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# Integrating Dietary Characterisation and Gut Microbiome Analysis in Norway Lobster (*Nephrops norvegicus*) Through Gut-Isotope-DNA (GID) Approach

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**Abstract**

The Norway lobster (*Nephrops norvegicus*) is an ecologically and commercially important species in the Mediterranean Sea and the North-East Atlantic. Therefore, studies on its trophic ecology are particularly relevant also for the purpose of sustainable management. This work aims to characterise, to the authors' knowledge for the first time in a crustacean, both gut microbial composition and feeding ecology through Gut-Isotope-DNA (GID) approach, which integrates gut content, stable isotopes and DNA metabarcoding analyses. Specimens were collected inside and outside the Pomo Pits Fishery Restricted Area (Adriatic Sea) in 2019 and 2023, in order to assess the spatial and temporal variability in diet and microbiome. Visually identified prey were dominated by the squat lobster *Iridonida speciosa*, and this was also confirmed through isotopic mixing models. However, DNA metabarcoding allowed a more exhaustive reconstruction of the mixed scavenger-predator behaviour of Norway lobster, with a higher dietary diversity, and greater contribution from finfish, recorded in 2019 than 2023, both inside and outside the Pomo Pits. Gut microbiome composition followed these trophic patterns, with significantly higher microbial diversity in specimens collected outside the Pits. The microbiome was dominated by Vibrionaceae, Erysipelotrichaceae and Mycoplasmataceae, and was able to distinguish both sampling area and years more effectively than strictly dietary analyses. While dietary composition and gut microbiome structure in Norway lobster appeared consistent, each technique homes in on different biological phenomena, requiring tailored interpretations and careful consideration when integrating evidence for the purpose of management.

**Keywords** DNA metabarcoding · GID approach · Gut microbiome · *Nephrops norvegicus* · Stable isotopes · Trophic ecology

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## Introduction

The Norway lobster (*Nephrops norvegicus* Linnaeus, 1758) is a burrowing decapod crustacean that inhabits the continental shelf of the northeast Atlantic Ocean and the Mediterranean Sea, typically found at depths ranging from 50 to 800 m [1]. This species holds significant commercial value throughout its distribution [1–3]. Within the Mediterranean Sea, the Adriatic Sea represents a key fishing ground for Norway lobster, although catches in the central-northern Adriatic have been declining since the early 2000 s [4]. In this area, the species primarily occurs at depths between 50 and 400 m, showing a preference for muddy substrates [5–8]. Several studies on Norway lobster in the Adriatic Sea have revealed regional variations in growth patterns and other biological traits [8–11]. Such spatial heterogeneity deserves further investigation through the lens of trophic ecology. The Pomo Pits area, in the central Adriatic Sea, has been identified as a key habitat for both spawning activities and nursery [7, 12, 13]. The peculiar hydrographic conditions characterising the area influence nutrient cycling, with consequences on trophic status of benthic communities [14]. Furthermore, fishing pressure and climate change could have modified local biodiversity [15–17] and potentially altered the trophic and ecosystem status [18–20]. Norway lobster is considered an euryphagous (i.e., characterised by a broad and generalist diet) and non-selective feeder, consuming a wide range of benthic and demersal prey, including crustaceans, fish, and molluscs, both through active predation and scavenging [1, 21–23].

Traditionally, studies on trophic ecology have relied on Gut Content Analysis (GCA) via stereoscopic analysis; while valuable for identifying consumed items and understanding diet, this method presents significant taxonomic limitations, particularly when prey items are highly digested [24]. In recent decades, the combination of stable isotope analysis (SIA) with GCA has greatly improved the understanding of prey contributions to diet composition [23, 25, 26]. The subsequent advent of DNA metabarcoding of gut contents provided higher taxonomic resolution, overcoming many of the constraints associated with prey recognition in GCA [27, 28]. The combination of these three methodologies, hereafter referred to as the GID (Gut, Isotope, DNA) approach offers a comprehensive and detailed perspective on trophic ecology. A similar approach has been tested with excellent results in the Red Sea and Atlantic Patagonia on reef fish and green crab respectively [29, 30]. Furthermore, in this study, DNA metabarcoding was applied to both diet reconstruction and the characterisation of the intestinal microbiome, representing, to the authors' knowledge, the first integrated application of this approach in a crustacean species.

The gut microbiome of decapod crustaceans plays a crucial role in host physiology, immunity, and nutrition [31], yet remains underexplored compared to that of vertebrates [32]. Meta-analyses across decapod taxa have revealed habitat-dependent core bacterial genera, with distinct compositional patterns across freshwater, marine, and deep-sea habitats. *Vibrio*, which is ubiquitous in marine environments and commonly found in marine decapod guts, illustrates how environmental conditions and dietary inputs jointly shape the gut microbiome [33–35]. Microbial communities contribute to the digestion of complex substrates, pathogen resistance, and metabolic homeostasis, underscoring their ecological relevance in both wild and cultured crustaceans [32, 36]. However, evidence that links trophic ecology with gut microbiome structure in wild decapod populations remains limited. Most recent gut microbiota studies in decapods have focused on cultured species, including some penaeid shrimps [37, 38]. In Norway lobsters, temporal variability has been documented in both feeding ecology and gut bacterial communities. Seasonal changes in the consumption of major prey, particularly decapod crustaceans and fish, have been observed in the Adriatic Sea and were associated with reproductive-related changes in energetic requirements [23]. Similarly, marked temporal shifts in gut bacterial composition have been reported, these changes were suggested to reflect temporal variation in food supply [39]. Experimental studies have further demonstrated that feeding conditions can alter gut bacterial diversity and select for specific microbial taxa in farmed Norway lobster [32]. Overall, these findings suggest that temporal changes in food availability and feeding habits may be accompanied by changes in gut microbiome composition, highlighting the importance of considering temporal variability when studying trophic-microbiome relationships in Norway lobster populations. More recent

work has further supported the existence of geographic differentiation in the gut microbiome of Norway lobsters [35].

Examining feeding ecology provides valuable insights into the species' behaviour and associated microbial communities, allowing the investigation of potential links between dietary composition and gut microbial biodiversity in different environmental contexts. The Pomo Pits area provides a valuable environment for investigating variations in diet composition and gut microbiome. This area encompasses differences in depth and fishing pressure. This further spatial complexity offers the opportunity to delve deeper into feeding habits, as well as the structure of the gut microbial community.

The integration of the information described above is particularly relevant in wild populations, where spatial and temporal fluctuations in prey availability are difficult to assess but heavily influence both foraging dynamics and gut microbial communities. In the Pomo Pits area, such an integrated framework may provide a more comprehensive assessment of the feeding ecology of a very relevant species. Furthermore, combining diet and microbiome data offers an opportunity to investigate whether shifting nutritional patterns are accompanied by a restructuring of the internal microbial community. To address this, the present study employs an integrated GID approach to characterize the trophic ecology and gut microbiota of the Norway lobster, tracking potential variations across two different time points within the region.

## Materials and Methods

### Study Area

The Pomo Pits area consists of three depressions delineated by the 200 m bathymetric contour, in the central Adriatic Sea. The area's complex topography, combined with the oceanographic dynamics of the Adriatic Sea, creates a unique environmental setting where the water remains for periods longer than a year [40]. This area used to serve as one of the primary fishing grounds in the Adriatic, exploited by both Italian and Croatian fleets [5, 6]. The area is also the main nursery ground for *Merluccius merluccius* (Linnaeus, 1758; European hake) in the Adriatic Sea [41–43], and is characterised by fluctuations in *Parapenaeus longirostris* (Lucas, 1846; Deep-water rose shrimp) and *Micromesistius poutassou* (Risso, 1827; Blue whiting; [44, 45]). These species are of commercial relevance and can contribute to defining the trophic strategies of the Norway lobster. Additionally, since 2003, a species replacement occurred, with *Munida intermedia* (Milne-Edwards and Bouvier, 1899) supplanted by *Iridonida speciosa* (von Martens, 1878; [17, 46]). The presence of muddy seabed and other environmental factors creates an optimal habitat for Norway lobsters [1]. The Pomo Pits area is characterised by a subpopulation of Norway lobsters that reach maturity at relatively small sizes [9, 10, 45], such pattern may result from the specific hydrographic conditions of the site combined with sustained fishing pressure over time. Although growth differences have been reported in the area, genetic analyses indicate no significant differentiation between the Pomo Pits and other Adriatic populations, suggesting high gene flow [47]. The Pomo Pits area represents a vulnerable marine ecosystem and an essential fish habitat, and for this reason, a series of management measures were implemented over time [45, 48, 49], which led to the establishment, in 2017, of a Fishery Restricted Area (FRA; [50]). The Pomo Pits FRA became permanent with GFCM (General Fisheries Commission for the Mediterranean) Recommendation 44/2021 [51]. The FRA is divided into three zones: "Zone A", which is closed to all fishing activity, "Zone B" and "Zone C", where bottom trawling is regulated with licenses and number of fishing days allowed (Fig. 1). The European Parliament and Council ratified the FRA with Regulation 2019/982 [52]. The sampling area of this study encompasses the "Zone B" of the FRA, in which fishery is subject to limitations, and an external adjacent buffer area located to the south-west ("ext ITA"), where no fishery limitations were implemented (except for the yearly seasonal trawl fishery closures regulated by national governments; [45]). These two zones represent two different depth layers: "Zone B", > 200 m, inside the western pit and "ext ITA", < 200 m, outside the pit (Fig. 1).

