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Age and environment are the main drivers shaping the wild common sole (*Solea solea*) microbiota

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Abstract
Microbiota plays an essential role in sh growth and health and may be in uenced by the changing environmental conditions. Here, we explored the microbiota of wild common sole, one of the most important shery resources in the Mediterranean Sea, collected from di erent areas in the North Adriatic Sea. Our results show that the sole microbiota di ers from that of the surrounding environment and among the di erent body sites (gill, skin and gut). Gut microbiota composition showed to be strongly related to sh age, rather than maturity, sex or sampling site. Age-related shifts in gut microbial communities were identi ed, with increased abundances of Bacteroidia and Desulfobacteria, unveiling potential microbial proxies for age estimation crucial for sheries management. Our results expand the limited knowledge of the wild common sole microbiota, also in the light of the potential usefulness of the sh microbiota as a tool for future stock identi cation and connectivity studies.

Keywords Solea solea, Wild sh microbiota, Adriatic Sea

Introduction

Fish farming, health, management, and habitat restoration are among the potential downstream uses of microbiome research in sh ecology [1]. Host-associated microbiomes present inside, as well as on body surfaces, can in uence a broad range of host immunological, evolutionary, and ecological processes [2]. e microbiome is increasingly recognized as a crucial yet poorly explored tool for managing sh resources, since it represents an e ective way to understand how sh respond to environmental changes [2–6]. erefore, exploring sh microbiota may represent a new strategy for promoting sh health and fundamentally improving how we safeguard and manage aquatic resources for future generations [1].

It has been emphasized that, in order to provide robust baseline data for both comparative purposes and a better understanding of sh biology, more studies on wild marine teleost sh species should be performed [7, 8]. is holds true also for the wild common sole, *Solea* (*S.*) *solea* (Linnaeus, 1758), a demersal species that is particularly abundant on relatively low-depth sandy and muddy bottoms in the Mediterranean Sea and the North–Eastern Atlantic [9]. It represents a commercially relevant sh resource in the Mediterranean Sea [10] and is one among the most valuable new candidates for aquaculture practices [11, 12]. Most of the common sole catches

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in the Mediterranean Sea are provided by shing activities carried out in the Central and Northern Adriatic basin [10, 13] e species spatial distribution extends from shallow to 90-100 m deep waters. It is characterized by ontogenetic migrations, with adult individuals moving toward deeper waters [14, 15] and eggs/larvae returning to western coastal waters and lagoons, generally following the basin circulation [16]. So, juveniles (0 years) are mostly concentrated along the Italian side up to 30 m depth around the Po River mouth. From Trieste to Ancona, 1-2 years old sole individuals are observed along the entire coast, in the central part of the basin, and (only partially) close to the Croatian coasts. A certain number of old spawners (age ≥ 3 years) inhabits a portion of the o shore waters southwest of the Istrian peninsula, characterized by the presence of a peculiar benthic community dominated by holothurians and bryozoans [17]. Since these benthic species led to a lower trawl shing e ort, damaging the catch and reducing its commercial value [18–21], this area serves as a refuge, protecting big and older spawners from trawling activities. For this reason, this area was de ned as a "*Sole Sanctuary*" (Fig. 1A) [10, 16, 22]. A particular ecological interest is being paid to this species due to an alarming overexploitation of sole stocks observed in the previous decade [10]. In addition, the species faces both indirect and direct anthropogenic impacts, such as contaminants, disrupted seabed, changing ocean conditions including decreased food availability, and juvenile exploitation by certain shing techniques (e.g., *rapido* trawling), especially in the Adriatic Sea [10, 23–25], that are critical factors in uencing the stock dynamics. Surprisingly, despite extensive research has been performed so far on the biology and ecology of this demersal species, microbiome studies in wild specimens of *S. solea* are still completely lacking, with the only available studies performed so far on farmed *Solea senegalensis* [26].

Here, in order to increase our understanding of this shery resource, we lled this knowledge gap by exploring, for the rst time, the microbiota of wild *S. solea* individuals collected in the Central and Northern Adriatic Sea. Microbial communities were sampled from three body sites, including gill, skin and gut. Representative marine sediment samples were also collected at each sampling zone to compare sh microbial communities with the surrounding environmental sources and to test whether the sh microbiota is in uenced by the host habitat. is study is the rst to explore the diversity and composition of the microbiota in wild common sole. Our ndings should help direct future actions for stock management, as well as to guide aquaculture applications of this species.

