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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 fish growth and health and may be influenced by the changing environmental conditions. Here, we explored the microbiota of wild common sole, one of the most important fishery resources in the Mediterranean Sea, collected from different areas in the North Adriatic Sea. Our results show that the sole microbiota differs from that of the surrounding environment and among the different body sites (gill, skin and gut). Gut microbiota composition showed to be strongly related to fish age, rather than maturity, sex or sampling site. Age-related shifts in gut microbial communities were identified, with increased abundances of Bacteroidia and Desulfobacteria, unveiling potential microbial proxies for age estimation crucial for fisheries management. Our results expand the limited knowledge of the wild common sole microbiota, also in the light of the potential usefulness of the fish microbiota as a tool for future stock identification and connectivity studies.

**Keywords** *Solea solea*, Wild fish microbiota, Adriatic Sea

## Introduction

Fish farming, health, management, and habitat restoration are among the potential downstream uses of microbiome research in fish ecology [1]. Host-associated microbiomes present inside, as well as on body surfaces, can influence a broad range of host immunological, evolutionary, and ecological processes [2]. The microbiome is increasingly recognized as a crucial yet poorly explored

tool for managing fish resources, since it represents an effective way to understand how fish respond to environmental changes [2–6]. Therefore, exploring fish microbiota may represent a new strategy for promoting fish 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 fish biology, more studies on wild marine teleost fish species should be performed [7, 8]. This 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 flatfish 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 fishing activities carried out in the Central and Northern Adriatic basin [10, 13]. The 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  $\geq 3$  years) inhabits a portion of the offshore 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 fishing effort, 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 defined 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 fishing techniques (e.g., *rapido* trawling), especially in the Adriatic Sea [10, 23–25], that are critical factors influencing 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 fishery resource, we filled this knowledge gap by exploring, for the first 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 fish microbial communities with the surrounding environmental sources and to test whether the fish microbiota is influenced by the host habitat. This study is the first to explore the diversity and composition of the microbiota in wild common sole. Our findings should help direct future actions for stock management, as well as to guide aquaculture applications of this species.