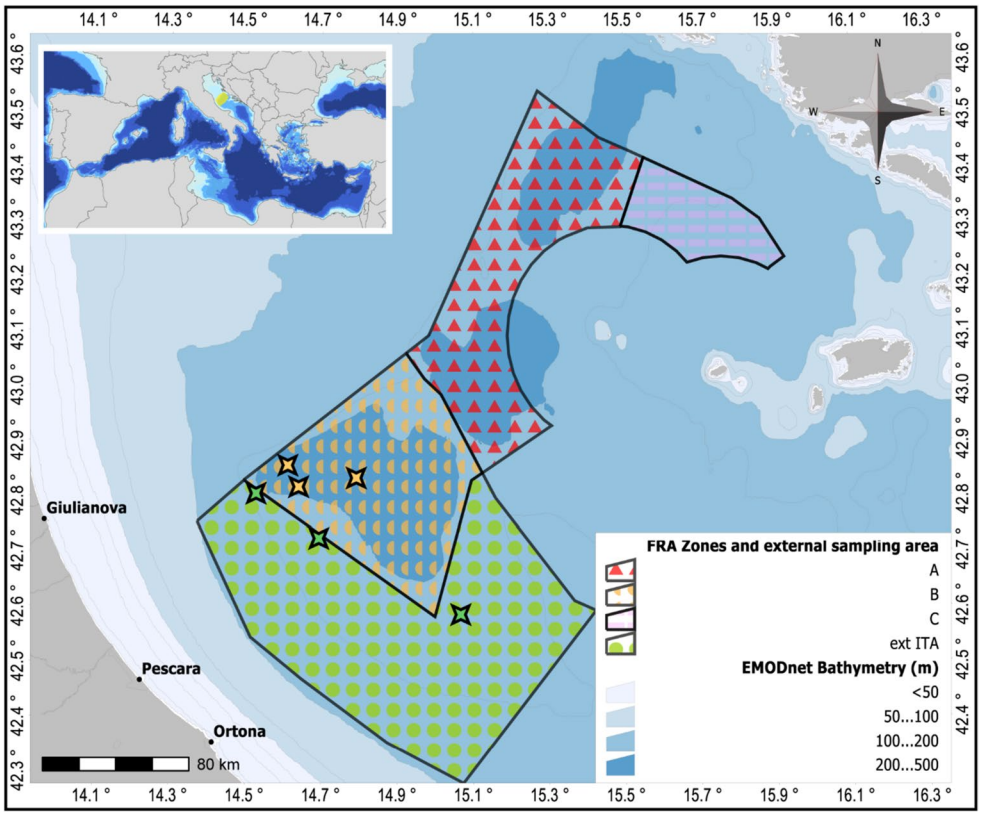



**Fig. 1** The location of the study area within the Mediterranean Sea is indicated in yellow in the top-left polygon; the main map shows the bathymetry of the Central Adriatic Sea [53] and the Pomo Pits Fishery Restricted Area, Zones "A", "B", "C", and the "ext ITA" sampling area. Orange crosses represent the sampling stations inside "Zone B" and the green ones in "ext ITA"

## Sample Collection

A total of 382 Norway lobsters were sampled in "Zone B" of the Pomo Pits FRA (128 in 2019 and 50 in 2023) and in "ext ITA" external buffer area (101 in 2019 and 103 in 2023) [45]. Sampling was conducted through experimental trawling operations carried out on board the 35.7 m research vessel *Dallaporta* in October 2019 and April-May 2023, during the "ScamPo" and "UWTV" (UnderWater TeleVision) surveys respectively [44, 54]. Trawling operations were performed at sunrise and sunset using an experimental net with a 22 mm mesh size in the body and 12 mm in the cod end [44]. For each specimen, carapace length (CL) was measured to the nearest millimetre, and weight (g), sex, and fishing depth were recorded. A subset of 80 Norway lobster, evenly distributed per year, sex and area were involved in the analysis of gut microbiota. Among these samples, 40 were also used to characterise the diet through DNA metabarcoding. Only in the 2023 sampling survey, bottom seawater and surface sediment samples were collected at each of the six sampling stations used for Norway lobster sampling (three in "Zone B" and three in "ext ITA"), with one bottom-water and one surface-sediment sample collected at each station. These environmental samples were collected as comparators for gut microbiome analyses. 3 L of seawater were filtered using 0.22 µm cellulose nitrate membrane filters (Sartorius, ø 47 mm) and preserved at −20 °C until processing. Sample workflow, subset overlap, and the number of individuals included in each analysis are shown in Supplementary Fig. 1.

## Gut Content Analysis (GCA)

GCA was performed specifically on stomach contents. A total of 382 samples for stomach content were examined to assess diet. Stomach contents were everted in a Petri dish and then examined under a stereoscopic microscope. Prey were identified to the lowest possible taxonomic level based on diagnostic morphological structures. Specifically, fish were identified from vertebrae, otoliths, bone elements, and scales; bivalves from shell fragments;

crustaceans from characteristic appendages and exoskeletal fragments such as claws, dactyls, and pleopods; cephalopods from beaks and associated tissue remains; and polychaetes from bristles. The contribution of each prey to the total stomach contents was calculated using the subjective point approach [55], as many prey items were crushed and heavily digested, making it difficult to weigh them separately. Frequency of prey occurrence (%F = [number of stomachs containing prey/total number of stomachs] × 100), the numerical abundance (%N = [number of prey/total number of prey] × 100) and the prey biomass (%W = [wet weight of prey/total weight of prey] × 100) of prey were then computed. The index of relative importance (IRI) for each prey group, according to [56], was then calculated as IRI = %F × (%N + %W). This index was expressed as %IRI = (IRIi/ΣIRI) × 100.

Univariate analyses were conducted to assess differences in fullness indices across area, year and sex. Initially, Levene's test was applied to evaluate whether the data met the assumption of homoscedasticity. Normality of residuals was also evaluated using the Shapiro-Wilk test. Three-way ANOVAs were performed to examine the individual effects of Area, Year and Sex on each dependent variable, as well as their interaction. Welch's t-test, was utilised to determine whether significant differences in mean CL existed between males and females. The univariate analyses were conducted using the RStudio version 4.5.0 (RStudio Team, 2020), with R packages "dplyr" (1.1.4), "ggplot2" (4.0.2), and "car" (3.1.2) [57, 58]. Variations in diet across depth classes (<and >200 m) were analysed using multivariate methods based on a two-way factor design including area and year. First, a nonmetric multidimensional scaling (nMDS) analysis was performed on the Bray-Curtis resemblance matrix of fourth-root-transformed prey biomass data. Taxa displayed as vectors in the biplots were fitted using the envfit function (vegan package 2.6.6), and their significance was assessed using permutation tests (999 permutations). Only taxa showing significant correlations with the ordination ($p < 0.05$) were retained. Then, a two-way permutational multivariate analysis of variance (PERMANOVA) was conducted to assess variations between the levels of depth and year with statistical significance assessed using 1,500 permutations. Similarity percentage analysis (SIMPER) was used to identify the taxa contributing most to similarity and dissimilarity within and between groups. To visualise separation among samples based on the hypothesised variables, the factor identified as significant in PERMANOVA was subjected to canonical analysis of principal coordinates (CAP) [59]. Post-hoc comparisons were obtained from estimated marginal means using "Emmeans" (1.1.1) and pairwise contrast was tested with Benjamini–Hochberg correction [60]. The R packages "vegan" (2.6.6) and "pairwiseAdonis" (0.4.1) were used for multivariate analyses [61, 62].

## Stable Isotope Analysis (SIA)

For SIA, a total of 16 individuals, sampled in 2023, equally divided per depth and sex were analysed. A piece of muscle tissue (from the abdomen without exoskeleton) was dissected from each specimen and oven dried at 60 °C for at least 24 h [63]. To determine $\delta^{13}C$ and $\delta^{15}N$ values, approximately 0.5–1 mg of dried tissue was weighed into tin capsules and analysed using an elemental analyser (Thermo Flash EA 1112) coupled to an isotope-ratio mass spectrometer (Thermo Delta Plus XP). Isotopic values were expressed in ‰ relative to Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric $N_2$ for nitrogen. Analytical accuracy, based on IAEA internal standards (IAEA-CH-6, IAEA-NO-3 and IAEA-N-2), ranged from 0.10 to 0.19‰ for $\delta^{13}C$ and from 0.02 to 0.08‰ for $\delta^{15}N$. Because lipids were not removed prior to analysis, $\delta^{13}C$ values were normalised for samples with C: N ratios > 3 following, applying the following formula; $\delta 13C_{normalised} = \delta 13C_{untreated} - 3.32 + 0.99 \times C{:}N$ [64].

