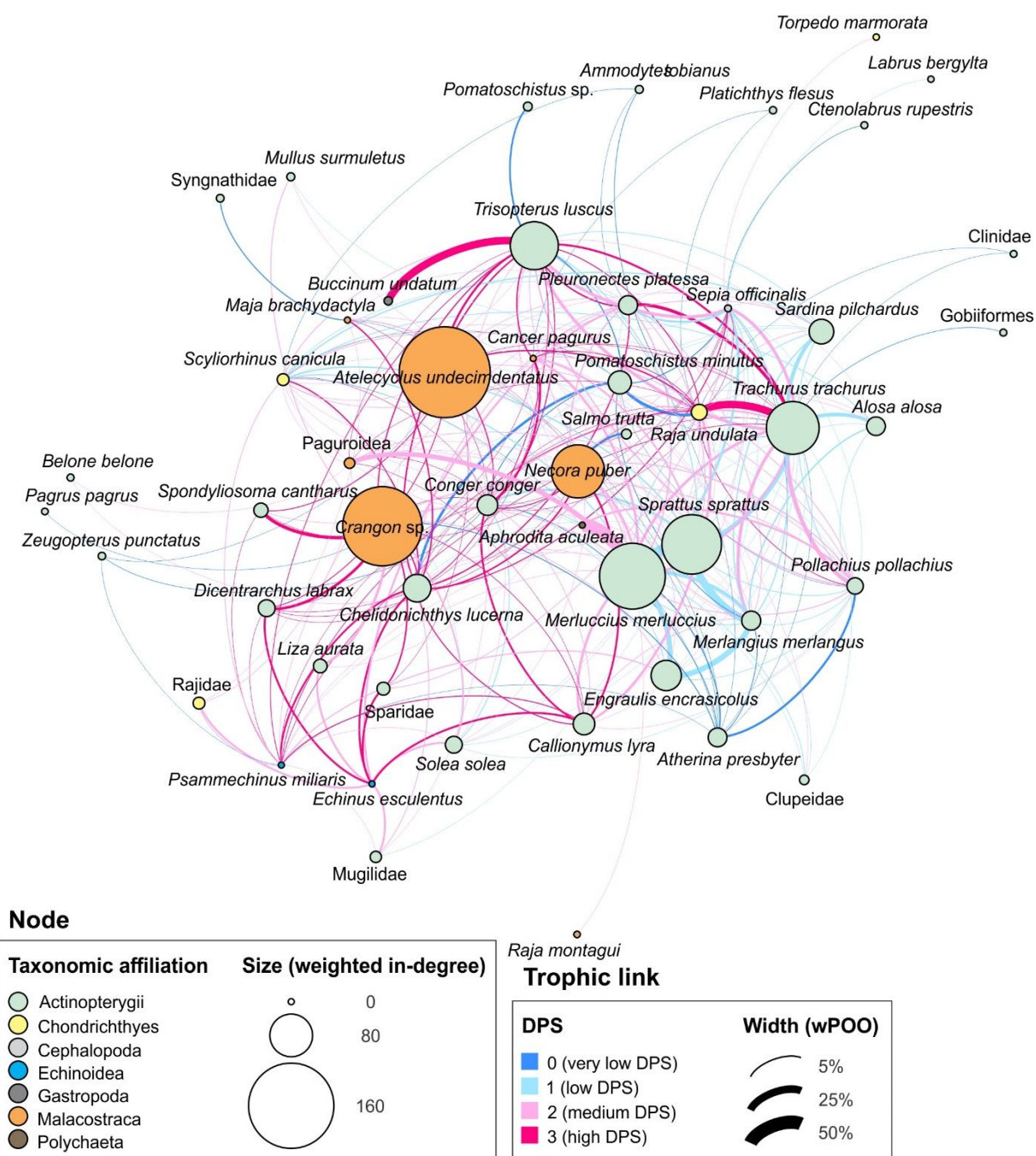
404 reliance on prey with DPS = 3 relative to their whole diet (0-6.6% wPOO). Conversely,
405 echinoids and all other decapods relied importantly on prey with DPS = 3, reaching 15.5 to
406 34.9% of their whole diet. The gastropod *B. undatum* relied exclusively on fish with DPS = 3,
407 constituting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