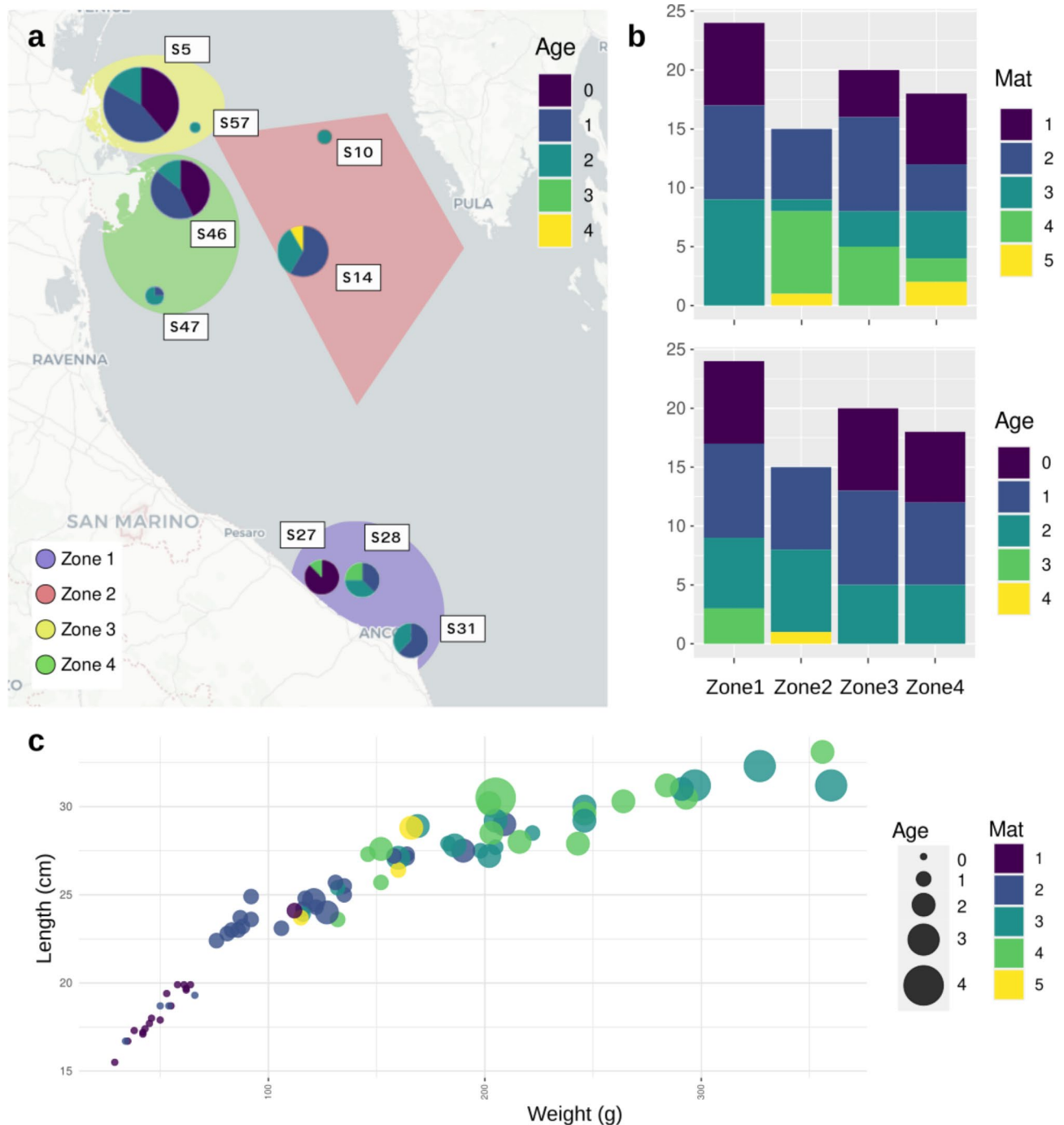
## Methods

### Sampling area

*S. solea* individuals were collected during the SoleMon *rapido* trawl survey 2019. This scientific fishing activity is carried out annually in fall according to a depth-stratified 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 fishery resources [27]. Samples were collected from 9 sampling stations, classified into 4 different ‘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). The 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 defined following a modified 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 fish 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 insufficient yield of extracted DNA, only 8 out of 77, samples (all collected at Zone 1) were analyzed as described later. All fish tissue samples were obtained by aseptic dissection of fish specimens using sterile scalpels and scissors. After collection, all tissue samples were immediately placed in sterile tubes rinsed with sterile phosphate buffer solution to remove possible loosely attached microorganisms and stored at  $-20^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$  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 different 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 reflected light against a black background (5X and 10X magnification) [31]. When the reading was doubtful, the burning and

sectioning techniques were used to improve the observation of ring deposition [32]. The 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 flatfishes in the Adriatic Sea are characterized by an opposite pattern of deposition compared to

that of other fishes 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 final age classification, 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 fish 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  $-20^{\circ}\text{C}$  until processing. After quantification, the PCR amplification 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 purified as described in Palladino et al. (2021). Nextera library indexing and preparation and Illumina MiSeq sequencing ( $2\times 300$  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 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; <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. The occurrence of statistical differences among richness values in the different types of samples was assessed with ANOVA test (*stats* package, version 4.4.0) considering all possible comparisons (sediment vs. fish 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. Significant differences in prokaryotic community composition between sample types (i.e., fish vs. environmental) as well as among fish tissues (i.e., skin, gut) were calculated by using ANOSIM through the *anosim* function (*vegan* package). A “Linear Discriminant Analysis Effect Size (LEfSe)” to find 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 fish collected in the Adriatic Sea. Biometric measures per each specimen are reported in Fig. 1 and Table S1. Briefly, sole individuals included 50 juveniles with an estimated age of 0–1 years, and 27 adults older than 2 years (2–4 years). Sampled fishes displayed a mean length of  $24.7\pm 4.6$  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 classified as males and 59 as females; all males were identified 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 significantly correlated. Similarly, fish size (i.e., length and weight) was not correlated to fish age and sexual maturity (Fig. 1C).

The number of initial raw reads and those passing the quality filtering performed by DADA2 are reported in Figure S2. Briefly, 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.