To assess isotopic niche width across depth, Bayesian statistical methods were applied using the R package "SIBER" (2.1.9) to construct standard ellipse areas (SEAs) for $\delta^{13}C$ and $\delta^{15}N$ values and the posterior distribution of the covariance matrix for each group was obtained through a Bayesian SEA analysis [65]. To account for limited sample size, the corrected standard ellipse area (SEAc) was used to estimate the isotopic niches of each species. The R package "tRophicPosition" (0.8.0) was employed to calculate the trophic position [66], incorporating isotopic data from potential prey sampled within the same area, Thaliacea and Calanus, with a mean $\delta^{15}N$ value of 3.78 ± 0.57‰, were incorporated into the model. The baseline was assigned a trophic level of $\lambda = 2$, applying

a nitrogen trophic discrimination factor of $\Delta^{15}N = 3.4 \pm 0.98‰$. Before conducting mixing models to identify the primary dietary contributors for Norway lobster, a simulation was performed using the R package "splancs" (2.1.45) [67]. This simulation involved calculating the mixing polygon for each iteration of source data, with each point within the polygon determining whether consumers fell within its boundaries or at its periphery. Iterations continued until the variance of the mixing polygon's area stabilised [68]. This process helped identify the most appropriate food sources for inclusion in the mixing model. The mixing model itself was constructed using the R package "SIMMR" (0.5.1.217) [69] and was used to estimate the relative contributions of different prey items to the diet of Norway lobster. Trophic discrimination factors were set to $\delta^{13}C = 0.40‰$ and $\delta^{15}N = 2.70‰$. This approach effectively differentiates among various food sources by analysing isotope values extracted from tissue samples [69]. A sensitivity analysis on sample size was conducted using R package "SIMMR" to assess the effect of the number of consumers on model accuracy. For sample sizes less than or equal to the observed sample size ($n = 4, 8, 12, 16$), datasets were generated using random subsampling. For larger sample sizes ($n = 20, 30, 50$), datasets were generated using bootstrap resampling of consumers. Accuracy was quantified as the mean width of the 95% credible intervals of source contribution estimates across prey groups.

The chosen species were also selected based on the GCA and dietary DNA metabarcoding results, as well as the prey availability, in order to include the most representative prey taxa. All isotopic values of the prey included in the mixing models were obtained from specimens collected in the same fishing ground and during the same sampling surveys as the Norway lobster. For the mixing models, prey items were categorised into four groups: *"Iridonida speciosa*", "Demersal shrimps", including *P. longirostris* and *Plesionika heterocarpus* (Costa, 1871), "Actinopterygii", including *Callionymus maculatus* (Rafinesque, 1810), *M. merluccius*, and *Lepidorhombus boscii* (Risso, 1810) and "Cephalopods", including *Illex coindetii* (Vérany, 1839) and Sepiola spp.

### DNA Extraction and High-Throughput Sequencing (HTS)

Intestine samples were selected for HTS in order to use the same biological matrix for both dietary DNA metabarcoding and gut microbiome analyses. Furthermore, the separate processing of the stomach and intestine limited potential contamination. Tweezers and scissors were sterilised before processing each specimen by immersion in a 10% bleach solution, followed by rinsing in 99% molecular biology-grade ethanol and flame sterilization. Intestinal samples for molecular analyses were processed under a biological safety cabinet (Biohazard Hood Cabinet Type II A/B3, BIO ACTIVA 180 PLUS). The DNA was extracted from 0.25 g of homogenised intestine (previously finely minced with sterile scissors) using the DNeasy PowerSoil Kit (Qiagen) following the manufacturer's instructions; extracted DNA samples were stored at −20 °C until processing. Extracted DNA was quantified with the Qubit 4.0 fluorometer using the Qubit dsDNA HS Assay Kit (Invitrogen), using the dsDNA HS Assay kit. Sequencing of the bacterial 16 S rRNA gene was performed targeting the V3–V4 (~460 bp) region using the Illumina primer pair 341 F (5′-CCTACGGGNBGCASCAG-3′) and 785R (5′-GACTACNVGGGTATC TAATCC-3) [70] as described in [71, 72]. A 313 bp fragment of the cytochrome oxidase I (COI) gene was also amplified for each sample with the primers mlCOIintF-XT (5′-GGWACWRGWTGRACWITITAYCCYCC-3′) [73] and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) [74]. In detail, each sample was amplified in triplicate, and the replicates were subsequently pooled. PCR reactions (20 µL total) contained 1 µL of each forward and reverse primer (0.5 µM), 2 µL of DNA template (0.5–10 ng/µL), 10 µL of MiFi mix (Bioline), 0.16 µL of Bovine Serum Albumin and 5.84 µL of molecular grade $H_2O$. Thermocycling conditions were: initial denaturation at 94 °C for 10 min; 35 cycles of 94 °C, 45 °C, and 72 °C for 1 min each; final extension at 72 °C for 5 min. Two negative (purified water) and one positive (iridescent catfish, *Pangasianodon hypophthalmus* Sauvage, 1878) controls were also amplified and sequenced. Amplicon quality was checked by electrophoresis on 2% agarose gels stained with SYBR Safe (Invitrogen), including the positive and negative controls. All PCR products were purified with magnetic beads (0.8×, Omega Mag-Bind). Cleaned PCR products were quantified using the Qubit dsDNA HS Assay and pooled according to their measured concentrations to normalise amplicon input prior to library preparation. Following the manufacturer's protocol, 3 µg of total DNA was used to prepare Illumina

libraries with the Perkin-Elmer NEXTFLEX® Rapid DNA-Seq Kit 2.0. Libraries were quantified by qPCR with the NEBNext Library Quant Kit for Illumina, normalized to 15 pM, spiked with 10% PhiX, and sequenced on an Illumina MiSeq (v2 chemistry; 2 × 300 bp paired-end). The sequences detected in the negative controls were used to correct for background contamination by subtracting, for each MOTU, the corresponding number of detected reads. The same method was applied to extract DNA from sediment (1 g) and seawater samples (0.22 µm filters).

### Bioinformatic and Data Analyses

The COI dietary metabarcoding dataset was analysed using a Molecular Operational Taxonomic Units (MOTUs) based workflow, whereas the 16 S gut microbiome dataset was processed separately using an ASV-based approach. Raw sequence reads of the eukaryotic COI gene were first quality-checked with FastQC. Paired-end reads were then merged using VSEARCH [75], and primers and sample tags were removed during demultiplexing with Cutadapt [76]. Demultiplexed reads were quality-filtered, converted to fasta format, pooled across samples, and dereplicated using VSEARCH, by removing sequences with expected error > 1.0, reads shorter than 300 bp or longer than 325 bp, and reads containing ambiguous bases. Chimeric sequences were identified and removed using UCHIME-*de novo* approach implemented in VSEARCH. The resulting dataset was then clustered into MOTUs using SWARM v3 with a local clustering parameter of d = 13 [77]. To generate the MOTU abundance table, quality-filtered reads were subsequently mapped back to the SWARM-defined representative MOTU sequences using VSEARCH with a minimum pairwise sequence identity threshold of 97%. This step was used only for read counting and was not used to redefine, merge, or recluster MOTUs. Taxonomic assignment of MOTUs was performed against the MIDORI2 COI reference database [78] using both SINTAX [79] and BLASTn (v2.11.0). SINTAX was initially run with a confidence cutoff of 0.7 to retrieve preliminary taxonomic assignments; this value represents classifier confidence and not sequence identity. Taxonomic assignments were subsequently manually curated using more stringent criteria, retaining SINTAX assignments with confidence scores ≥ 0.97 and BLASTn matches with ≥ 97% sequence identity, while assignments below these thresholds were excluded from downstream dietary analyses. To compare MOTU richness, rarefaction and extrapolation analyses were performed using the iNEXT package [80]. Raw sequences of the bacterial 16 S rRNA gene were processed using Cutadapt for primer and adapter removal. Paired-end reads were then imported and analysed using the DADA2 package (version 1.36). Quality check and trimming of the reads were performed following the package instructions (max estimated error > 3 and 3 per 100 bp for forward and reverse reads respectively, trimming at 260 and 220 bases per forward and reverse reads). Paired-end reads were subsequently merged, and amplicon sequence variants (ASVs) were inferred at single-nucleotide resolution, such that sequences differing by even a single nucleotide were considered distinct ASVs. Chimeric sequences were identified and removed from the dataset. Finally, prokaryotic taxonomy was assigned using a native implementation of the naive Bayesian classifier method against the SILVA database (v138.1; https://www.arb-silva.de/documentation/release-138/). Chloroplast and eukaryotic sequences were removed from the ASV table obtained from DADA2; [81]. Out of the initial subset of 80 Norway lobster samples selected for gut microbiome analysis, samples with fewer than 5,000 sequencing reads were excluded after processing the sequencing output. This threshold was determined based on inspection of rarefaction curves, which indicated that samples with ≥ 5,000 reads approached a plateau in observed ASV richness, indicating sufficient sequencing depth to capture the majority of bacterial diversity. Additional sensitivity analyses using alternative thresholds (e.g., 1,000 and 2,000 reads) were performed, confirming that a cutoff of 5,000 reads maximised sample retention while maintaining stable alpha- and beta-diversity estimates. A total of 32 samples were retained. Data were scaled to the median values of the read counts and subsequently transformed into relative abundances. All subsequent analyses were conducted using relative abundance data to account for variation in sequencing depth across samples.

