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Gut content metabarcoding of six crustaceans provides detailed diet description and insights into their roles as predators and scavengers

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Abstract

While many studies have analysed the diet of marine consumers using traditional morphology-based methods, the integral diet of many species of functionally important groups such as benthic invertebrates remains poorly resolved. This hinders our ability to precisely establish trophic links among species, which is necessary to understand food webs and ecosystem dynamics. Here, we applied DNA metabarcoding to obtain a high resolution picture of the animal fraction of the diet of six species of crustaceans in the Bay of Biscay; the edible crab *Cancer pagurus*, the velvet crab *Necora puber*, the circular crab *Atelecyclus undecimdentatus*, the spider crab *Maja brachydactyla*, the shrimp *Crangon crangon*, and the hermit crab *Pagurus bernhardus*. We used two pairs of primers targeting the 18S rDNA (generalist) and 12S rDNA (fish-specific) regions to identify prey that makes up the animal fraction of the diet. Based on occurrence data, 54 prey taxa belonging to 5 different phylum mostly represented by teleost fishes and malacostracans were identified. Through a high-level of prey detection, results indicate a scavenging strategy across all consumers mostly based on the consumption of preys likely originating from fishing discards. This study also reveals patterns of niche overlaps among consumers differentiating *C. crangon* from others and suggesting high overlap in animal resource use between *N. puber* and *M. brachydactyla* as well as *C. pagurus*. High resolution diet description revealed the complexity of trophic interactions on a local scale.

Keywords: North-east Atlantic, DNA molecular analysis, diet composition, benthic invertebrates, crustacean decapods, trophic ecology.

1. Introduction

Trophic interactions are based on a large number of ecological dynamics such as community structure, predator-prey relationships as well as species functional roles (Duffy 2002; Schmitz 2007; Schmitz 2017). These interactions participate in the formation of complex and more or less stable networks (Montoya et al. 2006). In a context where the pressures on marine ecosystems are multiple and act simultaneously (Beauchesne et al. 2021), the potential cumulative effects on food web dynamics are particularly difficult to predict and estimate (Crain et al. 2008; Côté et al. 2016; Lewis et al. 2022), in particular as biotic interactions are often poorly resolved (e.g. among benthic crustaceans or within scavenger communities) (Boudreau and Worm 2012; Depestele et al. 2019). Thus there is a need to better characterize biotic interactions, including predator-prey relationships (Worm and Duffy 2003). Understanding species' dietary profiles and their ecological implications has become essential for ecosystem-based management (Jennings and Kaiser 1998; Tam et al. 2017). While extensive research has focused on the dietary strategies of key consumers, particularly within exploited fish communities (Berry et al. 2015) and large apex predators (Machovsky-Capuska and Raubenheimer 2020), the role of invertebrates such as decapod crustaceans which can represent an important part of the biomass of marine ecosystems remains less understood (Boudreau and Worm 2012).

Crustaceans typically play a structural role in marine ecosystems through their intermediate position between benthic and pelagic compartment (Boudreau and Worm 2012; Szaniawska 2018). In food webs, they may play a key role, both as important prey for diverse vertebrate and invertebrate species (Boudreau and Worm 2012; Szaniawska 2018), and as predators exerting a significant structural influence on species composition (Reis 1979; Raffaelli et al. 1989). In addition, crustaceans may influence ecological dynamics including predator-prey interactions through various behaviours such as their burrowing or engineering activities (Martinetto et al. 2005). Indeed, by

modifying the habitat structure such as in the case of the colonization of the tubiculous *Haploops sp.* amphipods (Chaalali et al. 2017), or even the turbidity through burying in the soft substrate (Macia et al. 2003), crustaceans could directly or indirectly influence predator-prey interactions among other species (Martinetto et al. 2005). Most of decapod crustaceans are known to be omnivorous, and feed on a variety of preys such as molluscs, crustaceans, echinoderms, polychaetes, or algae depending on the natural and anthropogenic drivers in the local environment (Boudreau and Worm 2012). Although crustaceans exhibit a wide range of feeding strategies and behaviours, most species are also facultative scavengers, feeding on naturally dead organisms or even on fishing discards (i.e. bycatches returned to the sea) (Boudreau and Worm 2012; Lejeune et al. 2023). However, the importance of such scavenging behaviour in marine food webs may frequently be overlooked, especially with respect to the taxonomic and trophic diversity of species which may exploit fishing discards (Wilson and Wolkovich 2011; Lejeune et al. 2023).

The diet composition of a consumer is usually explored using traditional gut content observations. Nevertheless, gut content analysis may be much more demanding for small predators, where a plethora of prey items may be present, with only a small fraction recognizable due to digestion or destruction by prey capture mechanism and trophic structures (Symondson 2002; de Sousa et al. 2019). Indeed, crustaceans tend to macerate and crush their prey (Zipser and Vermeij 1978; Gibson et al. 1995; Asahida et al. 1997). As a result, conventional gut content analysis is unlikely to provide sufficient levels of prey detectability in crustaceans, especially for soft body preys (Symondson 2002). As an alternative, DNA metabarcoding of gut content (i.e. the identification of multiple prey taxa in the gut of consumers using New Generation Sequencing combined with DNA barcoding) is promising as it can provide a high taxonomic resolution of prey determination and high processing speed (da Silva et al. 2019). In this regard, metabarcoding has proven to be a powerful tool for studying the diet composition of a variety of marine consumer

species (Berry et al. 2015; van der Reis et al. 2020; de Jonge et al. 2021; Tamara et al. 2023; Shum et al. 2023).