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

656 **References**

- 657 Allesina, S. & Pascual, M. (2009) Googling food webs : can an eigenvector measure species'
658 importance for coextinctions ? *PloS Computational Biology*, **5**, e1000494.
- 659 Arroyo, N.-L., Safi, G., Vouriot, P., López-López, L., Niquil, N., Le Loc'h, F., Hattab, T. &
660 Preciado, I. (2019) Towards coherent GES assessments at sub-regional level: signs of

- 661 fisheries expansion processes in the Bay of Biscay using an OSPAR food web indicator,
662 the mean trophic level. *ICES Journal of Marine Science*, **76**, 1543–1553.
- 663 Bascompte, J., Melián, C.J. & Sala, E. (2005) Interaction strength combinations and the
664 overfishing of a marine food web. *Proceedings of the National Academy of Sciences*,
665 **102**, 5443–5447.
- 666 Bastian, M., Heymann, S. & Jacomy, M. (2009) Gephi: an open source software for exploring
667 and manipulating networks. *International AAAI Conference on Weblogs and Social*
668 *Media*
- 669 Bluhm, B.A. & Bechtel, P.J. (2003) The potential fate and effects of seafood processing
670 wastes dumped at sea: a review. *Advances in Seafood Byproducts, Alaska Sea Grant*
671 *College Program* (ed P.J. Bechtel), pp. 121–140. University of Alaska, Fairbanks, AK.
- 672 Boudreau, S.A. & Worm, B. (2012) Ecological role of large benthic decapods in marine
673 ecosystems : a review. *Marine Ecology Progress Series*, **469**, 195–213.
- 674 Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P. & Coissac, E. (2015) OBITools: a
675 unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*,
676 **16**, 176–182.
- 677 Bozzano, A. & Sardà, F. (2002) Fishery discard consumption rate and scavenging activity in
678 the northwestern Mediterranean Sea. *ICES Journal of Marine Science*, **59**, 15–28.
- 679 Calderón-Sanou, I., Münkemüller, T., Boyer, F., Zinger, L. & Thuiller, W. (2019) From
680 environmental DNA sequences to ecological conclusions : How strong is the influence of
681 methodological choices ? *Journal of Biogeography*, **47**, 193–206.
- 682 Casey, J.M., Meyer, C.P., Morat, F., Brandl, S.J., Planes, S. & Parravicini, V. (2019)
683 Reconstructing hyperdiverse food webs : Gut content metabarcoding as a tool to
684 disentangle trophic interactions on coral reefs. *Methods in Ecology and Evolution*, **10**,
685 1157–1170.