For the analysis of alpha diversity, ASV richness was calculated using the vegan package [62]. Non-metric multidimensional scaling (nMDS) was performed using a Bray-Curtis dissimilarity matrix and average linkage approach and plotted with the ggplot2 package. The igraph [82] package was used for the visualization of the

microbial communities, this was performed from the presence/absence matrix (ASVs × samples). A core microbiome analysis was performed identifying ASV present in at least 80% of samples with minimum of 0.01% of relative abundance in each sample. To identify the taxa most strongly driving community ordination, the envfit function was applied separately to the GCA content and HTS datasets. Taxa with the highest correlation ($R^2$) values at a stringent significance threshold ($p < 0.001$) were selected as the most representative for each dataset and were then overlaid as vectors on the NMDS plots. PERMDISP (betadisper function in vegan package) and then PERMANOVA (with permutations $n = 999$) were used for statistical differences among groups (sampling area and years). Spearman correlation test was implemented on 15 specimens, of which were present DNA eukaryotic and prokaryotic results, to investigate association between prey and microbiome. Alpha diversity was assessed using the Shannon-Wiener index for both the COI dietary metabarcoding dataset and the 16 S microbiome dataset. Prey items were classified as likely derived from active predation or scavenging behavior based on ecological characteristics. Benthic organisms were considered likely targets of active predation, while pelagic or highly mobile species, or taxa not typically associated with the seabed, were interpreted as more likely acquired via scavenging. This classification represents a probabilistic ecological inference rather than a direct behavioral observation.

## Results

### Length Frequency Distribution (LFD)

Summary statistics of carapace length (CL, mm) of Norway lobster by year, sex, and area are reported in Supplementary Table 1. In 2019, females ($n = 93$) ranged from 18.7 to 43.0 mm CL, whereas males ($n = 136$) ranged from 20.9 to 55.7 mm CL. In 2023, females ($n = 85$) ranged from 11.0 to 36.0 mm CL, whereas males ($n = 68$) ranged from 13.0 to 50.0 mm CL. LFDs were significantly different between sexes (ANOVA; Sex $F(1,374) = 62.25$, $p = 3.37 \times 10^{-14}$), years (ANOVA; Year $F(1,374) = 133.6$, $p < 2.2 \times 10^{-16}$) and areas (ANOVA; Area $F(1,374) = 8.88$, $p = 0.003$). Post-hoc comparisons (Tukey-adjusted) confirmed that males were consistently bigger than females across years x areas with differences of 10.42 mm CL in 2019 in "ext ITA" ($p < 0.0001$), 7.94 mm in 2019 in "Zone B" ($p < 0.0001$); whereas in 2023 the differences were less pronounced, amounting to 2.53 mm in "ext ITA" ($p = 0.0495$), and 3.74 mm in "Zone B" ($p = 0.0495$). Overall, both sexes showed a decrease in mean size from 2019 to 2023, with the reduction more evident in males (Supplementary Fig. 2).

### Diet Characterisation of Norway Lobster, Gut Content and Stable Isotopes Analysis

Out of the 382 analysed stomachs, 103 were empty. GCA showed that Norway lobster consumes principally crustaceans, fish, and cephalopods. Malacostraca represented the most important resource for the diet in all the areas and across the years. Significantly different dietary patterns were observed depending on both the area and the year considered. Malacostraca strongly dominated in "Zone B" (greater depth and strictly regulated fishing activities, 71%and 73% of the total prey weight respectively in 2019 and 2023), whereas in "ext ITA" (shallower depths and likely higher fishing pressure) a more diversified diet was observed, even if Malacostraca remained the main component (54% in 2019 and 61% in 2023; Fig. 2). The other main groups were, for "Zone B", Actinopterygii 20% in 2019 and 12% in 2023, Cephalopoda 3% in 2019 and 6% in 2023; for "ext ITA" 21% in 2019 and 17% in 2023 and 15% in 2019 and 11% in 2023 respectively. Diet composition differed significantly between areas (PERMANOVA; $F(1,279) = 27.35$, $p = 0.001$) and years (PERMANOVA; $F(1,279) = 13.88$, $p = 0.001$), while there was no significant difference between sexes (PERMANOVA; $F(1,279) = 1.98$, $p = 0.102$).

SIMPER analysis showed that in 2019 dietary dissimilarities were explained by *I. speciosa* (21.6%), Actinopterygii (12.4%), Peracarida (10.4%), Cephalopoda (9.7%). In 2023, differences were explained by *I. speciosa* (15.1%), Actinopterygii (14.9%), Peracarida (9.4%) and Cephalopoda (7.2%). Comparing Norway lobster diet

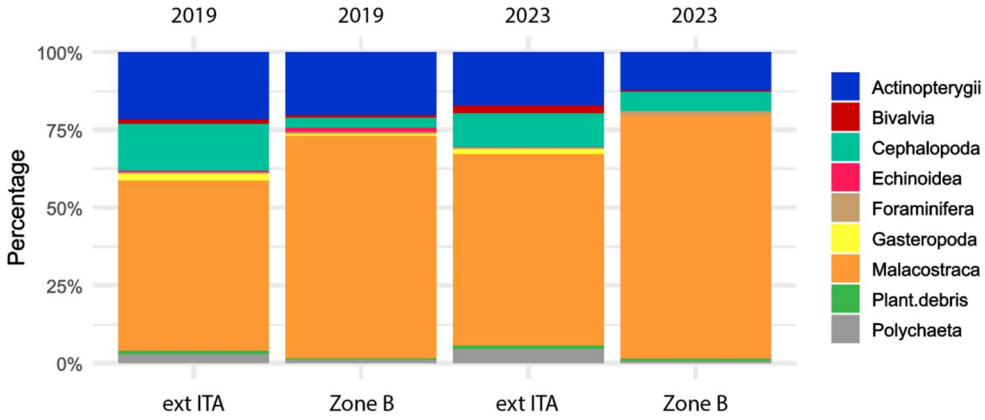



**Fig. 2** Dietary composition of Norway lobster in terms of prey wet weight

between the areas, it can be observed that the diet appears to have a similar composition in terms of genus/species recognised prey, with *I. speciosa* representing the more relevant prey in both the areas and years. In particular, an increase of *I. speciosa* was evident in "Zone B" compared to "ext ITA" in both years (Supplementary Fig. 3).

The specimens selected for SIA had a mean (±SD) CL of 25.7±3.7 mm for females and 34.2±4.3 mm for males. $\delta^{13}C$ values ranged from −18.62 to −17.92‰. The $\delta^{15}N$ values fluctuated between 6.18 and 7.76‰. For $\delta^{13}C$, significant differences were detected between areas (ANOVA; $F(1,12)=9.81$, $p=0.026$), with individuals from "Zone B" showing more enriched values, whereas no significant effect of sex was observed. However, in "ext ITA" $\delta^{15}N$ increased significantly with CL (ANOVA; $F(1,5)=8.89$, $p=0.031$). No significant differences in trophic position were observed between areas and sex; an overall trophic position of 3.09 (±0.15) was estimated. The mixing polygon simulation indicated that consumer values fell within the 95% mixing region (represented by the outermost contour), allowing the selection of prey for subsequent mixing models (Supplementary Fig. 4). All isotope data obtained and used in these analyses are provided in Supplementary Table 2. Analysis of uncertainty related to the consumer sample size showed a marked decrease in the mean width of the 95% credible intervals as the sample size increased, with a marked improvement up to the observed sample size of 16 individuals (Supplementary Fig. 5).

According to SIMMR results, in the "ext ITA" the most relevant dietary component in terms of isotopic contribution was *I. speciosa*, followed by Actinopterygii, Demersal Shrimps and Cephalopoda (Fig. 3), in "Zone B" the main component was *I. speciosa* followed by Demersal Shrimps, Cephalopoda and Actinopterygii. In "ext ITA", although *I. speciosa* showed a high contribution, the other prey categories were also well represented, while in "Zone B" its contribution was much stronger.