By using the DNA metabarcoding method on gut contents, the present study aims to investigate the detailed dietary composition of six common crustacean species in the Bay of Biscay (Northeast Atlantic): the edible crab *Cancer pagurus*, the velvet crab *Necora puber*, the circular crab *Atelecyclus undecimdentatus*, the spider crab *Maja brachydactyla*, the shrimp *Crangon crangon*, and the hermit crab *Pagurus bernhardus*. We focus specifically on the animal fraction of the diet of these crustaceans in order to better understand their role as predators; an aspect of their ecology for which data is limited in terms of resolution and ecosystem effect (Boudreau and Worm 2012). Beyond the objective of filling a knowledge gap of the diet of these species by providing a detailed description of the latter, the present study aims to highlight their trophic niche relationships.

2. Materials and methods

2.1 Data collection

Data were acquired in April 2019 in the Bay of Bourgneuf (Bay of Biscay, France, Northeast Atlantic) which is a sheltered ecosystem supplied with organic matter led by the Loire river. The bay of Bourgneuf covers 320 km^2 and presents a variety of sedimentary structures from intertidal mudflat to patchy rock area (Méléder et al. 2007). Sampling took place from a 10.95 m long commercial trawler. Six species of crustaceans were collected; the edible crab *Cancer pagurus*, the velvet crab *Necora puber*, the circular crab *Atelecyclus undecimdentatus*, the spider crab *Maja brachydactyla*, the brown shrimp *Crangon crangon*, and the hermit crab *Pagurus bernhardus*. Individuals collected from the trawl were handled with sterilized gloves and immediately frozen on board to be dissected in a dedicated laboratory in sterile conditions (all surfaces, materials and equipments were sterilized

by bleaching and flame sterilization between each dissection). The digestive contents were scraped along the entire length of the digestive tract and homogenized to be dried and preserved in silica gel for metabarcoding analysis.

2.2 DNA extraction, amplification and sequencing

Extractions and Amplifications were conducted by Argaly SA (Sainte-Hélène-du-LacSavoie, France; https://www.argaly.com) in dedicated, separate rooms. DNA extraction was performed using NucleoSpin Soil kit (Macherey–Nagel, Düren, Germany) following the manufacturer's instructions (final volume of 0.5 mL). Two primer pairs were used for identifying animal prey; the generalist marker "Euka02" (TTTGTCTGSTTAATTSCG and CACAGACCTGTTATTGC; (Guardiola et al. 2016) which target the 18S rDNA region, and "Tele02" (AAACTCGTGCCAGCCACC and GGGTATCTAATCCCAGTTTG; Taberlet et al. 2018) which target mitochondrial 12S rDNA and is more specific to ingested fish. Concerning DNA amplification, each DNA extract underwent amplification in four separate PCR replicates for each marker. Replicates were differentiated by incorporating unique eight-nucleotide tags at the 5′ end of both forward and reverse primers. These tags, featuring at least five nucleotide differences (Coissac 2012), were supplemented with an extra two to four random nucleotides for increased sequence diversity and detection of clusters. PCR amplification were performed with a reaction mixture of 2 μL of DNA extract, 0.4 μmol L^{-1} of each primer, 10 μL of AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, California, USA) and 0.16 μL of 20 mg mL^{-1} bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland) in a 20-μL volume. PCR profile included denaturation, hybridization and elongation steps detailed in Lejeune et al. (2022). PCR products were purified using the MinElute PCR purification kit (Qiagen, Hilden, Germany), and gel electrophoresis was performed using the E-Gel Power Snap system (Invitrogen, Carlsbad, California, USA) to assess the success of amplification and purification. Preparation of the library and sequencing were done by Fasteris SA (https://www.fasteris.com/dna/). Two libraries were constructed for each marker using the PCR-free

MetaFast protocol of Taberlet et al. (2018). All amplicons were sequenced on a NextSeq 500 sequencing platform (Illumina, San Diego, California, USA), using the rapid run mode generating 250 bp paired-end sequences. To control for potential contaminations and false positives caused by tag jumps (Schnell et al. 2015), we included negative controls at the extraction step (one blank by extraction batch) and at the PCR step (one or two blanks by PCR plate, along with 12 unused tag combinations by PCR plate).

2.3 Bioinformatic sequence analysis

For each marker, raw sequence reads were analyzed using version 2 of the OBITools (https://git.metabarcoding.org/obitools/obitools; Boyer et al. 2016). For each sequencing library, paired-end reads were first assembled with the program *illuminapairedend*, considering a quality score > 40, which corresponds to an exact overlap of paired-end reads of at least 10 bases. The retained sequences were then assigned to the corresponding PCR replicate using the program *ngsfilter*, by allowing two and zero mismatches on primers and tags, respectively. At this stage, the two files obtained for each marker were concatenated before dereplication using *obiuniq*. Bad-quality sequences (i.e. containing undefined nucleotides), sequences shorter than the minimum amplicon length observed in *silico* (< 26 bp for Euka02 and < 85 bp for Tele02, without primers), and sequences observed only once in the entire dataset were discarded in a first round of filtering. A 97% clustering was applied to the remaining sequences using the *sumaclust* program (Mercier et al. 2013), to obtain Molecular Operational Taxonomic Units (mOTUs). The most abundant sequence within a cluster was chosen as the mOTU representative of the cluster. Only mOTUs observed at least ten times in at least one PCR replicate were retained for the remainder of the analyses.