Methods

Sampling area

S. solea individuals were collected during the SoleMon *rapido* trawl survey 2019. is scienti c shing activity is carried out annually in fall according to a depth-strati ed sampling scheme with random allocation of stations within the GFCM Geographical Sub-Area 17 (GSA 17: Central and Northern Adriatic Sea) to collect updated data on demersal and benthic shery resources [27]. Samples were collected from 9 sampling stations, classi ed into 4 di erent 'Zones' (3 outside and 1 inside the "*Sole Sanctuary*") as follows: 'Zone 1' was located outside Ancona; 'Zone 2' corresponded to the '*Sole sanctuary*'; 'Zone 3' and 'Zone 4' were located above and below the Po River mouth, respectively (Fig. 1). e selection of these zones was based on available information on the species spatial distribution in the Adriatic Sea [10, 28] to investigate the microbiota community at the different ontogenetic phases and the association with the surrounding habitat. Fish individuals for biometric, otolith and microbiota analyses were randomly collected for each Zone.

Sample collection

A total of 77 wild *S. solea* individuals were collected between November 14th to December 1st during 2019 (Table S1). From each Zone, a total of 24, 15, 20 and 18 sole individuals were collected, respectively. For each of the 77 specimens, total length (TL, to the nearest millimeter below), weight (g), sex and macroscopic maturity stage were measured and recorded. Gonadal maturity was de ned following a modi ed ICES 5 stage maturity scale [27, 29, 30]. Sagittal otoliths were extracted from the inner ear, cleaned and stored dry in tubes for the following age analyses. For microbiota analyses, for each separate sh specimen, samples of skin (a 2 cm square from the left side), gut (tissue and inner digesta), and gill (second gill arch on the left gill) were collected. Only for gill samples, due to an insu cient yield of extracted DNA, only 8 out of 77, samples (all collected at Zone 1) were analyzed as described later. All sh tissue samples were obtained by aseptic dissection of sh specimens using sterile scalpels and scissors. After collection, all tissue samples were immediately placed in sterile tubes rinsed with sterile phosphate bu er solution to remove possible loosely attached microorganisms and stored at −20 °C until further analyses. Representative sediment samples for each Zone (Table S2) were collected through a box corer. Sediment aliquots (1 g) for microbiota analyses were collected using sterile tools, placed in sterile plastic tubes and stored at −20 °C until DNA extraction.