The analysis of microbial communities associated with sole tissues and environmental samples showed a significant difference in alpha diversity (i.e., ASV richness and Shannon index) between fish and sediment samples (ANOVA,  $p<0.001$ ), with higher mean richness in sediments (avg. ASV richness  $1,135.57\pm 256.4$ ) than in soles (avg. ASV richness  $597.76\pm 353.3$ ) (Table S3, Figure S4). Alpha diversity showed no significant difference between sediments collected at different zones. With

the exception of gills, where richness was statistically lower than in the other fish tissues (avg. ASV richness  $421.37 \pm 56.8$ , ANOVA,  $p < 0.01$ ), similar alpha diversity values were found among sole tissues' communities, i.e. gut (avg. ASV richness  $572.3 \pm 326.6$ ) and skin (avg.  $640.8 \pm 388.8$ ) (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 sufficient to allow for a statistical comparison for each group, thus hindering the possibility to clarify the significance 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 fish were particularly enriched in Gammaproteobacteria (avg.  $49.19 \pm 23.52\%$ ), followed by Alphaproteobacteria (avg.  $8.67 \pm 5.89\%$ ). Sole microbiota also displayed high relative abundances of Firmicutes (avg.  $9.88 \pm 11.35\%$ ), Bacteroidota (avg.  $7.6 \pm 5.2\%$ ), Planctomycetota (avg.  $4.0 \pm 5.3\%$ ), and Actinobacteriota (avg.  $3.5 \pm 3.1\%$ ). Higher proportions of Desulfobacterota ( $13.9 \pm 4.3\%$ ), Bacteroidota ( $20.1 \pm 5.4\%$ ), and Planctomycetota ( $7.5 \pm 4.5\%$ ) were overall observed in sediment rather than in fish samples, as well as a lower contribution of Gammaproteobacteria ( $25.62 \pm 4.95\%$ ) and Firmicutes ( $0.65 \pm 0.54\%$ ). Gammaproteobacterial proportions represented the main difference between gut and skin microbiota ( $39.88 \pm 21.86\%$  and  $60.95 \pm 18.59\%$ , respectively; ANOVA,  $p < 0.001$ ). Gut samples showed higher levels of Firmicutes than skin samples (respectively,  $12.7 \pm 12.09$  and  $5.3 \pm 5.9\%$ ; ANOVA,  $p < 0.001$ ), as well as of Actinobacteriota ( $5.6 \pm 3.1$  and  $1.7 \pm 1.5\%$ ), Verrucomicrobia ( $3.9 \pm 3.0$  and  $1.3 \pm 1.0\%$ ), Planctomycetota ( $6.7 \pm 6.7$  and  $1.5 \pm 1.4\%$ ) and Chloroflexi ( $2.6 \pm 1.5$  and  $0.5 \pm 0.6\%$ ) (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 differed when compared to the other ones, with the highest abundances of Firmicutes ( $27.20 \pm 18.42\%$ , Kruskal-Wallis  $p < 0.001$ ), Bacteroidota ( $13.72 \pm 8.91\%$ ), and Cyanobacteria ( $13.25 \pm 9.93\%$ , Kruskal-Wallis  $p < 0.001$ ) (Fig. 2A). Taxa contributing to the differences in community composition between sole and environmental microbiota were also explored through differentially abundant taxa analysis (Figure S5 and Fig. 2B and C). This analysis corroborated the abovementioned results, indicating Clostridia/Clostridiaceae, Acidimicrobiia, Verrucomicrobiae, Planctomycetes, Anaerolineae, Desulfobacterota and Burkholderiales as the most differentially abundant taxa in gut samples. Gammaproteobacteria (Alteromonadales, Pseudomonadales, Pseudoalteromonadaceae/*Pseud*

*alteromonas*, Moraxellaceae, *Psychrobacter*, *Vibrio* and *Acinetobacter*), and Desulfobacterota/Desulfobacteriales characterized skin samples. Gill microbiota was characterized by Bacilli, Bacteroidia (Flavobacteriales), and Mycoplasmataceae (*Mycoplasma*) (Fig. 2).