Two different reference databases of full-length metabarcode sequences were used for taxonomic assignment of the mOTUs observed for each marker. A local reference database, containing metabarcode sequences obtained for the purpose of this study with the Euka02 or Tele02 primer pair

on 24 and 21 tissue samples, respectively. A second global reference database, containing the same set of sequences as well as metabarcode sequences extracted from the public sequence database GenBank (version 249) with the *ecoPCR* program (Ficetola et al. 2010). *EcoPCR* conducted an in *silico* PCR on GenBank sequences using either the Euka02 or Tele02 primers, allowing for up to three mismatches per primer. The resulting sequences were then curated to retain only those with a taxonomic assignment at the family, genus, and species levels, which were then used to build the reference database. For each marker, taxonomic assignment of each mOTU was performed twice, once with the local reference database, and once with the global database, using the *ecotag* program of the OBITools. The final taxonomic assignment retained for the ecological analyses was that obtained with the global reference database, unless there was a strict match (100% sequence identity) between the query mOTU and the local reference.

2.4 R Data filtering

After taxonomic assignment, additional data filtering was conducted in R (R Core Team 2020) with the *metabaR* package (Zinger et al. 2021), to remove artifactual or contaminant mOTUs that are common in metabarcoding datasets, and that may bias ecological conclusions drawn from such data (Calderón-Sanou et al. 2019). Three types of mOTUs were sequentially excluded from the datasets: (1) mOTUs with a best identity with GenBank metabarcodes < 85% for Euka02 or < 95% for Tele02, as they are likely to be sequence chimeras since they are too divergent; (2) mOTUs that were more numerous in the negative control replicates than in true PCR replicates (*contaslayer* function with the "max" method), as they most likely contaminants; and (3) mOTUs with a relative frequency within a PCR replicate < 0.5% of that over the entire dataset (*tagjumpslayer* function), as these are likely to be "tag jumps" (Schnell et al. 2015). In addition, mOTUs assigned to non-metazoan taxa (Euka02) and remaining mammalian mOTUs (Euka02 and Tele02) were discarded. PCR replicates with less than 100 sequences were also eliminated, before aggregation of replicates belonging to the

same sample with the *aggregate_pcrs* function. Finally, the abundance of mOTUs observed less than 10 times per sample was set to zero.

After removal of sequences not pertinent to this study, 18 947 327 number of reads were assigned to a PCR replicate for Euka02 and 11 829 818 for Tele02 (see details of the remaining total read counts following the main filtering steps in **Table S1**). Self-hits have been removed from the dataset beforehand (*Atelecyclus undecimdentatus;* 117 633 ± 65 460, *Crangon crangon;* 67 677 ± 60 540, *Pagurus bernhardus;* 73 930 ± 70 727) with a remaining total number of sequences of 5 060 768 for Euka02 and 2 572 212 for Tele02. Variation in total reads abundance among the different crustacean species are presented for each marker in **Table S2**. Rarefaction curves showed that the sequencing depth was sufficient for all but 6 individuals belonging to the species *A. undecimdentatus* (Euka02) (Online resource, **Fig. S1**). Thus, these 6 samples were removed for further analysis. After these curation steps, each resulting dataset containing mOTUs raw read abundances (one for Euka02 and one for Tele02) were first expressed as relative read abundances and then converted to presence/absence data. To normalize detection across samples with different sequencing depths and avoid rare-item inflation that can bias diet estimation, we used a threshold of minimum sequence abundance to consider a mOTU as an occurrence. The threshold was set at ≥ 1% of the total number of sequences amplified in the gut content (i.e., for each individual's gut content, mOTUs representing < 1% of the total number of sequences amplified were removed; Deagle et al. 2019). Finally, both molecular marker datasets were merged to form a consensus diet following da Silva et al. (2019). The consensus diet contained a total of 54 identified mOTUs across all samples.

2.5 Diet analysis

Pagurus bernhardus had 1 empty digestive tract that was removed from the dataset for further analysis (Vacuity rate: 7 %). For both markers, a total of 93 digestive tracts were taken for analysis, including 93 digestive tracts for Euka02 marker (*Cancer pagurus;* n = 17, *Necora puber;* n = 16,

Atelecyclus undecimdentatus; n = 14, *Maja brachydactyla;* n = 20, *Crangon crangon;* n = 13, and *P. bernhardus;* n = 13) and 50 digestive tracts for Tele02 marker (*C. pagurus;* n = 12, *N. puber;* n = 10, *A. undecimdentatus;* n = 9, *M. brachydactyla;* n = 4, *C. crangon;* n = 12, and *P. bernhardus;* n = 3).

The average difference of mOTU richness between consumers was tested using non-parametric Kruskal-Wallis test followed by post-hoc Pairwise Wilcoxon-Mann-Whitney test. Niche breadth was estimated for each consumer through the calculation of the Normalized Shannon Index (NSI) (Shannon 1948; Colwell and Futuyma 1971). This index varies between 0 (i.e. fully specialist diet) and 1 (i.e. fully generalist diet). For this index, prey occurrences were first normalized by calculating their relative frequencies (occurrence is divided by the sum of occurrences of each prey taxa over the total number of prey). Normalized Shannon Index is calculated as follow:

$$h_i = -(\ln N)^{-1} \sum_{j=1,N} Foccu_{i,j} \ln(Foccu_{i,j})$$

Where N is the total number of prey taxa groups and $Foccu_{i,j}$ is the frequency of occurrence of prey taxon j in the diet of consumers species i.