- 686 Castro, M., Araújo, A. & Monteiro, P. (2015) Fate of discards from deep water crustacean
687 trawl fishery off the south coast of Portugal. *New Zealand Journal of Marine and*
688 *Freshwater Research*, **39**, 437–446.
- 689 Catchpole, T. & Frid, C.L.J. (2006) Importance of discards from the English *Nephrops*
690 *norvegicus* fishery in the North Sea to marine scavengers. *Marine Ecology Progress*
691 *Series*, **313**, 215–226.
- 692 Coissac, E. (2012) Oligotag: a program for designing sets of tags for next-generation
693 sequencing of multiplexed samples. *Data Production and Analysis in Population*
694 *Genomics, Methods in Molecular Biology Series* (eds F. Pompanon & A. Bonin), pp. 13–
695 31. Humana Press, Totowa, New Jersey.
- 696 Cornou, A., Quinio-scavinner, M., Sagan, J., Cloâtre, T., Dubroca, L. & Billet, N. (2021)
697 *Captures et Rejets Des Métiers de Pêche Français. Résultats Des Observations à Bord*
698 *Des Navires de Pêche Professionnelle En 2019*. Obsmer.
- 699 Deagle, B.E., Clarke, L.J., Thomas, A.C., McInnes, J.C., Vesterinen, E.J., Clare, E.L.,
700 Kartzinel, T.R. & Eveson, J.P. (2019) Counting with DNA in metabarcoding studies :
701 How should we convert sequence reads to dietary data ? *Molecular Ecology*, **28**, 391–
702 406.
- 703 Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F. & Taberlet, P. (2014) DNA
704 metabarcoding and the cytochrome c oxidase subunit I marker : not a perfect match.
705 *Biology letters*, **10**, 20140562.
- 706 Depestele, J., Feekings, J., Reid, D.G., Cook, R., Gascuel, D., Girardin, R., Heath, M.,
707 Hernvann, P.-Y., Morato, T., Soszynski, A. & Savina-Rolland, M. (2019) The Impact of
708 Fisheries Discards on Scavengers in the Sea. *The European Landing Obligation:*
709 *Reducing Discards in Complex, Multi-Species and Multi-Jurisdictional Fisheries* (eds
710 S.S. Uhlmann, C. Ulrich & S.J. Kennelly), pp. 129–162. Springer International

- 711 Publishing, Cham.
- 712 Elhessa, R., Kahveci, T. & Baiser, B. (2017) Motif centrality in food web networks. *Journal*
713 *of Complex Networks*, **5**, 641–664.
- 714 European Union. (2013) Regulation (EU) No 1380/2013 of the European parliament and of
715 the council of 11 December 2013 on the Common Fisheries Policy amending Council
716 Regulations (EC) No 1954/2003 and (EC) No 1224/2009 and repealing Council
717 Regulations (EC) No 2371/2002 and (EC). *Official Journal of the European Union*,
718 **L354**, 22–61.
- 719 European Union. (2019) Commission delegated regulation (EU) 2019/2237 of 1 October 2019
720 specifying details of the landing obligation for certain demersal fisheries in South-
721 Western waters for the period 2020-2021. *Official Journal of the European Union*, **L336**,
722 26–33.
- 723 Evans, P.L., Kaiser, M.J. & Hughes, R.N. (1996) Behaviour and energetics of whelks,
724 *Buccinum undatum* (L.), feeding on animals killed by beam trawling. *Journal of*
725 *Experimental Marine Biology and Ecology*, **197**, 51–62.
- 726 Ficetola, G.F., Coissac, E., Zundel, S., Riaz, T. & Shehzad, W. (2010) An In silico approach
727 for the evaluation of DNA barcodes. *BMC Genomics*, **11**, 434.
- 728 Fricke, R. (1986) Callionymidae. *Fishes of the North-eastern Atlantic and the Mediterranean*
729 (eds P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese), pp.
730 1086–1093. UNESCO, Paris.
- 731 Garthe, S., Camphuysen, K.C. & Furness, R.W. (1996) Amounts of discards by commercial
732 fisheries and their significance as food for seabirds in the North Sea. *Marine Ecology*
733 *Progress Series*, **136**, 1–11.
- 734 Gauduchon, T., Cornou, A., Quinio-Scavinner, M., Goascoz, N., Dubroca, L. & Billet, N.
735 (2020) *Captures et Rejets Des Métiers de Pêche Français. Résultats Des Observations à*

