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Host Species and Body Site Explain the Variation in the Microbiota Associated to Wild Sympatric Mediterranean Teleost Fishes

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► To cite this version:

M. Ruiz-Rodríguez, M. Scheifler, S. Sanchez-Brosseau, E. Magnanou, N. West, et al.. Host Species and Body Site Explain the Variation in the Microbiota Associated to Wild Sympatric Mediterranean Teleost Fishes. *Microbial ecology*, inPress, 10.1007/s00248-020-01484-y . hal-02451111

HAL Id: hal-02451111

<https://hal.sorbonne-universite.fr/hal-02451111>

Submitted on 23 Jan 2020

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1 **Host species and body site explain the variation in the microbiota associated to wild**
2 **sympatric Mediterranean teleost fishes**

3

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18 **ABSTRACT**

19 Microorganisms are an important component shaping the evolution of hosts and as such,
20 the study of bacterial communities with molecular techniques is shedding light on the
21 complexity of symbioses between bacteria and vertebrates. Teleost fish are a
22 heterogeneous group that live in a wide variety of habitats, and thus a good model group
23 to investigate symbiotic interactions and their influence on host biology and ecology.
24 Here we describe the microbiota of thirteen teleostean species sharing the same
25 environment in the Mediterranean Sea, and compare bacterial communities among
26 different species and body sites (external mucus, skin, gills and intestine). Our results
27 show that *Proteobacteria* is the dominant phylum present in fish and water. However, the
28 prevalence of other bacterial taxa differs between fish and the surrounding water.
29 Significant differences in bacterial diversity are observed among fish species and body
30 sites, with higher diversity found in the external mucus. No effect of sampling time nor
31 species individual were found. The identification of indicator bacterial taxa further
32 supports that each body site harbours its own characteristic bacterial community. These
33 results improve current knowledge and understanding of symbiotic relationships among
34 bacteria and their fish hosts in the wild, since the majority of previous studies focused on
35 captive individuals.

36 **Keywords:** Bacteria, Metabarcoding, Mediterranean Sea, Microbiota, Teleost fish

37 INTRODUCTION

38 Symbiosis among microorganisms and hosts are driving forces of evolution. Among
39 microorganisms involved in symbioses with animals, bacteria are the most widespread
40 and ubiquitous, shaping the evolution of their hosts by contributing both positively (basic
41 functions) and negatively (pathogenicity) to their fitness [1]. In-depth knowledge of the
42 taxonomic composition of the microbiota, and their relationships with their hosts, can
43 provide insights into both the function and dysfunction of the host organisms [2], due to
44 the important role that host-microbe interactions play in the physiology and performance
45 of animals [3].

46 Fish constitute about half of living vertebrate species [4], but the majority of studies on
47 microbiota have been conducted in mammals [5, 6]. High throughput 16S rRNA gene
48 sequencing is being increasingly employed to investigate the fish microbiota (e.g., [7, 8,
49 9,]), producing high-resolution descriptions of their community structure and diversity [2,
50 10], and providing further understanding of the consequences of symbioses with bacteria
51 [11]. Despite progress, important gaps remain in our current knowledge regarding the
52 factors that shape fish microbiota [12], such as environmental or phylogenetic variables,
53 whose influences could vary among species and / or habitats. Furthermore, most of the
54 information available is focused on the gut of captive species used in aquaculture, i.e.,
55 fish of economic significance and commercial interest [8, 13], or from model organisms
56 such as the zebrafish [14].

57 Wild teleost fishes exhibit a broad variety of morphologies, physiologies, ecologies and
58 natural histories [11, 15]. Therefore, they represent a good model group to investigate the
59 factors that shape host-bacterial assemblages in nature [16]. Fishes are in intimate contact
60 with the surrounding water, and bacterial colonisation from the environment is thought
61 to be one of the primary mechanisms of microbiota acquisition for fishes [16, 17]. Hence,
62 environmental factors influencing water microbial reservoirs, such as water salinity,
63 could ultimately influence fish microbiota [11, 18]. The water environment also hosts
64 high concentrations of potentially pathogenic microorganisms [19] that may enter the fish
65 body. Therefore, the dynamics and diversity of the microbial communities originating
66 from the surrounding water could influence significantly the individuals' fitness [20].

67 The external surfaces of fishes, such as the skin and gills, are coated in a mucus secretion
68 that hosts an indigenous microbiota dominated by bacteria [10, 21, 22] that acts as a
69 protective barrier against pathogens [23-26]. The digestive tract of fish also receive water

70 and food that are populated with microorganisms that will undoubtedly affect the resident
71 microbiota.

72 Besides the surrounding water, host-related factors may also shape fish-associated
73 microbial communities [18, 27]. In some teleost species, the skin bacterial diversity is
74 very different from that of the surrounding bacterioplankton, and varies among different
75 parts of the host body [28]. In addition, different species reared in the same water
76 environment vary in their gut [18, 29] and gills [9] microbiota.

77 We hypothesize that wild teleost microbiota could be influenced by abiotic environmental
78 factors and host-related factors. If host-related factors are more important than abiotic
79 factors in determining the fish microbiota, we would expect to find specific microbiota
80 from each part of the body, and in different fish species, independently of when the fishes
81 were captured. To test this hypothesis, and evaluate the importance of these different
82 factors, multiple individuals of 13 species of teleost were collected at the same location
83 in the shallow Mediterranean Sea on four different occasions and four body sites were
84 sampled (mucus, skin, gills, and gut). The microbial community composition of these
85 samples was characterized by 16S rRNA gene sequencing and their alpha and beta
86 diversity compared. We also tried to determine indicator bacterial taxa that could be
87 representative for each teleost species and body site, being both unique to a given group
88 (exclusivity) and occurring in all sample units within a group (fidelity) [30, 31].