Prevalence of mOTU in the gut content of each consumer was assessed by calculating the frequencies of occurrence in percentage. It was computed for each consumer as the number of occurrences of a mOTU divided by the total number of gut contents sampled for that consumer species. Frequencies of occurrence were then normalized by the sum of occurrence frequencies for each consumer. Nonmetric multidimensional scaling (nMDS) was performed on the Sørensen-Dice distance matrix to visualize the niche breadth and potential overlapping of the different consumers. For this analysis, mOTUs were grouped into categories of prey that combine (1) the position in the water column (benthic, pelagic or demersal), as obtained from the fishbase (Froese and Pauly 2023), SeaLifeBase (Palomares and Pauly 2023), WoRMS databases (WoRMS Editorial Board 2023), and (2) the phylum to which they belong. Prey categories and taxonomic classification of each mOTU are available in Online resource **Table S3**. To fix the issue of undefined resemblances in samples

with complete absences, a dummy variable was added to each observation. This operation involves the addition of a column (dummy prey) with identical value across all sampled guts (+1) in order to regularize distances when samples have only one or no observations (Clarke et al. 2006). A permutational analysis of multivariate dispersion (PERMDISP) was conducted to evaluate if the dispersion between groups was homogeneous before the analysis of variance (Anderson et al. 2006). Permutational Multivariate analysis of variance (PERMANOVA) with 999 permutations was performed to test the difference of diet composition between consumers. nMDS, PERMANOVA and PERMDISP analyses were conducted in R (Version 4.3.2) using the vegan package (Oksanen et al. 2022). Finally, a Pairwise adonis test based on Euclidian distance with Holm correction was used to determine which consumers' niches are significantly different from the others using the pairwiseAdonis package (Martinez Arbizu 2020).

3. Results

In total, 54 mOTUs were identified in the gut contents of the six crustaceans studied. Kruskal-Wallis test indicated a difference in the average mOTU richness among the six consumers (n = 93, H (5) = 22.34, $p < 0.0005$). *Crangon crangon* had the highest mean mOTUs richness (mean ± SD = 7.8 ± 4.3) whereas *Maja brachydactyla* had the lowest mOTU richness (2.8 ± 1.9). Pairwise Wilcoxon-Mann-Whitney tests confirmed that mOTU richness in *M. brachydactyla* guts was significantly inferior to that of *C. crangon* ($p = 0.004$), and inferior to that of *Cancer pagurus* (p = 0.05). Normalized Shannon Index (NSI) showed a similar trend with the highest value found for *C. crangon* (0.66), indicating that this species is more generalist, and a lowest NSI (0.28) for *M. brachydactyla*, indicating a more specialized diet. The NSI values of *N. puber*, *P. bernhardus, A. undecimdentatus* and *C. pagurus* were intermediate and highly similar, ranging from 0.51 to 0.58.

Overall, analyses of the relative frequencies of occurrence (%) of the different prey indicate that arthropods and chordates were the major prey group across all consumers (**Fig. 1a**). Concerning

the fish fraction in diets, all consumers ingested a significant proportion of teleosts fish (**Fig. 1b**). The most important contribution of fish in crustacean's diet is observed for *Crangon crangon* (79.77 % of teleosts), mostly composed of Eupercaria (26.97 %) and Gadiforms (10.11 %), as well as Mugiliformes (8.99 %) and Pleuronectiformes (7.87 %) to a lesser extent (Online Resource, **Table S4**). Among these prey, the most representative prey for *C. crangon* is *Dicentrarchus labrax* (10.11 %). Teleosts also represent a considerable proportion of the diet of others consumers; *Atelecyclus undecimdentatus* (53.22 %), *Cancer pagurus* (50.86 %), *Necora puber* (49.99 %), *Maja brachydactyla* (22.24 %) and *Pagurus bernhardus* (20.70 %). Across these diets, gadiforms are the most represented prey; *Merluccius merluccius, Pollachius pollachius* and *Trisopterus luscus* probably originating from fisheries discards (Online Resource, **Table S4**). Other fish mOTUs globally represent various proportions of the consumer's diet, such as *Salmo trutta* in the diet of *N. puber* (9.09 %), *Conger conger* in the diet of *A. undecimdentatus* and *C. pagurus* (8.51 % and 7.02 % respectively), or *Sprattus sprattus*, *Belone belone* and *C. conger* in the diet of *P. bernhardus* (3.45 % each).