## Prey Composition of Norway Lobster Through High-Throughput Sequencing Data Analysis

Host DNA reads, as well as those from microalgae, fungi, Trematoda, and Nematoda, were excluded from dietary analyses. In the context of this study, their presence in gut contents may reflect environmental contamination, incidental ingestion, parasitism, or secondary predation rather than intentional diet. After the non-target DNA removal, five Norway lobsters were excluded because no dietary DNA remained in the gut samples, leaving 35 individuals for analysis. In terms of MOTUs richness, the diet varied considerably, with significant differences detected between years but not between areas. Lower biodiversity was observed in 2023 compared to 2019, confirmed by Shannon-Wiener indices (0.75±0.57 and 1.28±0.82 respectively). Even if there were no statistically significant differences, biodiversity indices were also calculated considering the area factor, showing higher values in "ext ITA" than in "Zone B" in both years (Shannon-Wiener: 1.39±0.69 (2019) and 0.86±0.52 (2023) for "ext ITA"; 1.19±0.94 (2019) and 0.63±0.62 (2023) for "Zone B". Rarefaction and extrapolation curves (Supplementary Fig. 6) showed consistently higher MOTU richness in 2019 compared to 2023 in both depth strata. In particular, samples collected in "Zone B" in 2019 exhibited the highest estimated MOTU richness,

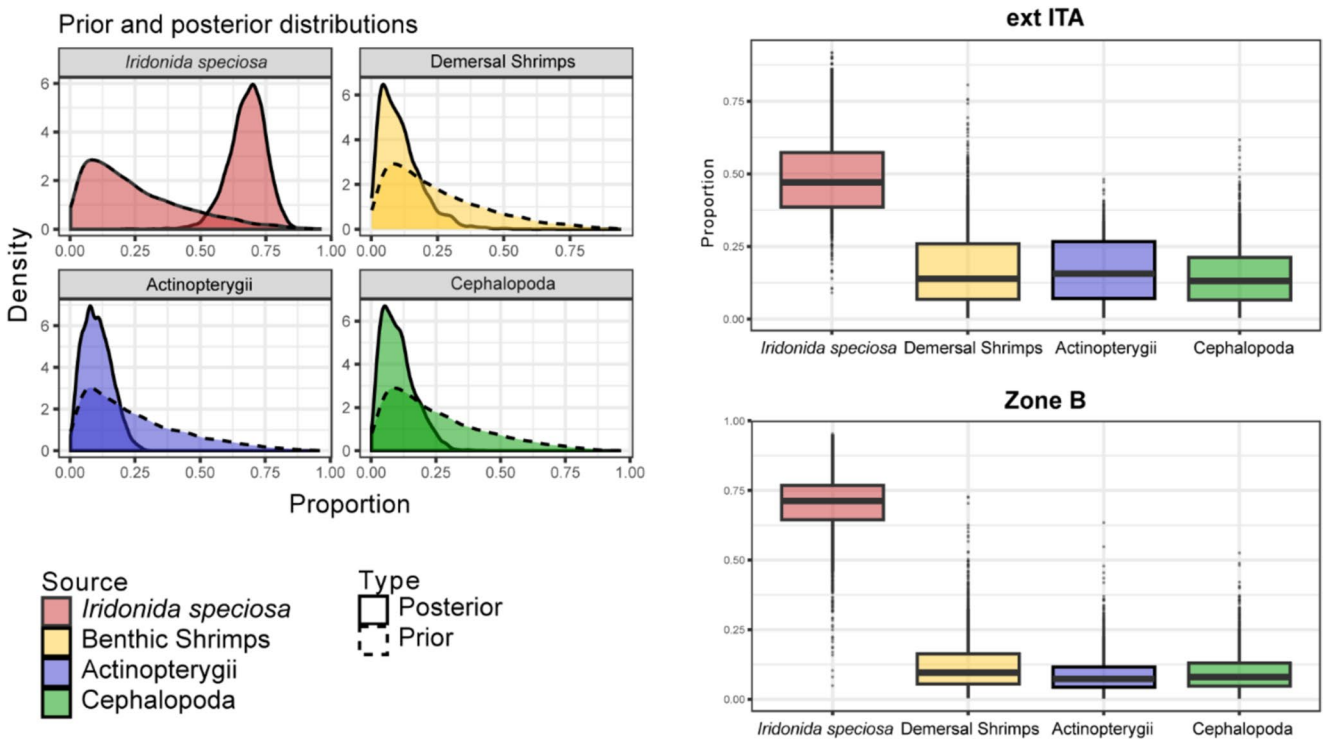



**Fig. 3** Prior and posterior distributions of various food sources for Norway lobster in the considered areas

whereas "ext ITA" samples in 2023 showed the lowest values. Overall, all the samples from 2019 showed higher MOTU richness than those of 2023. The most relevant prey classes for each area were Actinopterygii in 2019 and Malacostraca in 2023. Although confidence intervals partially overlapped at higher extrapolated sample sizes, the overall pattern suggests a reduction in prey diversity in 2023 across both areas. However, considering both areas and years, Actinopterygii, Malacostraca, and Polychaeta were the most representative classes. Individually, consistent results with a core of Malacostraca are observed across years and between areas, as well as increased diversity in 2019 and in "ext ITA" compared to 2023 and "Zone B" (Supplementary Fig. 7).

SIMPER analysis indicated that annual dietary differences were mainly driven by a limited number of species. In "ext ITA" area, the main contributors to dissimilarity were *Pontophilus spinosus* (Leach, 1816), *I. speciosa*, *P. longirostris*, *Pista lornensis* (Pearson, 1969), and *Scorpaena scrofa* (Linnaeus, 1758), whereas in "Zone B" they were *P. spinosus*, *I. speciosa*, *P. longirostris*, and *Aetideus* spp. Furthermore, analysis of species composition across areas and years allowed the identification of prey species detected in Norway lobster intestines. In "ext ITA", 10 species were unique in 2019 and 5 in 2023, with 8 species shared across the years within this area (Fig. 4).

For "Zone B" the unique species in 2019 were 15, while only 3 in 2023, with 5 shared species (Fig. 5).

## Gut and Environmental Microbiome

After sequence pre-processing and quality filtering, only 43 samples (32 Norway lobster guts, 5 sediments and 6 bottom waters) showed a sufficient number of reads, while maintaining an approximately balanced distribution among the groups, as shown in Supplementary Table 3, resulting an average of 20,483 reads per Norway lobster sample; while an average of 66,347 reads per environmental sample was obtained. Rarefaction analysis (Supplementary Fig. 8) indicated sufficient coverage of the bacterial community within these samples. No archaeal reads were found in the dataset. Since benthic organisms are susceptible to environmental microbial contamination,

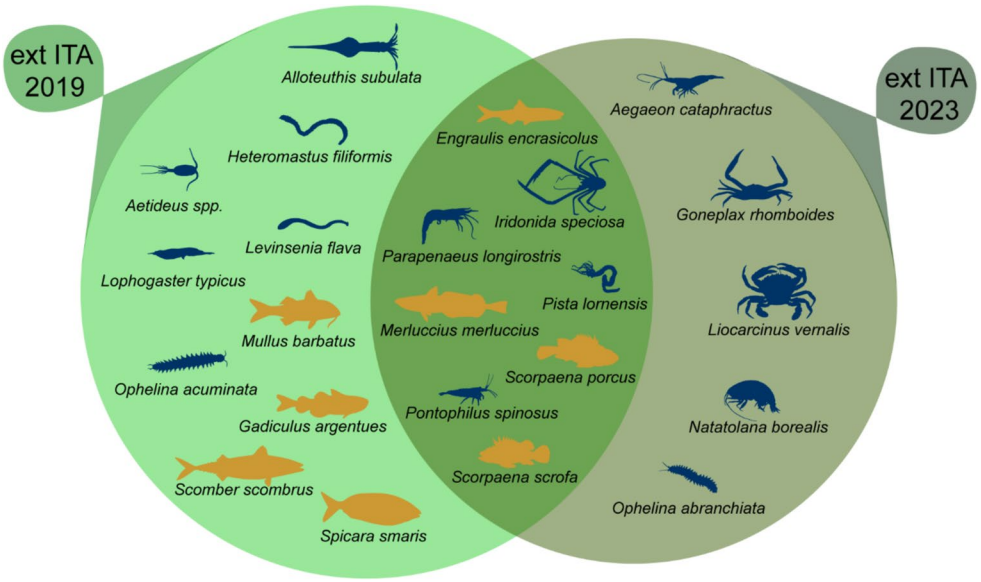



**Fig. 4** Euler–Venn diagram showing species composition based on metabarcoding of the diet of Norway lobster in "ext ITA" in 2019 (light green) and 2023 (dark green); shared species are shown in the overlap. Silhouettes are coloured according to more probable feeding strategy: dark blue indicates active predation, and dark yellow scavenging