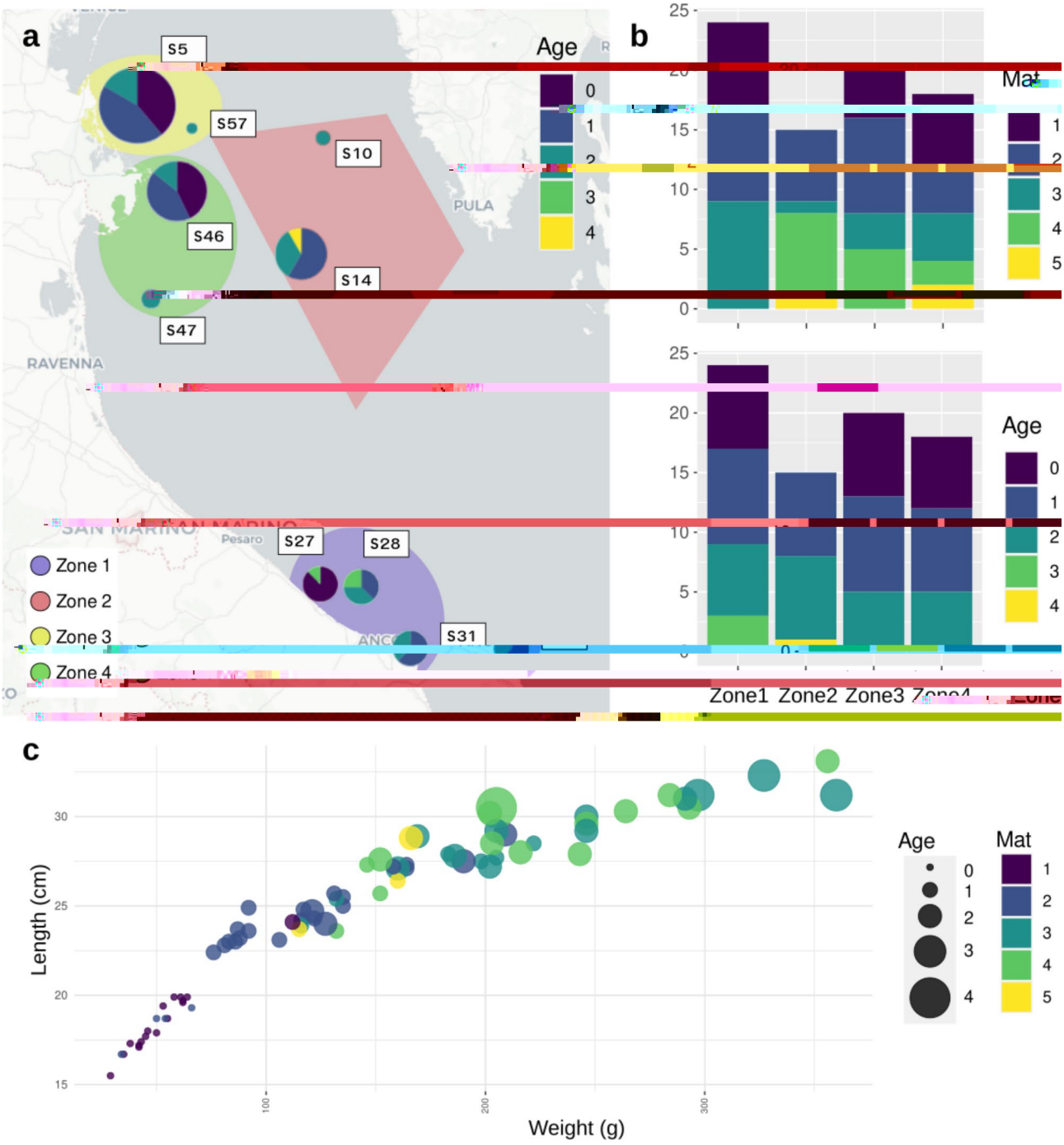

Fig. 1 **(A)**. Map showing the sampling areas of the common sole individuals analyzed in this study. Per each sampled area (colored circles and polygon) the proportion of specimens at di□erent ages is reported. The pink polygon indicates the "Sole Sanctuary". **(B)**. Sexual maturity ("Mat") and age ("Age") of sole individuals collected in each sampling Zone. Please note that "Age 0" means that sole individual's age was < 1 year old. **(C)**. Plot showing the relationship between weight and length of sampled common sole individuals and their age (bubble size) and sexual maturity (color gradient)

Otolith analysis

Whole sagittal otoliths were immersed in ethanol with the distal surface up (*sulcus acusticus* downwards) and read under a stereomicroscope using re ected light against a black background (5X and 10X magni cation) [31]. When the reading was doubtful, the burning and sectioning techniques were used to improve the observation of ring deposition [32]. e age was estimated by three readers separately by counting the opaque rings from the core to the edge (Figure S1) and then compared. Soles and other at shes in the Adriatic Sea are characterized by an opposite pattern of deposition compared to

that of other shes of temperate and cold waters, as the opaque ring is laid down in winter/spring and the translucent ring in summer/autumn [31]. One opaque and one translucent ring were considered an annual growth (*annulus*) If the disagreement persisted after a further reading, the otolith was discarded. For the nal age classi cation, the edge type, the date of capture and the theoretical date of birth (January 1st) were also considered [31].

DNA extraction and sequencing

From each type of sh tissue and sediments, DNA was extracted within 8 months from collection and using the DNeasy PowerSoil Kit (Qiagen) as described in Quero et al. [33]. Extracted DNA samples were stored at −20 °C until processing. After quanti cation, the PCR ampli cation of the V3-V4 hypervariable region of the 16 S rRNA gene was carried out using the primer pair 341 F-785R [34] and the PCR product puri ed as described in Palladino et al. (2021). Nextera library indexing and preparation and Illumina MiSeq sequencing (2×300 bp paired-end protocol) were performed as described in Palladino et al. [35].

Data analysis

Primer and adapter sequences were removed from raw reads with Cutadapt [36]. Paired-end reads were then imported and analyzed in RStudio version 4.4.0 (RStudio Team, 2020) using the DADA2 package (version 1.32) [37]. Quality check and trimming of the reads were performed following the package instructions (max estimated error>2 and 2 per 100 bp for forward and reverse reads, respectively). Paired-end reads were subsequently merged in amplicon sequence variants (ASVs, i.e., clusters sharing 100% sequence identity); chimeric sequences were identi ed and removed from the dataset. Finally, prokaryotic taxonomy was assigned using a native implementation of the naive Bayesian classi er method against the SILVA database (v138; https://www.arb-silva.de/documentation/release-138/). Chloroplast and eukaryotic sequences were removed from the ASV table obtained from DADA2; samples with low numbers of ASV were removed from the dataset (Supplementary Data File 1). Abundance values were normalized using the median value of the dataset with the *vegan* (version 6.1) and *phyloseq* (version 1.48) packages [38, 39] and transformed in relative abundances. For the analysis of alpha diversity, ASV richness was calculated using the *vegan* package.

e occurrence of statistical di erences among richness values in the di erent types of samples was assessed with ANOVA test (*stats* package, version 4.4.0) considering all possible comparisons (sediment vs. sh samples; gut vs. skin; gut vs. age; gut vs. maturity; skin vs. age; skin vs. maturity; gut vs. Zone; skin vs. Zone; sediment vs. Zone).