With the exception of *C. crangon* for which diet is mainly composed of teleosts fishes and copepods, other consumers ingested an important proportion of malacostracans (**Fig. 1b**); *A. undecimdentatus* (25.54 %), *P. bernhardus* (27.59 %), *C. pagurus* (29.81 %), *N. puber* (38.63 %), and *M. brachydactyla* (52.78 %). Concerning *M. brachydactyla*, diet is dominated by the circular crab *A. undecimdentatus* (52.78 %), which also represents a substantial part of the diet of the edible crab *C. pagurus* (24.56 %) and the velvet crab *N. puber* (34.09 %) and was often discarded from local fisheries at the time of sampling (Online Resource, **Table S4**). The Malacostraca fraction of the diet of *A. undecimdentatus* and *P. bernhardus* is mainly composed of the sub-order Pleocyemata (23.41 % and 20.69 % respectively), but cannot be identified at a more precise level. Finally, *P. bernhardus*'s diet is made up of a wider variety of prey, including copepods (27.60 %) and gastropods (10.35 %).
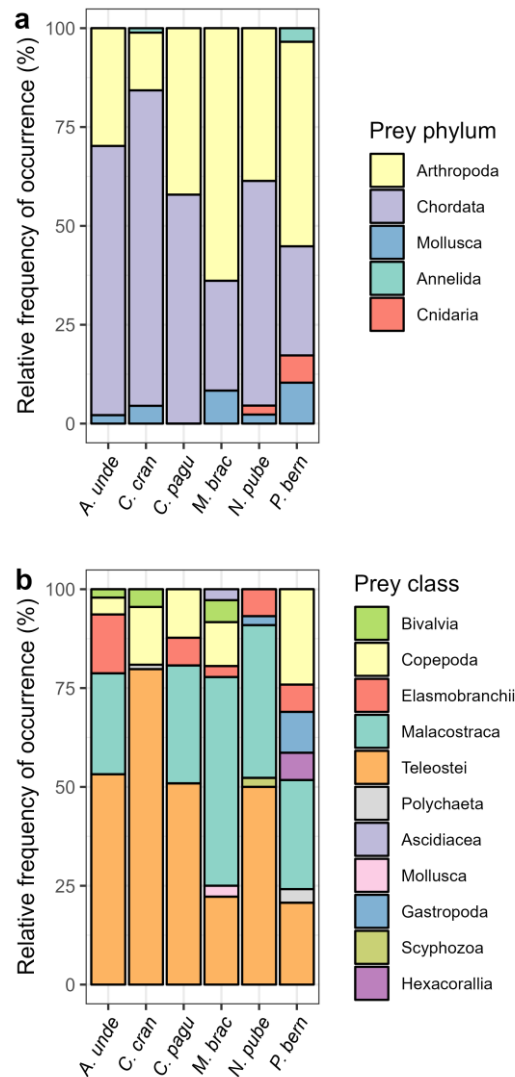
Fig. 1 Relative frequency of occurrence (%) of the different prey identified in the gut contents of six crustaceans. Prey are grouped at the phylum (**a**) and class (including subclass or infraclass) level (**b**). The x-axis labels correspond to the consumers; *A. unde*: *Atelecyclus undecimdentatus* (n = 14), *C. cran*: *Crangon crangon* (n = 13), *C. pagu*: *Cancer pagurus* (n = 17), *M. brac*: *Maja brachydactyla* (n = 20), *N. pube*: *Necora puber* (n = 16), *P. bern*: *Pagurus bernhardus* (n = 13).

nMDS revealed that the trophic niche of *Crangon crangon* is clearly different from that of *Maja brachydactyla* and *Necora puber* on MDS1 (**Fig. 2**). While the trophic niche of *C. crangon* is mostly influenced by the consumption of demersal and pelagic fish, the trophic niches of *M. brachydactyla* and *N. puber* are mainly influenced by benthic arthropods consumption, as well as

benthic chordates, molluscs and pelagic cnidarians. *M. brachydactyla, N. puber* and *C. pagurus* share similar trophic niches by mostly preying on benthic organisms. *Pagurus bernhardus*' niche position is intermediate, between *C. crangon* and *M. brachydactyla*. Niche location of *P. bernhardus* highlighted its relationship with the benthic cnidarians represented by the Hormathiidae family. The trophic niche of *C. pagurus* is large and overlaps the ones of *M. brachydactyla* and *N. puber*, as well as *P. bernhardus*. Segregation between *A. undecimdentatus* and *C. crangon* niches is noticeable along MDS2 axis. *A. undecimdentatus* seems to be closer to pelagic arthropods prey while *C. crangon* more related to prey belonging to the pelagic and dermersal chordates category. Graphic observations are confirmed by the PERMANOVA since at least one consumer's dietary niche differs from others (PERMANOVA; Pseudo-$F_{5,92}$ = 11.31, R^2 = 0.39, p = 0.001). Pairwise Adonis post-hoc test indicated significant trophic niche differences among all consumers (p <= 0.05) except between *M. brachydatyla* and *N. puber* (p = 0.096) and between *C. pagurus* and *N. puber* (p = 0.096) which share similar niche position in the multivariate space (Online resource, **Table S5**).
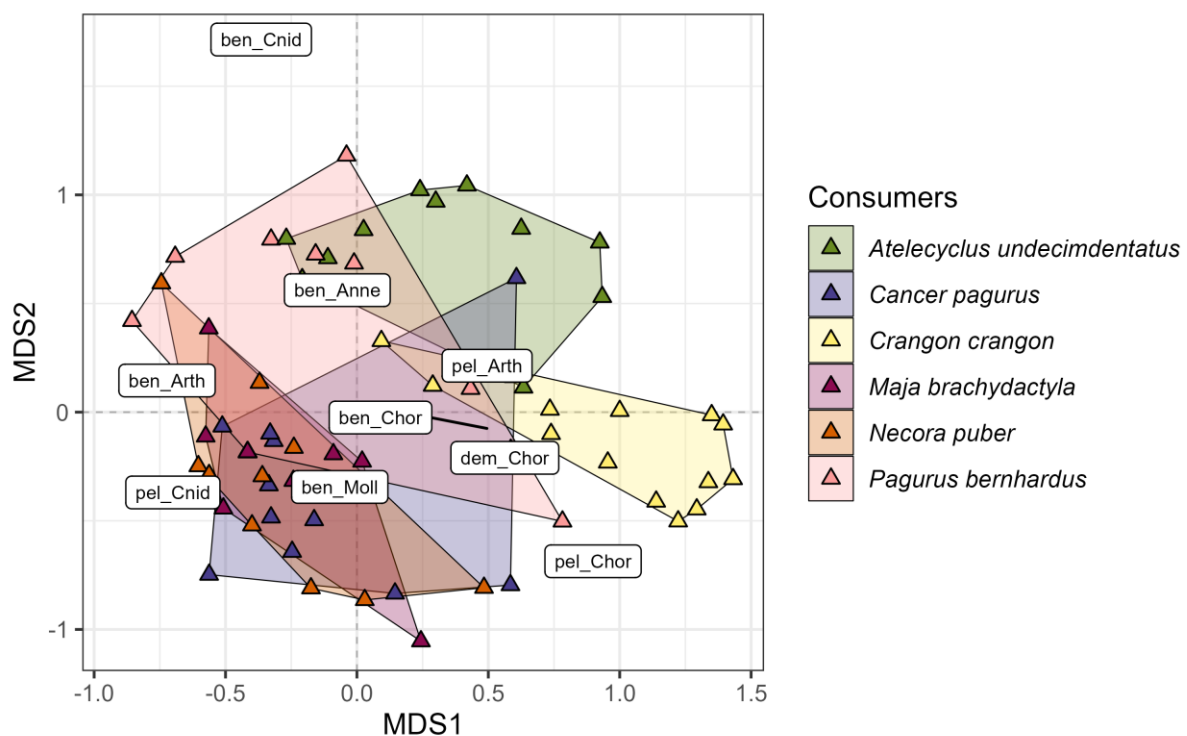
Fig. 2 Two-dimensional ordination of non-metric multidimensional scaling (nMDS) based on