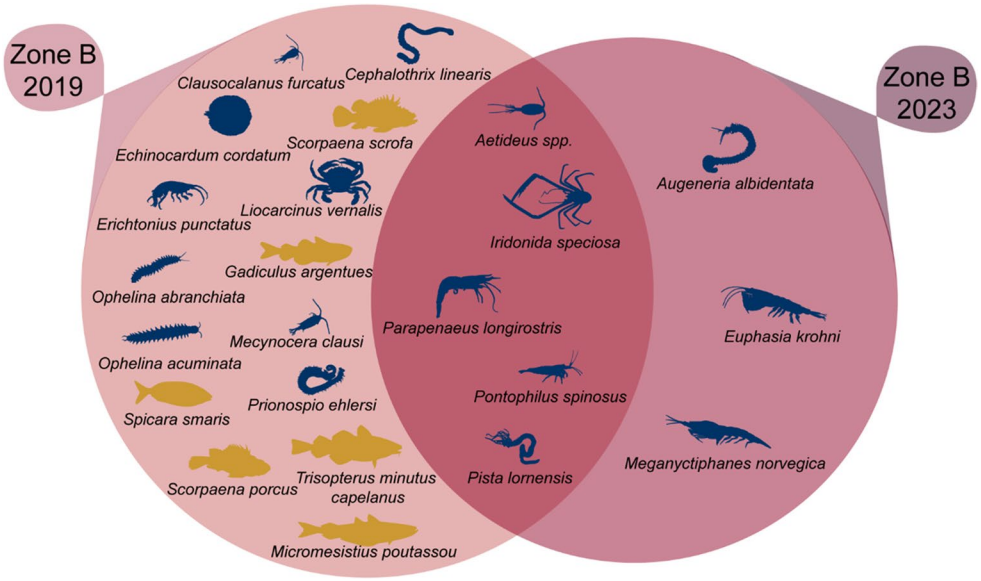



**Fig. 5** Euler–Venn diagram showing species composition based on metabarcoding of the diet of Norway lobster in "Zone B" in 2019 (light red) and 2023 (dark red); shared species are shown in the overlap. Silhouettes are coloured according to more probable feeding strategy: dark blue indicates active predation, and dark yellow scavenging

environmental samples were included as a comparator to confirm gut microbiome distinctness and specialisation. Alpha diversity, assessed using the Shannon index, firstly highlight the differences in terms of richness between the organism and the environmental samples (Fig. 6A, ANOVA; $F = 92.26$, $p = 1.04 \times 10^{-15}$), with sediment and bottom water showing higher values. Secondly it revealed significant spatial differences in the gut microbiome. Specifically, gut samples from "ext ITA" showed higher diversity compared to those from "Zone B" (ANOVA; $F = 8.81$, $p = 0.005$). No overall differences were observed when considering years. Beta diversity analysis performed on gut samples showed significant differences in microbial community structure when areas and years were considered together (PERMANOVA; Bray–Curtis dissimilarity: $R^2 = 0.20$, $F = 2.35$, $p < 0.001$). Sampling year explained a modest but statistically significant proportion of the variation in microbiome beta diversity (PERMANOVA; Bray–Curtis dissimilarity: $R^2 = 0.10$, $F = 3.63$, $p = 0.002$). However, because multivariate dispersion also differed significantly among years (PERMDISP: $F = 6.2$, $p = 0.01$), the temporal PERMANOVA effect should be interpreted with caution, as differences in within-year dispersion may contribute to the observed pattern. In contrast to the alpha diversity patterns, sampling year explained a modest but statistically significant ($p < 0.05$)

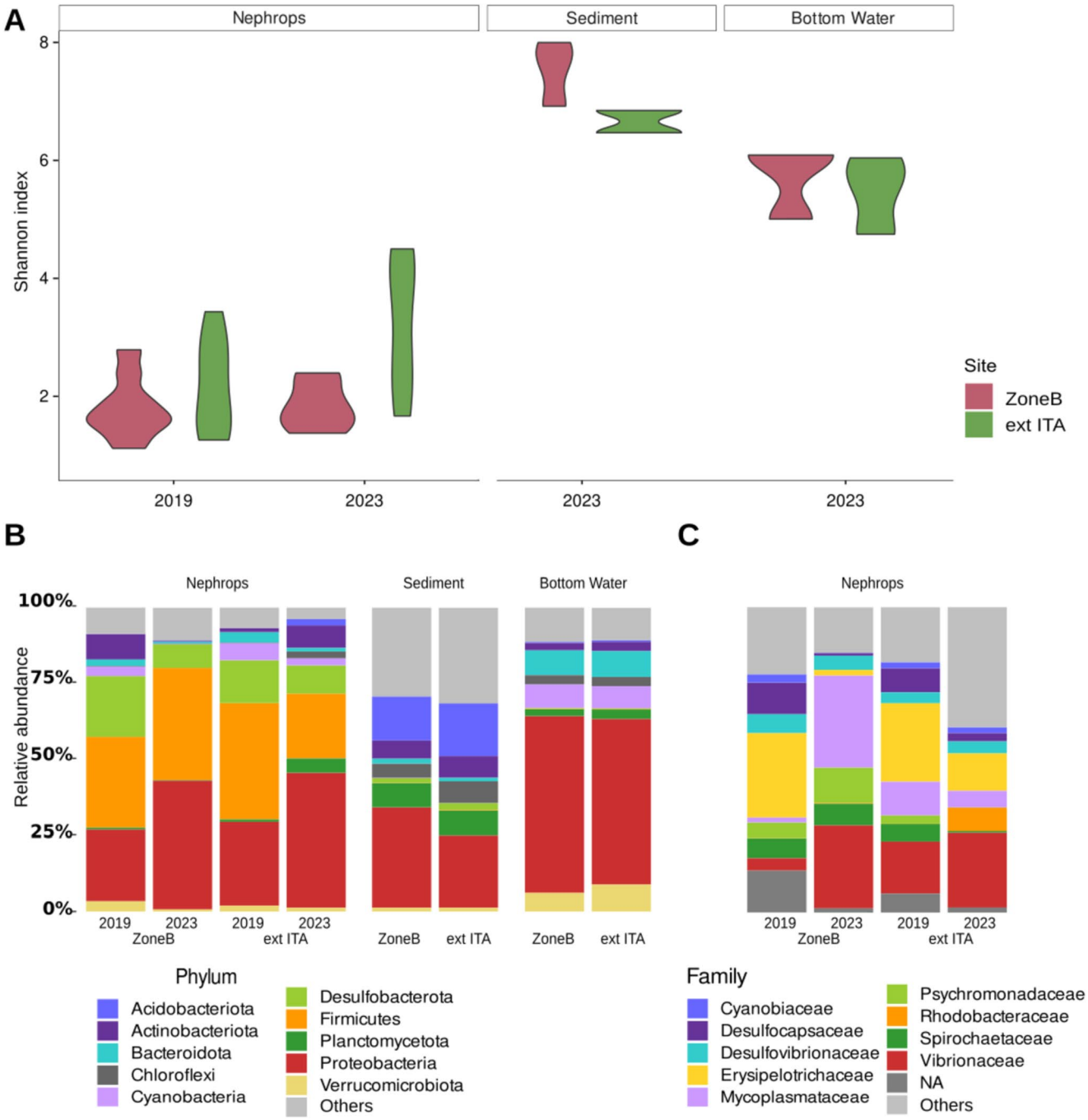



**Fig. 6** (**A**) Violin plot showing the Shannon index of the Norway lobster samples, and the environmental samples (Sediment and water) per years of sampling, and areas. Barplot showing the prokaryotic community composition (as relative abundance) at the Phylum (**B**); and Family level (**C**) separated according to sample years and areas (only the 10 most abundant taxa are shown)

proportion of microbiome beta-diversity variation, confirming that temporal factors contributed to compositional differences (PERMANOVA; Bray-Curtis dissimilarity: $R^2 = 0.1$, $F = 3.63$, $p = 0.002$). Overall, the gut microbiome did not display a stable or uniform structure across individuals (Supplementary Fig. 9), consistent with the observed heterogeneity in temporal dispersion and the modest explanatory power of the tested factors. Marked differences in microbial community composition between the environment and the organism were observed at

the phylum-level analysis (Fig. 6B). Despite the variability and the observed differences among years and site groups, in Norway lobster the microbial communities were consistently dominated at the phylum level (Fig. 6B) by Proteobacteria (33.64 ± 30.75%), Firmicutes (31.29 ± 26.38%), and Desulfobacterota (13.11 ± 17.13%), and, at the family level (Fig. 6C), by Erysipelotrichaceae (17.66 ± 23.76%), Vibrionaceae (17.33 ± 29.88%, mainly represented by the genus Photobacterium), and Mycoplasmataceae (11.16 ± 20.29%).