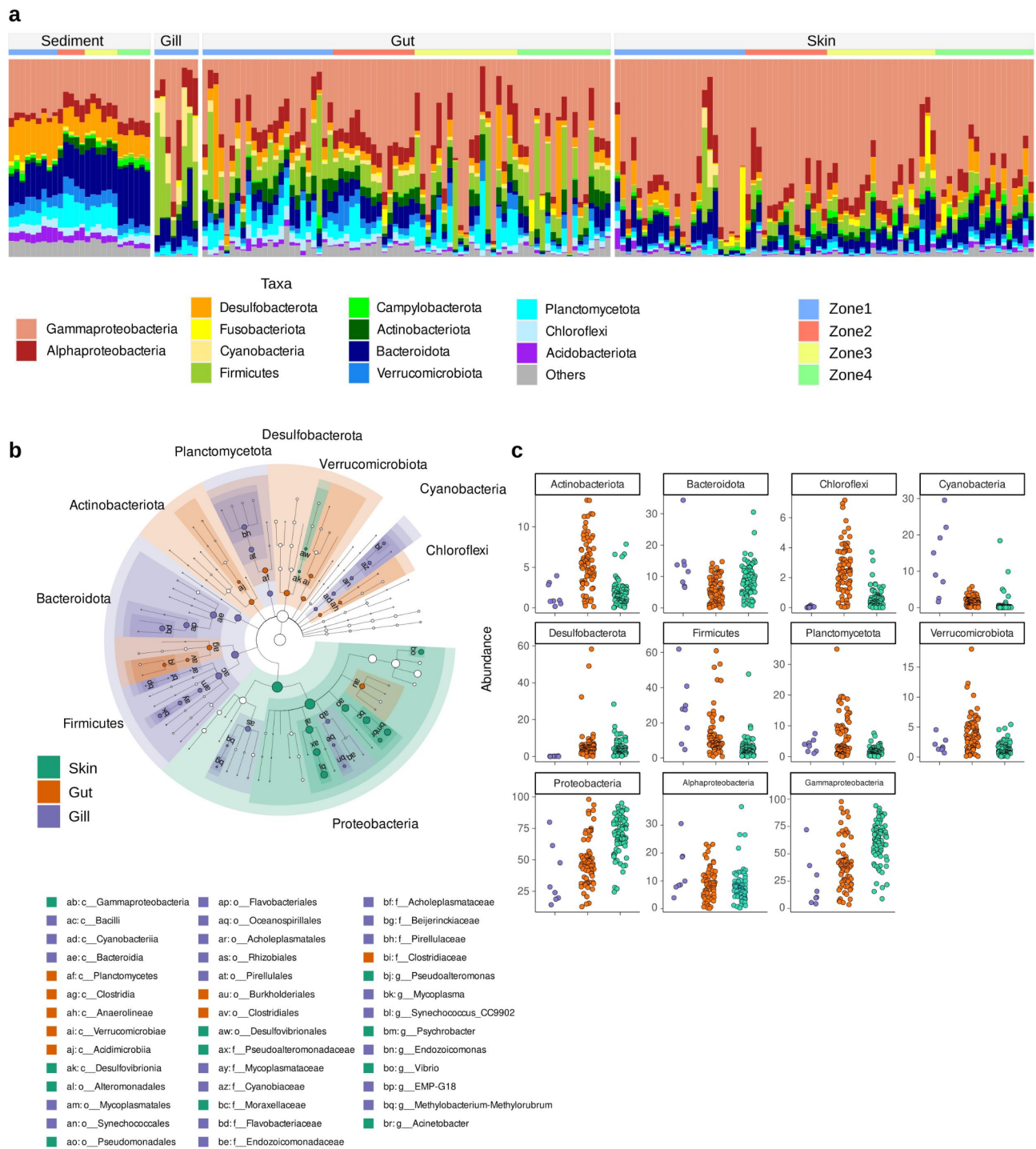
The community composition of fish- and sediment-associated microbiota was statistically different (ANOSIM,  $R = 0.4$ ). At the same time, non-metric multidimensional scaling (NMDS) highlighted a clear separation of sediment microbial communities according to the sampling Zone (ANOSIM,  $R = 0.8$ ) (Figure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