Sørensen-Dice distance matrix. Colored polygon represents the niche breadth of the six consumers,

triangle represents the digestive tracts, and label corresponds to the following prey categories;

ben_Anne: benthic annelids, ben_Arth: benthic arthropods, ben_Chor: benthic chordates, ben_Cnid:

benthic cnidarians, ben_Moll: benthic mollusc, dem_Chor: demersal chordates, pel_Arth: pelagic

arthropods, pel_Chor: pelagic chordates, pel_Cnid: pelagic cnidarians.

4. Discussion

Despite numerous studies investigating the dietary habits of decapod crustaceans using

traditional methods (Williams 1981; Choy 1986; Raffaelli et al. 1989; Ansell et al. 1999; Chartosia et

al. 2010), metabarcoding offers the potential for a more in-depth analysis by providing a high-level

of detection for the typically wide range of prey items characterizing the diet of such omnivorous

species (de Sousa et al. 2019). Indeed, gut content metabarcoding enables unprecedented taxonomic

resolution as compared to traditionally used morphological identification of gut contents, which are

often hindered by the state of prey alteration in guts (prey items may be crushed or digested beyond

recognition, especially soft-bodies) (Baker et al. 2014; Buckland et al. 2017). However, this

advantage in terms of resolution comes with a main limitation, which is the difficulty to go beyond

occurrences with DNA based methods. Indeed, presence/absence approach are less affected by

recovery bias (i.e. the fact that some DNA sequences are more easily recovered and amplified than

others) than relative read abundance approach (Deagle et al. 2019). In this study, we employed an

occurrence approach in a bid to minimize potential biases while maximizing biological information

accuracy (Elbrecht and Leese 2015). We focused on the animal fraction of the diet of the studied

crustaceans in order to better understand their role as predators; an aspect of their ecology for which

data is limited in terms of resolution and ecosystem effect (Boudreau and Worm 2012). Here we

expanded knowledge of the diets of six consumers, particularly *Atelecyclus undecimdentatus* for

which the diet was previously unknown. The results highlighted the importance of scavenging behaviour, mainly based on the ingestion of fishes and malacostracans with a differential level of opportunism depending on various intra- and inter-specific mechanisms.

Concerning the dietary profile of consumers, prior studies indicated that decapod crabs are omnivorous predators that favor macroinvertebrates such as crustaceans and mollusks (Shelton et al. 1979; Choy 1986; Chartosia et al. 2010; Boudreau and Worm 2012). Across the studied crabs, *Cancer pagurus*, *Necora puber*, and *Maja brachydactyla*, our results on the animal fraction of the diet highlight an important consumption of malacostracans, dominated by *A. undecimdentatus*. Contrary to earlier studies suggesting a generalist diet for *M. brachydactyla*, involving various low-mobility benthic invertebrates (Števčić 1967; Bernárdez et al. 2000), our results reveal that *M. brachydactyla* displays a more specialized diet than the other five crustaceans. Its diet is disproportionately influenced by circular crab *A. undecimdentatus* consumption relative to others, but this could potentially result from the influence of local factors such as a high abundance of circular crabs (dead or alive, further discussed below) and opportunistic feeding. While it has been possible to identify the preferred prey of *M. brachydactyla* at the taxonomic level of the species, the identification of the arthropod preys of *A. undecimdentatus* and *Pagurus bernhardus* is compromised by limited taxonomic resolution. Despite the broad coverage of prey provided by the 18S rDNA region, precise identification is not always possible. The use of the complementary Tele02 marker (12S region) enhances fish identification, but incorporating additional markers specific to arthropods such as IN16STK (16S region) or ZBJ (COI region) could further improve taxonomic resolution in arthropod prey (da Silva et al. 2019). It is also essential to acknowledge that, although cannibalism is common among many groups of decapod crustaceans (Romano and Zeng 2017), metabarcoding is not suitable for estimating it as the recovered genetic sequences can originate from both the degradation of consumer cells and ingested prey (Taberlet et al. 2018; Casey et al. 2019). Therefore, identifying the consumer DNA within its own gut does not necessarily imply cannibalism, but is in

fact likely to result from the consumer's own cell degradation. For this reason, "self-hits" (i.e. mOTUs with the same identity as the consumer in which they were identified) were removed from the metabarcoding dataset along the data curation steps, as cannibalism cannot be studied or taken into account on the basis of these results.