In terms of taxonomic sharing, ASVs and families detected in the gut microbiome were firstly compared with those present in bottom water and sediment samples collected in 2023 from the same areas (Supplementary Fig. 10). Although environmental samples exhibited higher overall diversity, Norway lobster gut microbiomes harboured 31% unique ASVs and 18% unique families, while the remaining taxa were shared with the surrounding environment. Differences were also observed across the Norway lobster samples: at family level, 22% of the detected families were shared among all groups, with the largest proportion of specific ASVs observed in the 2023 "ext ITA" group (Supplementary Fig. 10). While each of the other groups also retained distinct sets of specific ASVs and families only 2% of ASVs were shared (i.e., present in at least one sample per group) across all four year-site groups. Among the ASVs shared within all the samples, a core set was identified, consisting of ASVs associated with Erysipelotrichaceae, Spirochaetaceae, Marinilabiliaceae, and Cyanobiaceae.

### Eukaryotic and Microbial Community Patterns

Beta diversity analyses of GCA, gut eukaryotic, and gut microbiome data (Fig. 7) showed no consistent sample grouping by year or area, and overall heterogeneity in community dispersion. The high contribution of *I. speciosa* was also highlighted by the nMDS on GCA data, which showed a separation of Norway lobster samples collected in "Zone B" driven by the presence of this prey (Fig. 7A, B). This prey also emerged as a major contributor in the gut contents from DNA metabarcoding results (proportion of reads, Fig. 7C, D). In panels E and F, the microbial composition shift was significant considering year and area, principally driven by differences in the relative contribution of specific microbial taxa, such as Erysipelotrichales and Enterobacterales on the nMDS1 axis. Spearman correlation analyses (presence/absence) revealed moderate prey-specific associations between gut eukaryotic DNA and the microbiome, with some microbial taxa positively correlated with specific prey taxa (Enterobacterales - *Engraulis encrasicolus* (Linnaeus, 1758), $\rho = 0.69$, $p = 0.009$, padj = 0.69, Actinomarinales, Rhizobiales and Gaiellales – *E. encrasicolus*, $\rho = 0.59$, $p = 0.033$, padj = 0.69, Enterobacterales - *P. longirostris*, $\rho = -0.54$, $p = 0.057$, padj = 0.69 and *P. spinosus*, $\rho = -0.54$, $p = 0.059$, padj = 0.69). However, none of these associations remained significant after Benjamini–Hochberg correction for multiple testing, and they should therefore be interpreted as exploratory.

## Discussion

This study provides the first integrated characterisation of both trophic ecology and gut microbiome of Norway lobster from the Pomo Pits area (Adriatic Sea). The GID approach, integrating gut content analysis, stable isotopes and DNA metabarcoding, allowed the authors to describe the diet of Norway lobster across different temporal scales and to link it to the structure of the gut microbiome.

### Feeding Habits of Norway Lobster Through GID Approach

The sampling stations of "Zone B" and "ext ITA" differ both in depth and expected fishing pressure. This is a direct result of the FRA design and the long-term monitoring plan within which the surveys were conducted. This plan relied on established sampling stations and was primarily structured to evaluate the effects of management measures over time, rather than the effects of depth on individual species. Consequently, as it was not possible to sample neighbouring areas to serve as controls for isolating these factors, the results must be interpreted by

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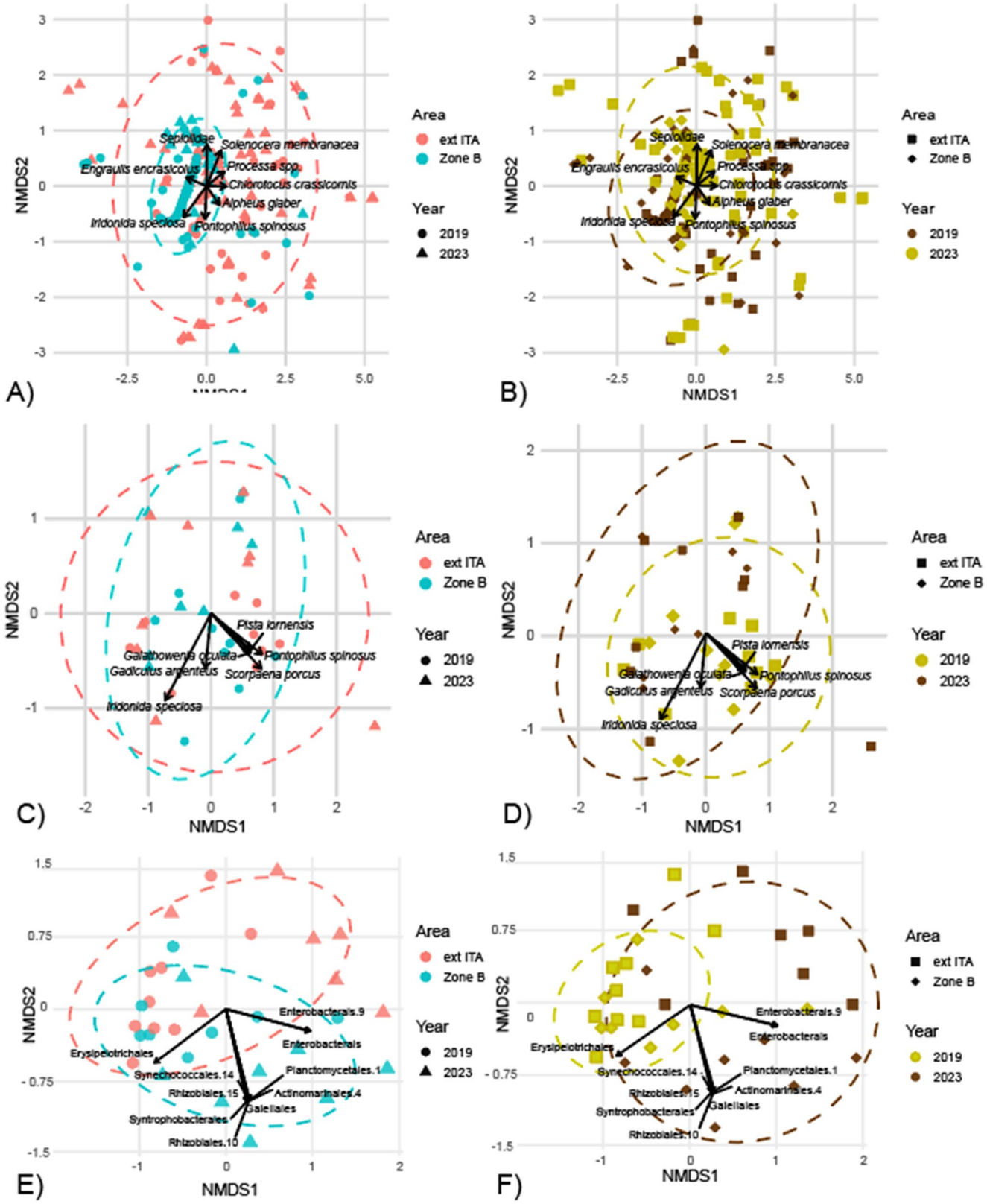



**Fig. 7** NMDS plots showing differences in Norway lobster GCA (**A**, **B**), gut eukaryotic DNA (**C**, **D**), and gut microbial community (**E**, **F**). In red are the samples from "ext ITA", in blue from "Zone B", with dots for the 2019 samples and triangles for 2023 for A, C and E. Ordinations were based on Bray-Curtis dissimilarity matrices. Squares indicate the samples from "ext ITA" and diamonds from "Zone B", in yellow for the 2019 and in brown for the 2023 for B, D and F