Non-metric multidimensional scaling (nMDS) was performed using a Bray–Curtis dissimilarity matrix and average linkage approach and plotted with the *ggplot2* package. Signi cant di erences in prokaryotic community composition between sample types (i.e., sh vs. environmental) as well as among sh tissues (i.e., skin, gut) were calculated by using ANOSIM through the *anosim* function (*vegan* package). A "Linear Discriminant Analysis E ect Size (LEfSe)" to nd biomarkers of each group was performed and plotted using the *ggplot2* package (version 3.5.1) [40].

Results

We analyzed the microbiota associated with the gut, skin and gills of a total of 77 wild common sole specimens sh collected in the Adriatic Sea. Biometric measures per each specimen are reported in Fig. 1 and Table S1. Brie y, sole individuals included 50 juveniles with an estimated age of 0–1 years, and 27 adults older than 2 years (2–4 years). Sampled shes displayed a mean length of 24.7±4.6 cm (within a range of 15.5–33.1 cm) and maturity class ranging from 1 to 5 (Fig. 1). In the entire dataset, 18 individuals were classi ed as males and 59 as females; all males were identi ed as <2 years and with a maturity stage of 1 or 2. Zone 2 was characterized by the absence of the youngest (<1 year) and immature (stage 1) organisms; in contrast, in the same zone, individuals with the highest maturity classes (4 and 5) represented more than half of collected samples. Furthermore, we observed that the youngest individuals (i.e., age 0) were distributed exclusively in the coastal stations, whereas adult samples were mostly collected from Pula and Ancona stations (Fig. 1). Age and sexual maturity were not signi cantly correlated. Similarly, sh size (i.e., length and weight) was not correlated to sh age and sexual maturity (Fig. 1C).

e number of initial raw reads and those passing the quality ltering performed by DADA2 are reported in Figure S2. Brie y, across the whole dataset, raw reads per samples ranged from 7,230 to 82,156 reads per sample, for an average number of 35,399 raw reads per sample. Rarefaction curves are reported in Figure S3. A total of 53,958 ASVs were observed across the entire dataset, of which 10,120 (18.8%) exclusive for sediment samples, 39,372 (73.0%) exclusive for sole samples and only 4,466 (8.3%) shared across all types of samples.

e analysis of microbial communities associated with sole tissues and environmental samples showed a signi cant di erence in alpha diversity (i.e., ASV richness and Shannon index) between sh and sediment samples (ANOVA, $p<0.001$), with higher mean richness in sediments (avg. ASV richness 1,135.57±256.4) than in soles (avg. ASV richness 597.76±353.3) (Table S3, Figure S4). Alpha diversity showed no signi cant di erence between sediments collected at di erent zones. With

the exception of gills, where richness was statistically lower than in the other sh tissues (avg. ASV richness 421.37±56.8, ANOVA, $p<0.01$), similar alpha diversity values were found among sole tissues' communities, i.e. gut (avg. ASV richness 572.3±326.6) and skin (avg. 640.8±388.8) (ANOVA, $p>0.5$). Overall, we observed higher variability in alpha diversity in younger samples (juveniles *versus* adults), with decreasing values observed with both increasing age and sexual maturity (Figure S4). However, the low number of samples belonging to older individuals as well as those with a higher maturity index was not su cient to allow for a statistical comparison for each group, thus hindering the possibility to clarify the signi cance of this trend.

At the phylum level, the common sole microbiota was dominated by Proteobacteria in all the analyzed tissues (Fig. 2A). Microbial communities associated with sh were particularly enriched in Gammaproteobacteria (avg. 49.19±23.52%), followed by Alphaproteobacteria (avg. 8.67±5.89%). Sole microbiota also displayed high relative abundances of Firmicutes (avg. 9.88±11.35%), Bacteroidota (avg. 7.6±5.2%), Planctomycetota (avg. 4.0±5.3%), and Actinobacteriota (avg. 3.5±3.1%). Higher proportions of Desulfobacterota (13.9±4.3%), Bacteroidota (20.1±5.4%), and Planctomycetota (7.5±4.5%) were overall observed in sediment rather than in sh samples, as well as a lower contribution of Gammaproteobacteria (25.62±4.95%) and Firmicutes (0.65±0.54%). Gammaproteobacterial proportions represented the main difference between gut and skin microbiota (39.88±21.86% and 60.95±18.59%, respectively; ANOVA, $p<0.001$). Gut samples showed higher levels of Firmicutes than skin samples (respectively, 12.7±12.09 and 5.3±5.9%; ANOVA, $p<0.001$), as well as of Actinobacteriota (5.6±3.1 and 1.7±1.5%), Verrucomicrobia (3.9±3.0 and 1.3±1.0%), Planctomycetota (6.7±6.7 and 1.5±1.4%) and Chloro exi (2.6±1.5 and 0.5±0.6%) (Fig. 2A). Despite only 8 samples of gills were available for microbiota analysis, all collected from Zone 1, our data showed that the community composition of this sole tissue di ered when compared to the other ones, with the highest abundances of Firmicutes (27.20±18.42%, Kruskal-Wallis $p<0.001$), Bacteroidota (13.72±8.91%), and Cyanobacteria (13.25±9.93%, Kruskal-Wallis $p<0.001$) (Fig. 2A). Taxa contributing to the di erences in community composition between sole and environmental microbiota were also explored through di erentially abundant taxa analysis (Figure S5 and Fig. 2B and C). is analysis corroborated the abovementioned results, indicating Clostridia/Clostridiaceae, Acidimicrobiia, Verrucomicrobiae, Planctomycetes, Anaerolineae, Desulfobacterota and Burkholderiales as the most di erentially abundant taxa in gut samples. Gammaproteobacteria (Alteromonadales, Pseudomonadales, Pseudoalteromonadaceae/*Pseudoalteromonas*, Moraxellaceae, *Psychrobacter*, *Vibrio* and *Acinetobacter*), and Desulfovibrionia/Desulfovibrionales characterized skin samples. Gill microbiota was characterized by Bacilli, Bacteroidia (Flavobacteriales), and Mycoplasmataceae (*Mycoplasma*) (Fig. 2).