Recognized as highly opportunistic (Lancaster 1988), *Pagurus bernhardus* targets various prey (i.e. diatoms, foraminifera, small crustaceans, polychaetes or bivalve) (Orton 1927; Lancaster 1988). The opportunistic nature of this species is reflected in our results concerning the animal fraction of the diet since it includes a wide diversity of prey including copepods, gastropods, malacostracans and teleost fishes. While the consumption of microscopic prey item such as copepods is consistent with the fact that this species has a feeble mouth part and a filter feeding activity (Gerlach et al. 1976; Lancaster 1988), malacostracans and teleosts are less documented prey items. The presence of benthic cnidarians in the diet of *P. bernhardus* represented by Hormathiidae anemones is likely due to their symbiotic relationship (Gusmão and Daly 2010). Concerning *Crangon crangon*, its generalist diet encompasses copepods and teleosts, consistent with Siegenthaler et al. (2019) metabarcoding findings, and diverging from earlier traditional gut content analyses that did not detect fish as principal prey (Raffaelli et al. 1989; Ansell et al. 1999; Oh et al. 2001). Overall, high consumption of teleost fishes was observed for most consumers ranging from 79.77 % of the diet of *C. crangon* to 20.70 % of the diet of *P. bernhardus*. This proportion of fish in the diet of all consumers is higher in our study compared to gut content method (Shelton et al. 1979; Choy 1986; Raffaelli et al. 1989; Bernárdez et al. 2000; Oh et al. 2001; Mascaró and Seed 2001), but consistent with other studies using baits and cameras documenting fish consumption across a wide variety of decapods (Ramsay et al. 1997; Groenewold 2000; Lejeune et al. 2023).

The importance of fish in the diet of the different crustaceans may be linked to the fact that sampling was conducted on a fishing ground. The Northeast Atlantic ocean is recognized as a 'discard hotspot' where discards can constitute up to 50 % of the catch in some fisheries, e.g. in the

Nephrops fishery in the Bay of Biscay (Cornou et al. 2021). The bay of Bourgneuf is extensively exploited by fisheries and is subjected to discarding practices (Cornou et al. 2021), which might significantly influence food web dynamics in some fishing areas (Lejeune et al. 2022). Discards are due to target species quota or minimum size restrictions, but also to by-catches of non-target species (Catchpole et al. 2005). Across all studied consumers, teleost fishes represent a substantial part of the diet. Ingested fish are mainly represented by gadiforms, especially hake *Merluccius merluccius,* pouting *Trisopterus luscus* and pollack *Pollachius pollachius*, as well as Eupercaria represented by seabass *Dicentrarchus labrax,* gilt-head seabream *Sparus aurata* and black seabream *Spondyliosoma cantharus*. Even if some decapods are considered as active predators which can prey on more than one trophic level (Boudreau and Worm 2012), decapods are unlikely to feed on large living fish whether in terms of size and accessibility (Choy 1986). As hake and pouting are common target species in Bay of Biscay and exhibit a reduced capacity to resist air exposure when discarded alive (Morfin et al. 2017; Tsagarakis et al. 2018), it is likely that the consumers fed on damaged fishing discards. On the other hand, other species such as seabass, which have a high survival rate when discarded (Lamb et al. 2022), are frequently eviscerated onboard fishing vessels and are therefore probably consumed as decomposed discards. Other species such as the circular crab are also heavily captured and discarded as by-catch in the study area. This latter species shows a higher vulnerability to trawling and handling compared to other crustaceans such as spider crab (Boussarie et al. 2020). Thus, given its significant contribution to the diet of *Maja brachydactyla* and, to a lesser extent, *Necora puber* and *Cancer pagurus*, circular crab likely serves as a preferred prey due to its accessibility and abundance. As a result, all the consumers may be more or less dependent on prey originating from fishing discards. Although fishing discards should decrease with fishery landing obligation policies (European Union 2013), they may often be found in larger quantities than benthic invertebrates can ingest after trawling (Depestele et al. 2019) and unevenly distributed in terms of space and frequency (Kopp et al. 2016). Furthermore, it's important to consider that despite a ban on

discarding, some species will continue to be eviscerated on board and therefore their viscera can still be discarded at sea (Lejeune et al. 2022). As well, by damaging sessile organisms, the passage of trawls could already provide a considerable source of food for scavengers (Jennings and Kaiser 1998). Nevertheless, an abrupt reduction in discards might destabilize predator-prey relationships by causing a shift in predation, increasing pressure on natural prey (Fondo et al. 2015; Kopp et al. 2016). While the effects of a discard ban could be very small on a large scale (Depestele et al. 2019), it is likely that on a finer scale and for certain species such as *Crangon crangon*, the effects of this measure will be more visible and may lead to cascading repercussions such as the modification of trophic levels or the increased competition on natural prey (Oro et al. 2013; Fondo et al. 2015). Lastly, it should be pointed out that metabarcoding provides a single snapshot of the diet, limiting the exploration of temporal variations in absence of repeated measurements (Casey et al. 2019). For instance, if sampling coincided with trawl activity, a higher prevalence of fishing discards might be observed depending on digestion time (McGaw and Curtis 2013).