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

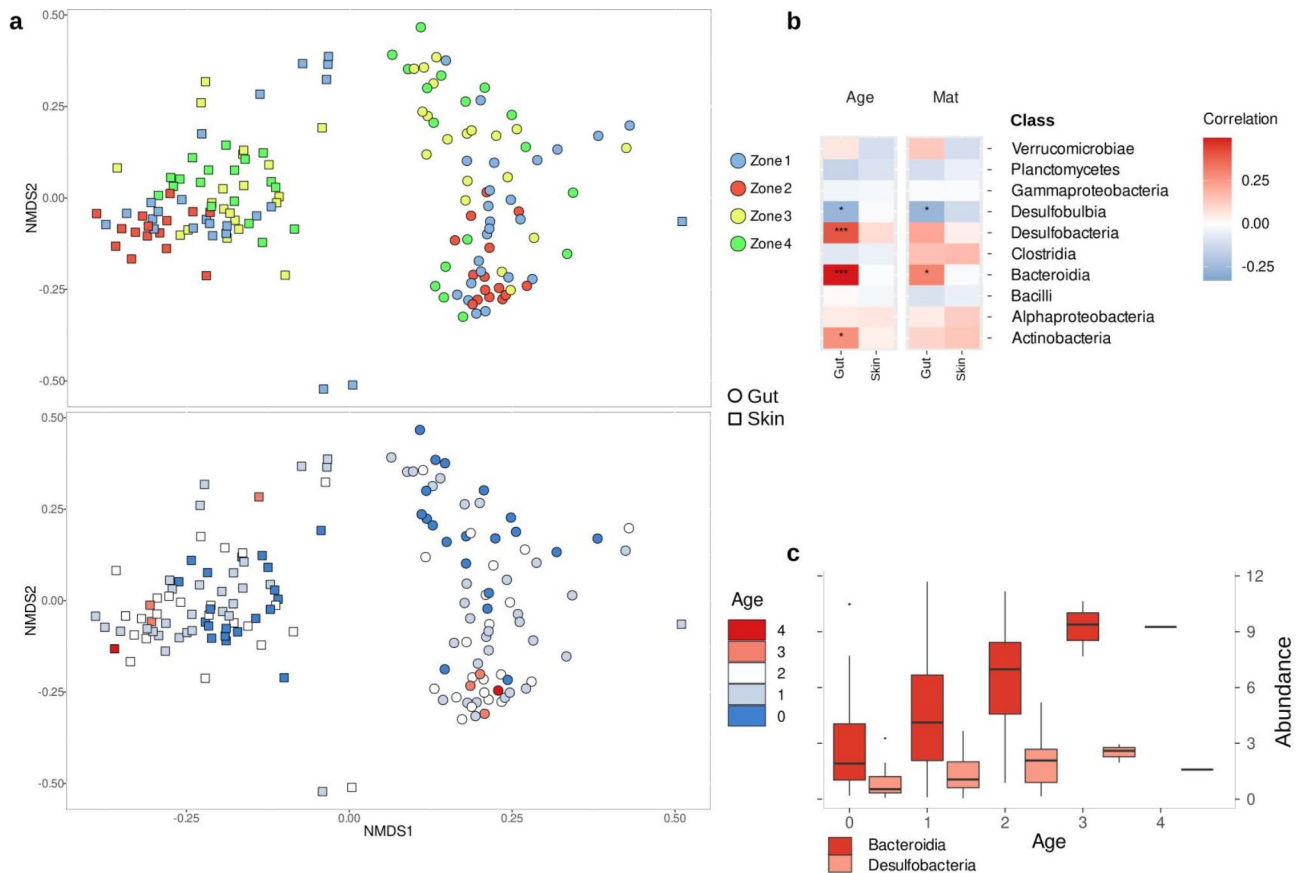
## Discussion

Considering both the commercial relevance and the ecological role of *S. solea*, as suggested in general for fisheries science [1], a deeper understanding of the wild common sole microbiome is prompted to enhance our knowledge of this fisheries 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 influencing 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 fish 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 fishing 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 significant 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 fish species [45–48].

The lack of significant differences among the microbiota of sole tissues in terms of alpha diversity along life stages, age and sexual maturity might indicate that changes among such different fish 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 fish, 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 significantly different 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 fish microbiota, indicating that the host plays a crucial role in selecting specific assemblages and represents the primary driver shaping fish microbiota [33, 45, 51, 52]. Additionally, the observed differences 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 fish 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 significantly influence microbiota, suggesting a higher influence of the fish 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 significantly affects the composition of the skin microbiota. This 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]. The skin mucus may likely trap microbes from the surrounding sediments. Considering the benthic lifestyle of this fish species and the substantial differences observed among sediments collected in different zones and sampling stations (likely reflecting 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 fisheries sector and stock exploitation [10]. More studies are needed to further investigate this topic.