e community composition of sh- and sediment-associated microbiota was statistically di erent (ANOSIM, $R=0.4$). At the same time, non-metric multidimensional scaling (NMDS) highlighted a clear separation of sediment microbial communities according to the sampling Zone (ANOSIM, $R=0.8$) (Figure S6). Considering only sh samples, microbiota associated with gut and skin were found to be signi cantly di erent (ANOSIM, $R=0.7208$, Signi cance=0.001) (Fig. 3A). Considering gut and skin microbial communities separately, we found that sampling Zone did not a ect community composition (ANOSIM, $R=0.27$ and 0.16 for gut and skin samples, respectively). On the other hand, sole skin microbiota signi cantly di ered according to the sampling stations (ANOSIM $R=0.42$, Signi cance: 0.001).

Gut microbiota composition was signi cantly and positively correlated with both age and sexual maturity (*envfit*, $p<0.005$), whereas no signi cant correlation was found considering the skin microbiota (Fig. 3B). e same analysis was not performed for gills due the low number of samples collected for this type of sh tissue. Examining the most enriched taxa in skin and gut communities, signi cant correlations were observed only in gut samples. In more detail, Bacteroidia and Desulfobacteria showed signi cant correlations with age, and a less signi cant correlation with sexual maturity (Fig. 3B and C).

Discussion

Considering both the commercial relevance and the ecological role of *S. solea*, as suggested in general for sheries science [1], a deeper understanding of the wild common sole microbiome is prompted to enhance our knowledge of this sheries resource and to guide future management decisions, as well as for potential aquaculture applications [41]. Despite extensive studies on the biology of this demersal species, to the best of our knowledge, no research has been conducted thus far to unveil the diversity and factors in uencing the wild common *S. solea* microbiome.

Fish microbiome plays a crucial role in enhancing the host immune system, promoting well-being, aiding in food digestion and facilitating the synthesis of vital nutrients [42–44]. Various environmental factors (e.g. seasonality, salinity, geographic location), dietary habit (e.g., carnivorous, herbivorous) and genetic variations (e.g., species, population and inter-individual variations), contribute to the diversity of sh microbiome across different tissues and organs [45 and references therein].