Trophic niches are influenced by various intra and inter-specific parameters, including predator mobility, detection ability, metabolism associated with prey decomposition level or size, as well as biotic interactions (Depestele et al. 2019). As well opportunism level may play an important role in patterns of niche overlaps among consumers living in sympatry. Existing trophic niche differences among studied species may be linked to different predation strategies, functional morphology or specific abilities. Although high fish consumption across consumers might be linked to fishing discards as previously discussed, the importance of fish prey items might also be linked to the specificity of trophic ecology of certain consumers (i.e. morphological, behavioral, and physiological traits) (Schmitz 2017). As an example, the trophic niche of *Crangon crangon* stands out from other species because of its substantial consumption of demersal and pelagic fish. This could be explained by the diversity of strategy employed by crangonid shrimps encompassing ambush tactics such as burial and changes in coloration to feed on small mobile prey (Gibson et al.

1995; Siegenthaler et al. 2018). The ingestion of pelagic or demersal fish might also be linked to the predation on early life stage individuals, including larvae, as observed in the case of *Pleuronectes platessa* (van der Veer and Bergman 1987; Bailey 1994). As well, if we consider that these pelagic prey are really dead or damaged prey originated from fishing discards, crangonid shrimps could also implement strategies by aggregating around a carcass, outnumbering potential competitors (Depestele et al. 2019). On the other hand, hermit crab *Pagurus bernhardus* display a weaker ability to penetrate fish skin due to their chelae unsuitable for cutting (Ramsay et al. 1997), which may explain its lower fish consumption compared to other consumers. *P. bernhardus* is more likely to feed on carrion at a more advanced stage of decomposition than predating living fish (Ramsay et al. 1997), but because carrion are often quickly consumed (Lejeune et al. 2023), scavenging opportunities may be less frequent for *P. bernhardus*.

In addition to individual parameters, biotic interactions, and in particular competition, represent an important driver of trophic niche distribution (Depestele et al. 2019). The overlapping trophic niches of *Maja brachydactyla*, *Necora puber* and *Cancer pagurus* could indicate a potential competition for shared resources. These species are opportunistic scavengers (Norman and Jones 1992; Ramsay et al. 1997; Davenport et al. 2016; Lejeune et al. 2023), and intra and inter-specific competition is widespread across crabs which may be aggressive towards one another when gathering around the same prey carcass (Amaral et al. 2009; Davenport et al. 2016; Lejeune et al. 2023). Consequently, negative effects of competition (including the risk of death) when preying could also represent important drivers of trophic niche distribution (Depestele et al. 2019). This potential competition among consumers might also explain the high proportion of ingested Pleocyemata (decapod suborder) since the aggregation of consumers around a carcass increase the risk of being attacked/killed by a superior competitor (Kaiser et al. 1998; Davenport et al. 2016; Depestele et al. 2019). A complex interaction among individual parameters, biotic interactions, as well as the prey decomposition state shapes chronological successions of scavengers (Bergmann et

al. 2002; Jenkins et al. 2004; Davenport et al. 2016; Quaggiotto et al. 2016). In a context of discard consumption, while crangonid shrimps and portunid crabs might be the first to feed on carrion when it reaches the bottom (Davenport et al. 2016; Lejeune et al. 2023), the scraps they leave might then benefit to other scavengers such as *P. bernhardus* which may migrate to recently exploited areas (Ramsay et al. 1996; Bergmann et al. 2002; Davenport et al. 2016). In this study, the use of fish-specific primers highlighted the consumption of fishing discards. Nevertheless, since significant differences in the detectability of fish have already been observed when using fish-specific primers or universal primers (Siegenthaler et al. 2019b), other markers could be used in future studies to complete our knowledge of the animal fraction of the diets. Finally, characterisation of the fauna on the site, environmental parameters as well as the proportion of plant prey items could be included in future studies in order to complete our understanding of the global diet of these species.

5. Conclusion

Metabarcoding of gut contents provided a detailed description of the animal fraction of diet of several crustacean species, which allowed a better understanding of their role as predators and scavengers and is a first step towards a better understanding of their global diet and detailed links with the various food sources they may potentially consume as omnivores. The importance of fish or other decapods suggest that scavenging may be a significant energetic pathway, reinforcing recent views of the potentially underappreciated role of scavenging in the structuration of marine communities (Wilson and Wolkovich 2011; Beasley et al. 2012). Scavengers' communities (i.e. mostly benthic invertebrates) feed on multiple prey taxa and trophic levels (Boudreau and Worm 2012; Lejeune et al. 2023) and are consumed by a wide diversity of predators (Boudreau and Worm 2012). Hence, they may contribute to increasing the number and the heterogeneity of trophic links leading to greater stability of the trophic network (Rooney et al. 2006). While our results suggest the prevalence of opportunistic and scavenging feeding behaviours among decapod crustaceans, the

contribution of this functional role to community structure and food web dynamics in the study area remains to be defined.

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Declaration of interests

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Highlights

- The dietary profiles of six crustaceans were studied.
- DNA metabarcoding on gut content provides an accurate detection of prey items.
- Teleost fishes and malacostracans were the main prey items across the consumers.
- *Necora puber* trophic niche is common to *Maja brachydactyla* and *Cancer pagurus* ones.
- Scavenging is a significant energetic pathway that probably structure communities.