The microbial community structure associated with the different common sole body sites was also investigated. Interestingly, members of Clostridiaceae and Verrucomicrobiae, particularly abundant in the gut samples, have been widely reported as dominant members of the animal gut microbiota, particularly in herbivorous fish [54, 55]. *Clostridium*, for example, is known for its ability in degrading cellulose [56], and contributing to host nutrition through the supply of fatty acids and vitamins [44]. Its presence in common sole can be linked to the dietary habits of this species, which feeds on relatively small benthic invertebrates, swallowing them with a huge amount of indigestible items, usually, sediment particles [57]. Therefore, such microbial community members may indicate the presence of microorganisms occurring both in the sediment and those associated with their prey, consisting of both animal and plant materials [58]. Beyond this natural symbiosis, some species of *Clostridium* have been proposed or successfully used as a probiotic in aquaculture [26, 59]. Regardless of fish host species and environment, Flavobacteriales and Bacillales are typically reported as components of the fish gill microbiota [60, 61], suggesting a potential role in this body site that deserves further investigations. Conversely, *Mycoplasma* is often referred to as a widely recognized gut microbiota member (or even symbiont) in several teleosts and invertebrates [35, 62, 63]. Despite being recognized as either parasitic or commensal to their host, several studies have underlined its positive role in fish hosts, such as in salmon, where its abundance of *Mycoplasma* has been suggested as a putative biomarker for monitoring performance and disease status in farmed salmonids [64]. Members of Alteromonadaceae (i.e., *Alteromonas* spp.) and Pseudomonadaceae (i.e., *Pseudomonas* spp.) have been found to be significantly abundant in the (healthy) fish skin microbiota [45].

The community composition of the common sole gut microbiota was found to be related to sole age data. This

linkage, already observed in other farmed and wild species such as surgeonfish [65], gilthead sea bream [66], southern catfish [67], and chinook salmon [68], suggests that age-related physiological changes may influence the common sole gut microbiota. Our results also show that the contribution of Bacteroidia and Desulfobacteria increases with fish age. These taxa could become helpful tools to combine with traditional age estimates through otolith analysis. However, further investigations based on a broader age range will be necessary to fully understand the potential of using the microbiota associated with age studies. Members of the Bacteroidia class have been reported to be normally mutualistic in the animal gastrointestinal flora, with a strong role in polysaccharides breakdown and metabolization [69], and proposed to promote carbohydrate fermentation in the fish intestine [70]. On the other side, paucity of knowledge is currently available on the presence and function of Desulfobacteria in fish microbiota, underscoring the need for detailed investigations on this aspect. However, Desulfobacteria are often associated with sulfate reduction and anaerobic environments [71] and, therefore, we hypothesize that such relation might indicate shifts in the gut environment or metabolic processes associated with fish age. Interestingly, microbial alpha-diversity didn't change across *S. solea* age categories, suggesting that microbiota set up in this species occurs mainly at the compositional level, rather than in terms of alpha-diversity.

## Conclusions

Our investigation provides the first exploration into the microbiota of wild *S. solea* in the Central and Northern Adriatic Sea, offering insights to decipher the association between microbial communities and various biometric parameters. The observed distinctions in microbial composition across different body sites and their correlation with fish age highlight the dynamic nature of the host-microbiome relationship in sole. Notably, the identification of Bacteroidia and Desulfobacteria as potential age-related microbial proxies opens avenues for integrating age estimates with otoliths in *S. solea*, promising practical applications in fisheries management. Additionally, the unique microbial signatures in skin microbiota, indicative of collection areas, suggest a potential new application to be explored for a variety of purposes, including fish traceability, ecological monitoring and movement ecology of this species. Overall, our study enhances our comprehension of the common sole microbiota, providing a foundation for future research on host-microbiome interactions in wild fish populations. Future surveys and additional large-scale microbiome data collection will certainly contribute to a better understanding and knowledge of this species and its ecology.



## 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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