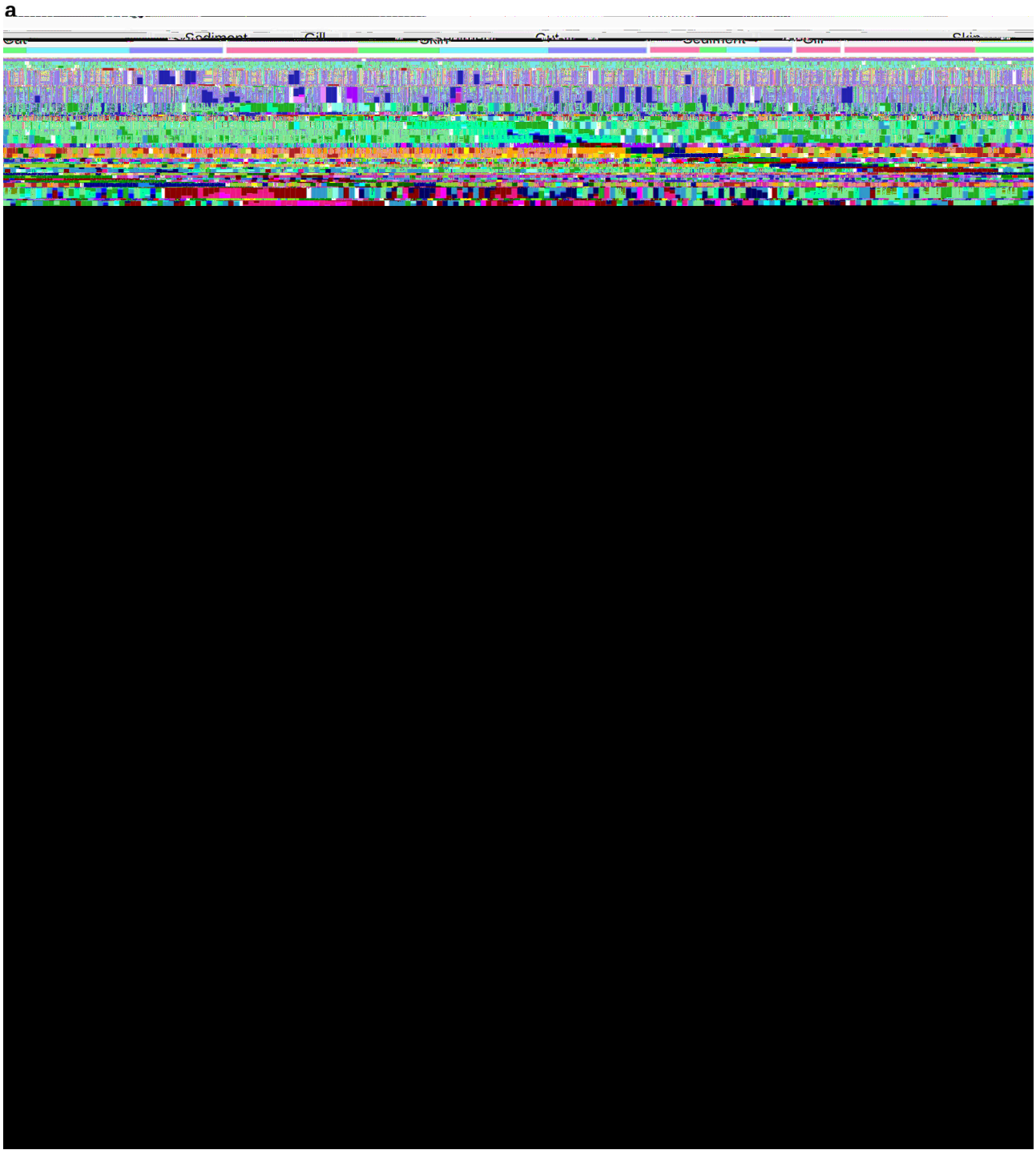

Fig. 2 **(A)** Barplot showing the prokaryotic community composition (as relative abundance) at the phylum and class level (for Proteobacteria only) separated according to sample type (sediment and fish gill, gut and skin) and sampling zone. Taxa with an average relative abundance < 1% across all samples were aggregated as "Others". **(B)** Taxonomic cladogram comparing skin, gill and gut microbiota using linear discriminant analysis effect size (LEfSe). Significantly discriminant taxon nodes are colored in green for skin, orange for gut and purple for gill samples; in addition, branch areas are shaded according to the highest ranked group for that taxon. A threshold of 3.2 was chosen for the logarithmic LDA scores. Not significantly discriminant taxa are represented in white. **(C)** The panel focuses on the most abundant and discriminant phyla (and class for Proteobacteria), grouped according to the type of fish tissue