- 736 *Bord Des Navires de Pêche Professionnelle En 2018*. Obsmer.
- 737 González-Irusta, J.M., Preciado, I., López-López, L., Punzón, A., Cartes, J.E. & Serrano, A.
738 (2014) Trawling disturbance on the isotopic signature of a structure-building species, the
739 sea urchin *Gracilechinus acutus* (Lamarck, 1816). *Deep-Sea Research Part II: Topical*
740 *Studies in Oceanography*, **106**, 216–224.
- 741 Groenewold, S. & Fonds, M. (2000) Effects on benthic scavengers of discards and damaged
742 benthos produced by the beam-trawl fishery in the southern North Sea. *ICES Journal of*
743 *Marine Science*, **57**, 1395–1406.
- 744 Guardiola, M., Uriz, M.J., Taberlet, P., Coissac, E., Wangensteen, S. & Turon, X. (2015)
745 Deep-sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine
746 canyons. *PLoS ONE*, **10**, e0139633.
- 747 Guillen, J., Holmes, S.J., Carvalho, N., Casey, J., Dörner, H., Gibin, M., Mannini, A.,
748 Vasilakopoulos, P. & Zanzi, A. (2018) A review of the European union landing
749 obligation focusing on its implications for fisheries and the environment. *Sustainability*,
750 **10**, 900.
- 751 Hill, B.J. & Wassenberg, T.J. (2000) The probable fate of discards from prawn trawlers
752 fishing near coral reefs A study in the northern Great Barrier Reef, Australia. *Fisheries*
753 *Research*, **48**, 277–286.
- 754 ICES. (1997) *Database Report of the Stomach Sampling Project 1991*.
- 755 Ifremer. Système d'Informations Halieutiques. (2020) *Quartier Maritime Noirmoutier. 2019*.
756 *Activité Des Navires de Pêche*.
- 757 Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J.,
758 Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange,
759 C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J. &
760 Warner, R.R. (2001) Historical overfishing and the recent collapse of coastal

- 761 ecosystems. *Science*, **293**, 629–638.
- 762 Jacomy, M., Venturini, T., Heymann, S. & Bastian, M. (2014) ForceAtlas2 , a continuous
763 graph layout algorithm for handy network visualization designed for the Gephi software.
764 *PLoS ONE*, **9**, e98679.
- 765 Jenkins, S.R., Mullen, C. & Brand, A.R. (2004) Predator and scavenger aggregation to
766 discarded by-catch from dredge fisheries : importance of damage level. *Journal of Sea
767 Research*, **51**, 69–76.
- 768 Karris, G., Rinis, V.K., Kalogeropoulou, A., Xirouchakis, S., Machias, A., Maina, I. &
769 Kavadas, S. (2018) The use of demersal trawling discards as a food source for two
770 scavenging seabird species : a case study of an eastern Mediterranean oligotrophic
771 marine ecosystem. *Avian Research*, **9**, 26.
- 772 Kelleher, K. (2005) *Discards in the World's Marine Fisheries. An Update*. FAO Fisheries
773 Technical Paper. No. 470, Rome.
- 774 Kopp, D., Lefebvre, S., Cachera, M., Villanueva, M.C. & Ernande, B. (2015) Reorganization
775 of a marine trophic network along an inshore–offshore gradient due to stronger pelagic–
776 benthic coupling in coastal areas. *Progress in Oceanography*, **130**, 157–171.
- 777 Kopp, D., Robert, M., Chauvelon, T. & Méhault, S. (2016) Some expected impacts of the
778 Common Fishery Policy on marine food webs. *Marine Policy*, **66**, 8–14.
- 779 MarLIN. (2006) BIOTIC - Biological Traits Information Catalogue. URL
780 www.marlin.ac.uk/biotic [accessed 4 February 2021]
- 781 Mcinnes, J.C., Jarman, S.N., Lea, M., Raymond, B., Deagle, B.E., Phillips, R.A., Catry, P.,
782 Stanworth, A., Weimerskirch, H., Kusch, A., Gras, M., Cherel, Y., Maschette, D. &
783 Alderman, R. (2017) DNA metabarcoding as a marine conservation and management
784 tool : A circumpolar examination of fishery discards in the diet of threatened
785 Albatrosses. *Frontiers in Marine Science*, **4**, 277.