considering both variables simultaneously. In both sampling areas ("ext ITA" and "Zone B"), the diet of Norway lobster was primarily based on crustaceans, followed by fishes, cephalopods and polychaetes. This pattern is consistent with previous descriptions of the species as an opportunistic and euryphagous feeder in the Adriatic Sea and other regions [1, 21–23]. Only minor differences emerged between results obtained with GCA and DNA metabarcoding: while the main prey categories were shared, the higher taxonomic resolution of metabarcoding revealed several additional prey groups. Crustaceans and in particular *I. speciosa* played a key role in the diet across the areas. In "ext ITA" the diet was more heterogeneous, although *I. speciosa* remained the principal prey, whereas in "Zone B" that species clearly dominated the diet. The spatial distribution of *I. speciosa* overlaps with that of Norway lobster and, regardless of management measures, its abundance generally seems higher in "Zone B" [45], making it plausible that the species dominates the diets of Norway lobster below 200 m. Metabarcoding results showed higher prey richness in 2019 compared to 2023 for both sampling areas, a trend reflected by the lower Shannon diversity values recorded in 2023. When interpreting these results, it should be taken into account that the observed differences between years may reflect not only interannual variation but also seasonal effects, as the two surveys were conducted in different periods (Autumn 2019 vs. Spring 2023). It is worth noting that the relative proportion of reads should not be interpreted as a direct proxy for prey biomass or numerical abundance, as taxon-specific differences in primer affinity and PCR amplification efficiency can limit its quantitative interpretation. Such seasonality likely influenced local community composition and prey availability. Further analyses integrating environmental parameters and benthic biodiversity from trawling surveys are ongoing. As previously reported for the Adriatic Sea [23], Norway lobsters show a clear preference for crustaceans but, being both a scavenger and an active predator, it also feeds on organisms, such as finfish, either dead on the seabed or captured alive in their early-life stages. The GCA and DNA metabarcoding were consistent in identifying the main prey groups in the diet of Norway lobster, namely crustaceans, fish and cephalopods. Several key prey taxa were detected by both methods, including *I. speciosa*, *P. longirostris*, *P. spinosus*, *Goneplax rhomboides* (Linnaeus, 1758), *Liocarcinus* spp., *E. encrasicolus*, *M. merluccius* and *Mullus barbatus* (Linnaeus, 1758). However, DNA metabarcoding detected several fish, crustacean and polychaete taxa, whereas GCA identified a smaller subset of prey and a few taxa not captured by metabarcoding (Supplementary Table 4), underscoring the value of the GID approach. The SIA confirmed the patterns observed with GCA and DNA metabarcoding, highlighting a strong isotopic contribution of *I. speciosa* in both areas and a more heterogeneous dietary contribution in the "ext ITA" area. Altogether, convergence of the three methods indicates that Norway lobster displays a broad, generalist diet that is strongly influenced by prey availability on the seafloor, affecting both the types of prey consumed and their relative importance. The trophic insights gained from this study provide valuable complementary data for Norway lobster assessment. In particular, tracking changes in the occurrence and relative importance of key prey taxa may help identify shifts in trophic relationships associated with changes in benthic and demersal community composition under climatic or anthropogenic pressures. This is especially relevant in regions like the Pomo Pits area, which are for example characterised by documented shifts in benthic communities, such as the replacement of *M. intermedia* by *I. speciosa*. Future studies should therefore integrate GID-based analyses with independent assessments of biomass and biodiversity, together with environmental variables. Standardised, integrated long-term monitoring of this kind would allow testing whether changes in dietary diversity are associated with changes in resource availability and community structure, ultimately supporting an ecosystem-based approach to fisheries management.

### Gut Microbiome of Norway Lobster and Relationship with Diet

The gut microbiome of Norway lobster collected across two sampling areas and two years in the Adriatic Sea was investigated and compared with gut-content data to unveil links between dietary habits and gut-associated microbial community composition. These findings must also be interpreted in light of the reduced final sample size: of the 80 specimens initially selected, only 32 were retained after quality filtering, which may have reduced statistical power and increased uncertainty in beta-diversity estimates. Nonetheless, the distribution of the samples and

the significant effect observed despite this reduction suggests that the core ecological signal is relatively strong. Notably, environmental samples were available for the 2023 survey only. The higher alpha diversity observed in sediment and bottom water collected during this survey, compared with the Norway lobster gut, is consistent with the generally greater richness of environmental microbial communities relative to host-associated microbiomes, which are constrained by host-specific selective pressures [83]. The marked taxonomic overlap between gut and environmental samples indicates that surrounding sediments and bottom waters act as an important microbial source for the gut, particularly for benthic, sediment-associated species [39]. However, the presence of 31% unique ASVs and 18% unique families in the gut suggests active host selection or enrichment of specific taxa, supporting the view that crustacean gut microbiomes reflect a balance between environmental acquisition and host-driven structuring [84, 85].

The spatial differences in gut microbiome structure and diversity were consistent with the dietary patterns identified by GCA and eukaryotic DNA metabarcoding. The greater dietary heterogeneity in "ext ITA", compared with the dominance of I. speciosa in "Zone B", may have provided different substrates and microbial inputs, potentially contributing to the observed differences in microbial diversity and composition as investigated in previous study [32]. Although prey-specific ASV correlations were not statistically significant, these patterns suggest that diet may contribute to site-specific microbiome structuring [35] and warrant further investigation through targeted feeding and functional studies. However, the interpretation of temporal differences requires caution, because multivariate dispersion differed significantly between years. Thus, temporal differences in the gut microbiome should be considered as exploratory patterns rather than evidence of a uniform year-driven shift in microbiome structure. One of the earliest experimental microbiome studies [32], demonstrated that dietary composition strongly modulates gut bacterial community structure in reared Norway lobster, with many diet-responsive taxa also present in natural populations. Although methodological differences prevent a direct comparison with the results of the present study, the principle that diet shapes the gut microbiome is consistent with the results of this study, which show similar patterns in dietary and microbiome diversity. Furthermore, in this study Spearman correlation analyses revealed prey-specific associations at the microbiome level, with individual ASVs showing moderate positive correlations with some prey, but without statistical significance and thus should be taken as an overview. The lower diversity values in gut microbiomes of individuals collected in the deepest area ("Zone B") fall within the range already reported for wild Norway lobster [36]. In contrast, the more heterogeneous diet observed in "ext ITA", supported by both GCA and metabarcoding, corresponded to greater gut microbial diversity compared to "Zone B", where the diet was largely dominated by *I. speciosa* and microbial diversity was significantly lower. The consistent dominance of *I. speciosa* across areas and years may contribute to maintaining a stable trophic environment, potentially favouring the persistence of a core gut microbiome. The composition of the gut microbiome herein observed is also consistent with the recent analyses of the gut microbiome of Norway lobster from three geographically distant locations (Greece, Italy and Sweden) [35], which demonstrated clear site-specific differences, with Italian specimens particularly enriched in Bacilli (Mycoplasmataceae) and Gammaproteobacteria (Vibrionaceae), just like in the present study. Furthermore, in both studies *Photobacterium* (family Vibrionaceae) is among the most abundant genera in Norway lobster guts. This genus was frequently reported in Norway lobster as well as other crustaceans [32, 34, 86]. *Photobacterium* is of ecological and functional interest because it can produce polyunsaturated fatty acids, lipases, esterases, and antimicrobial compounds, and it has been proposed as a candidate probiotic in fish aquaculture [86, 87]. Its consistent occurrence in the sampled individuals suggests a potentially stable and beneficial association within the Norway lobster gut environment. The findings of the present study also fit within broader decapod microbiome trends, confirming the prevalence of *Vibrio* and *Hepatoplasma* (Mycoplasmataceae) in saltwater decapods [34]. The high presence of taxon ZOR0006 (Erysipelotrichaceae) is consistent with observations in Norway lobster affected by advanced *Hematodinium* infection [36] and in *Litopenaeus vannamei* [88], where these opportunistic taxa increase under stress. These results suggest that this group represents a stable but opportunistic taxon that proliferates under specific physiological or environmental constraints. This may explain the variability in its abundance observed across samples. This study also contributes to deepen the understanding of the core microbiome in Norway lobster. Several of the

main members of the core microbiome (i.e., Erysipelotrichaceae, Spirochaetaceae, Marinilabiliaceae, Cyanobiaceae) support the findings from previous studies performed in crustaceans [35, 36]. The presence of these taxa across species and geographical regions indicates that they might play conserved functional roles in crustacean digestive systems, such as fermentation, polysaccharide degradation, and nutrient recycling, an aspect that is worth of further investigation.

## Conclusion

This study provides an integrated view of the trophic ecology and gut microbiome of Norway lobster dwelling in the Pomo Pits area, an important and vulnerable marine ecosystem and historically one of the most important fishing grounds within the Adriatic Sea. The application of the GID (Gut-Isotope-DNA) approach proved particularly effective in identifying the key trophic sources and linking feeding strategies with gut microbiome of a decapod species in the wild. Differences in prey diversity and composition were accompanied by changes in gut microbial diversity and composition, supporting the hypothesis that diet is a key driver of microbiome variability in Norway lobster. The overall composition of the gut microbiome is also governed by phenomena that transcend the organism's trophic choices, including local adaptative dynamics that are less influenced by short-term environmental fluctuations. Although this study highlights the value of integrative approaches to understand trophic ecology, host-microbe interactions, and ecosystem functioning, it also warrants a judicious approach to interpreting different lines of evidence, especially in the context of marine environmental management.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00248-026-02902-3.

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**Author Contributions** L: Z, M.M. and F.D. conceived the ideas and designed methodology; L.Z and F.D collected the data; L.Z., M.B., S.M. and M.L. analysed the data; L.Z., M.B., M.M., S.M., G.M.Q. and E.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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**Data Availability** All the obtained sequences of 16 S metabarcoding are submitted to the SRA Sequence Read Archive (BioProject PRJNA1432507), and the COI metabarcoding sequence, isotope, morphometric diet data, and the OTU and ASV table generated in this study have been deposited in a public repository and are available under the following DOI:10.5281/zenodo.18874392. Original R scripts are available at the following GitHub page: https://github.com/marco-basili/Nephrops.

## Declarations

**Competing interests** The authors declare no competing interests.

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