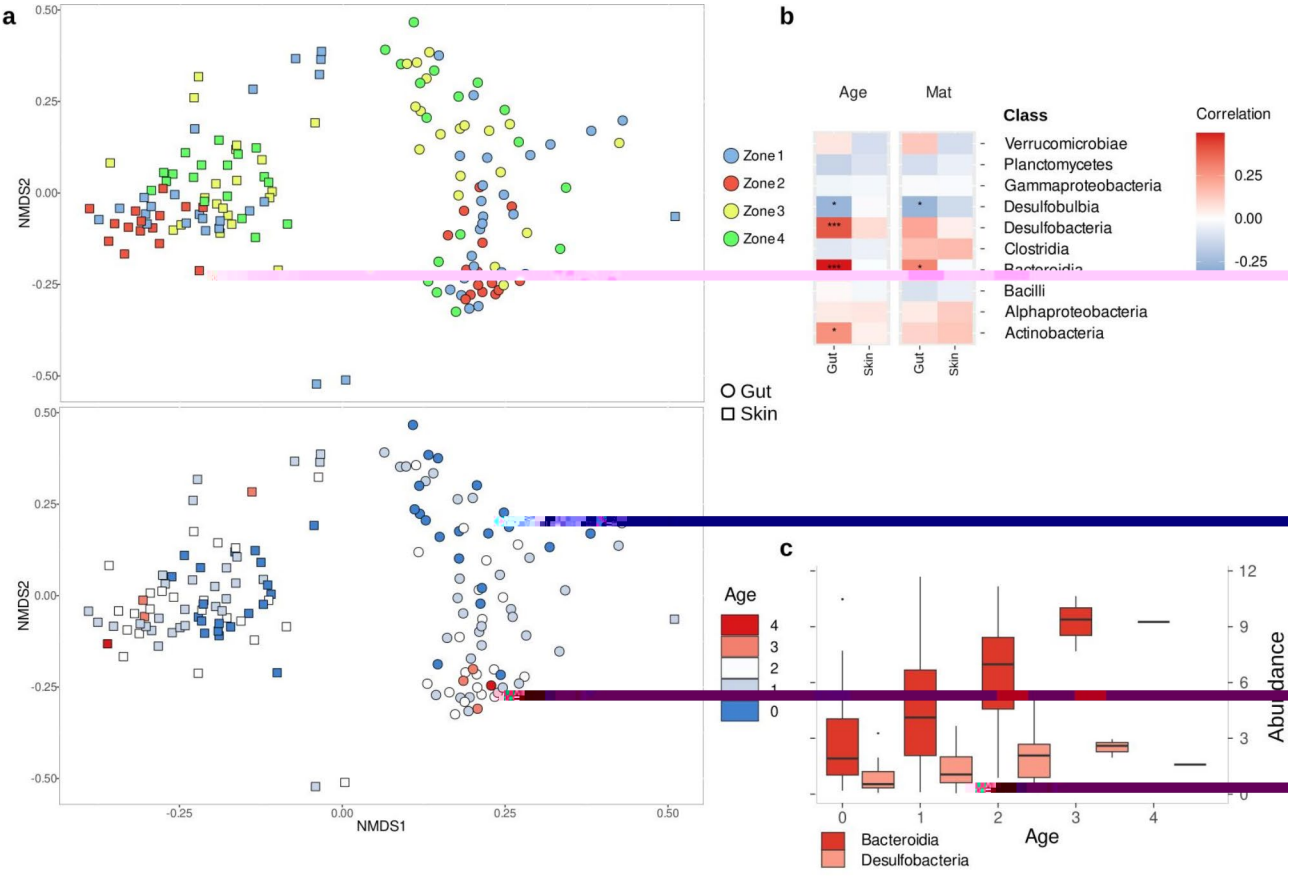

Fig. 3 **(A)** NMDS ordination (Stress = 0.2291931) based on Jaccard dissimilarity of gut (circles) and skin (squares) microbiota samples, colored in accordance to the shing area (upper panel) and the age of the sole (lower panel). **(B)** Pearson correlation analysis between the abundance of taxa (at Class levels) in gut and skin samples and the age and maturity stage of collected sole individuals; asterisks indicate signi cant correlations between the considered taxon and the biometric parameter (Pearson, *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$); **(C)** Box plots showing the increasing relative abundance of Bacteroidia and Desulfobacteria in gut samples with increasing sole age

Among other factors, age, sexual maturity and life stage have also been observed to be linked to microbiota diversity and composition in several sh species [45–48].

e lack of signi cant di erences among the microbiota of sole tissues in terms of alpha diversity along life stages, age and sexual maturity might indicate that changes among such di erent sh conditions occur mainly at the community composition level and, likely, at the functions exerted by the microbes inhabiting each tissue along sole individuals' life stages. Generally, the higher microbial diversity observed in sediment rather than in sh, as noted in our study, is now well-established and hypothesized to be linked to the higher loads of organic matter in sediments, and the high heterogeneity likely supports the growth and co-existence of a higher number of prokaryotic taxa [49, 50].

Here, we show that wild common soles collected in the Adriatic Sea exhibited a signi cantly di erent microbiota compared to the surrounding environment. At the same time, the microbiota of sole varied based on the analyzed body site. Our results support the large existing knowledge on sh microbiota, indicating that the host plays a crucial role in selecting speci c assemblages and represents the primary driver shaping sh microbiota [33, 45, 51, 52]. Additionally, the observed di erences in community composition between skin and gut microbiota suggest a clear distinction in the microbes inhabiting these two host compartments, likely related to the role such microbes play within each sh organ. Although limited in terms of data representativeness, gills microbiota also showed a distinction compared to skin and gut communities.