- 786 Meyer, J.M., Leempoel, K., Losapio, G. & Hadly, E.A. (2020) Molecular ecological network
787 analyses : an effective conservation tool for the assessment of biodiversity, trophic
788 interactions, and community structure. *Frontiers in Ecology and Evolution*, **8**, 588430.
- 789 Nielsen, J.M., Clare, E.L., Hayden, B., Brett, M.T. & Kratina, P. (2017) Diet tracing in
790 ecology: method comparison and selection. *Methods in Ecology and Evolution*, **9**, 278–
791 291.
- 792 Olaso, I., Sánchez, F., Rodríguez-cabello, C. & Velasco, F. (2002) The feeding behaviour of
793 some demersal fish species in response to artificial discarding. *Scientia Marina*, **66**, 301–
794 311.
- 795 Palomares, M.L.D. & Pauly, D. (2021) SeaLifeBase. World Wide Web electronic publication.
796 www.sealifebase.org, version (04/2021)
- 797 Pinnegar, J.K. (2014) *DAPSTOM - An Integrated Database & Portal for Fish Stomach*
798 *Records. Version 4.7*. Centre for Environment, Fisheries & Aquaculture Science,
799 Lowestoft, UK.
- 800 Ramsay, K., Kaiser, M.J., Moore, P.G. & Hughes, R.N. (1997) Consumption of fisheries
801 discards by benthic scavengers: utilization of energy subsidies in different marine
802 habitats. *Journal of Animal Ecology*, **66**, 884–896.
- 803 Scheffer, M., Carpenter, S. & de Young, B. (2005) Cascading effects of overfishing marine
804 systems. *Trends in Ecology & Evolution*, **20**, 579–581.
- 805 Schnell, I.B., Bohmann, K. & Gilbert, M.T.P. (2015) Tag jumps illuminated – reducing
806 sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology*
807 *Resources*, **15**, 1289–1303.
- 808 Sherley, R.B., Ladd-Jones, H., Garthe, S., Stevenson, O. & Votier, S.C. (2019) Scavenger
809 communities and fisheries waste : North Sea discards support 3 million seabirds, 2
810 million fewer than in 1990. *Fish and Fisheries*, **21**, 132–145.

- 811 da Silva, L.P., Mata, V.A., Lopes, P.B., Pereira, P., Jarman, S.N., Lopes, R.J. & Beja, P.
812 (2019) Advancing the integration of multi-marker metabarcoding data in dietary analysis
813 of trophic generalists. *Molecular Ecology Resources*, **19**, 1420–1432.
- 814 Taberlet, P., Bonin, A., Zinger, L. & Coissac, E. (2018) *Environmental DNA: For*
815 *Biodiversity Research and Monitoring*. Oxford University Press, Oxford, UK.
- 816 Takahashi, M., DiBattista, J.D., Jarman, S., Newman, S.J., Wakefield, C.B., Harvey, E.S. &
817 Bunce, M. (2020) Partitioning of diet between species and life history stages of
818 sympatric and cryptic snappers (Lutjanidae) based on DNA metabarcoding. *Scientific*
819 *Reports*, **10**, 4319.
- 820 Thompson, M.S.A., Pontalier, H., Spence, M.A., Pinnegar, J.K., Greenstreet, S., Moriarty,
821 M., Hélaouët, P. & Lynam, C.P. (2020) A feeding guild indicator to assess
822 environmental change impacts on marine ecosystem structure and functioning. *Journal*
823 *of Applied Ecology*, **57**, 1769–1781.
- 824 Tyson, C., Shamoun-baranes, J., Van Loon, E.E., Camphuysen, K.C.J. & Hintzen, N.T.
825 (2015) Individual specialization on fishery discards by lesser black-backed gulls (*Larus*
826 *fuscus*). *ICES Journal of Marine Science*, **72**, 1882–1891.
- 827 Votier, S.C., Bicknell, A., Cox, S.L., Scales, K.L. & Patrick, S.C. (2013) A bird's eye view of
828 discard reforms : Bird-borne cameras reveal seabird/fishery interactions. *PloS ONE*, **8**,
829 e57376.
- 830 Zeller, D., Cashion, T., Palomares, M.-L. & Pauly, D. (2018) Global marine fisheries
831 discards : A synthesis of reconstructed data. *Fish and Fisheries*, **19**, 30–39.

832