Overall, the sampling area did not appear to signi cantly in uence microbiota, suggesting a higher in uence of the sh host rather than the environment in shaping the associated microbiota [2, 33]. However, this was more evident for the gut and gill communities than the skin microbiota. Indeed, the skin-associated assemblages showed a higher similarity with sediment samples than the gut and gill, suggesting that the sampling station signi cantly a ects the composition of the skin microbiota.

is may be somehow expected, as the skin mucus, a

hydrated gel mainly composed of mucopolysaccharides, acts as a dynamic and semipermeable barrier with various functions in sole, including osmoregulation, respiration, nutrition or locomotion [53]. e skin mucus may likely trap microbes from the surrounding sediments. Considering the benthic lifestyle of this sh species and the substantial di erences observed among sediments collected in di erent zones and sampling stations (likely re ecting changing environmental conditions and gradients of anthropogenic impact), our data suggest the potential of common sole skin microbiota to act as a novel traceability tool, indicating the collection area for this species and supporting other control measures to achieve a healthy balance between sheries sector and stock exploitation [10]. More studies are needed to further investigate this topic.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-024-02303-5.

Supplementary Material 1: Table S1. Detailed data for each sole specimens and sample analyzed in this study. Per each Zone and station, reported are total length (TL), weight, length, sex, maturity stage and age. In the last three columns, reported are the full labels of the sample collected and analyzed per each sole individual at each sampling site. E.g. "Sole1-gut-S27": "Sole1" = sole individual #1, "gut" = DNA extracted from gut tissue, "S27" = station 27. Table S2. Detailed data for each sediment sample collected and analyzed in this study. Per each Zone and station, reported are Depth (meters), Longitude, Latitude, Sample number and sediment sample code. In the last column, reported is the full label of each sediment sample collected and analyzed. E.g. "Sediment1-S27": "Sediment1" = sediment sample #1, "S27" = station 27. Fig. S1. Age estimation of whole (A) and sectioned (B) otoliths of the common sole. Red dots indicate the opaque rings counted. Figure S2. Barplot showing the number of raw reads obtained after sequencing per each analyzed sample (total of green and red bars), number of reads removed after bioinformatic analysis (red bars) and number of reads which passed bioinformatic analyses and thus used for the study (green lines). Figure S3. Rarefaction curves of the analyzed sequenced samples. Different colors refer to different types of samples analyzed, as detailed in the legend. Table S3 Correlation analysis considering alpha diversity measures (i.e., Shannon index and ASV Richness) and age and maturity stage in gut and skin microbiota from common sole individuals analyzed in this study. Fig. S4. Alpha diversity analyses. (A) Box plots reporting Shannon index and ASV richness values calculated considering all sole versus sediment samples for each sampling zone; (B) Box plots reporting Shannon index and ASV richness values calculated considering all gut versus skin samples and grouped according to age and maturity stage ("Mat"). Fig. S5. Taxonomic cladogram comparing sediment, skin, gill and gut microbiota by linear discriminant analysis effect size (LEfSe). Significantly discriminant taxon nodes are colored in green for sediment, orange for skin, purple for gut and magenta for gill samples. Branch areas are shaded according to the highest ranked group for that taxon. A threshold of 3.2 was chosen for the logarithmic LDA scores. Not significantly discriminant taxa are represented in white. Fig. S6. NMDS ordination (Stress = 0.11) based on Jaccard dissimilarity of sediment microbiota samples, colored according to the sampling Zone. Supplementary Data File 1. ASV, Phylum, Class, Order, Family and Genus tables with taxonomy

Supplementary Material 2: Table of taxa abundance and identification at the ASV, Phylum, Class, Order, Family and Genus levels.

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Author contributions

GS, GML and GMQ conceived the study; GS and GML: resources; LS and GP: acquisition of samples; LS, GP, MB, NM, EM and FD: laboratory analyses; MB and GMQ: data analysis and interpretation; GMQ, MB and GML: manuscript drafting. All authors edited the manuscript and approved the final draft.

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Data availability

The dataset (raw sequences) supporting the conclusions of this article is available in the SRA—Sequence Read Archive (BioProject PRJNA1043696, BioSample SAMN38350398 to SAMN38350483) repository, (https://www.ncbi.nlm.nih.gov/sra). Original R scripts are available at the following GitHub page: https://github.com/marco-basili/Sole.

Declarations

Ethics approval and consent to participate

This study has been conducted in accordance with relevant guidelines and regulations. Fish specimens were collected by trawling within the SoleMon monitoring program set up by the Italian Ministry for Agriculture and Forestry (D.G. Fisheries and Aquaculture) and endorsed by the Italian Fishery Data Collection Programme (Programma Nazionale Raccolta Dati Alieutici). Sampling permission was issued by the 'CAPITANERIA DI PORTO ANCONA', permit number 06/03/26, NULLA OSTA N. 26/B/2019". To comply with ethical standards, cervical dislocation was used as a procedure, in accordance with the current European directive and national law (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, Decree Law n. 26 of 4 March 2014).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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