89 This study is novel in characterizing the bacteria associated with wild fish, while the
90 majority of previous studies addressed bacterial assemblages in captive fish.

91

92 **MATERIAL & METHODS**

93 Fish sampling

94 Fishes were captured in the NW Mediterranean Sea, Bay of Banyuls (Gulf of Lion,
95 Banyuls-sur-mer, SE France, 42°29'4.618''N, 3°8', 35.39''E) on 4 different days in
96 2017: June 21, June 26, July 18, and October 4, and several physicochemical variables
97 for each sampling were recorded (Table S1). We captured a total of 59 individuals
98 belonging to 5 families and 13 species (see Table 1). For all fish individuals except the
99 Family Gobiidae, one gill net was placed between 0 and 6 m depth during the entire night
100 period, and at sunrise, it was recovered with the fish (42°29'15.073''N, 3°7', 49.688''E).
101 Fish were collected dead, handled with gloves and stored into individual plastic bags.

102 They were immediately brought from the vessel to the laboratory. They were kept at 4 °C
103 until dissection, within the next 48 h, mostly within 6 h. Individuals from the Family
104 Gobiidae were captured with one net placed for just 2 hours close to the seashore.

105 All thirteen species share nearly the same environment since they were captured in the
106 same place at the bay, and most of these species have similar feeding habits. In general,
107 they are omnivorous, eating mainly small crustaceans, invertebrates and small fishes, but
108 they can also ingest, to different degrees, other particles such as algae and phytoplankton
109 [33]. There is an exception for the species *S. salpa*, in which juveniles are omnivorous
110 (mainly carnivores), but adults are exclusively herbivores [33]. The adult stage is reached
111 at about three years, when the body size is longer than 20 cm [34]. The length of
112 individuals belonging to *S. salpa* that were captured were shorter than 20 cm for four of
113 the five individuals (the lengths were 16.8, 17.2, 15.5 and 14 cm). The fifth individual
114 was of adult size (23.8 cm.) This is the only individual, from all fish investigated here,
115 whose feeding would be exclusively vegetarian. For the other 12 species, no significant
116 differences in feeding habits according to age are described [33].

117 Once in the laboratory, each sample was taken as follows: (i) the skin mucus was scraped
118 with a sterile scalpel from the entire body surface; (ii) when the mucus was completely
119 removed, a 3 cm² piece of skin was cut from the central part of the body, close to the
120 lateral line; (iii) then, one gill arch was cut from each side of the body; finally, (iv) 5 cm
121 of the intestine distal part was cut. We cut the final part of the gut because, due to
122 defecation, it reflects the bacterial community of the entire intestinal tract [35, 36].
123 Because of possible differences in bacterial communities among different parts of the
124 same organ [28], samples were always taken from the same part of the body in all the
125 different species, sterilizing the material by flaming before taking each part of the same
126 individual. Samples were immediately placed into sterile Eppendorf tubes and kept at -
127 80 °C until DNA extraction. In the case of the species *Scorpaena notata*, there was no
128 mucus on the skin, so the skin piece was cut directly.

129 At each sampling site, 2 litres of seawater were taken from the same place where the nets
130 were installed and the water stored in a sterile glass bottle. Briefly, in the lab, the water
131 was vacuum filtered through a 47 mm diameter membrane filter with a pore size of 0.2
132 µm (Fisherbrand, Thermo Fisher Scientific, France). This filter was kept at -80 °C prior to
133 DNA extraction.

134

135 DNA sequencing

136 A total DNA extraction was performed using the Kit Quick-DNA Fecal/Soil Microbe
137 MiniPrep Kit (Zymo Research, Orange, California), following the manufacturer's
138 instructions. The V4-V5 regions from the 16S rRNA-encoding gene of isolated DNA
139 were amplified by means of PCR, using the universal primers 515F-Y (5'-
140 GTGYCAGCMGCCGCGGTAA) and 926R (5'-CCGYCAATTYMTTTRAGTTT)
141 [37]. The PCR mix contained 5 µl of KAPA 2G, 0.2 µl of each primer, 3.6 µl of ultrapure
142 water and 1 µl of DNA for a final volume of 10 µl. After 3 min of initial denaturation at
143 95 °C, the following conditions were applied: 22 cycles of 95 °C for 45 s (denaturation),
144 50 °C for 45 s (annealing) and 68 °C for 90 s (extension), ending with a final extension
145 at 68 °C for 5 min. For each sample, three PCRs were performed in the same conditions,
146 to increase the DNA quantity, while minimizing cycle numbers to avoid PCR bias [38].
147 The product of each PCR was verified by agarose gel electrophoresis and triplicate
148 reactions were pooled.

149 A second PCR was performed to attach the Illumina adapters and 8-bp barcodes for
150 multiplexing. They were added in a reaction mix in which barcode sequences were
151 individually added for each sample. The mix for this second PCR contained 12.5 µl of
152 KAPA 2G, 0.5 µl of each barcode primer (Nextera Index Sequences in
153 <http://seq.liai.org/204-2/>), 10.5 µl ultrapure water and 1 µl of DNA for a final volume of
154 25 µl. PCR conditions were as follows: initial denaturation at 98 °C for 30 s, 8 cycles of
155 98 °C for 10 s, 60 °C for 20 s, 72 °C for 30 s, and a final extension at 72 °C for 2 min.
156 PCR products were purified using the USB ExoSAP-IT PCR Product Cleanup
157 (Thermofisher, France) kit by incubating the samples at 37 °C for 30 min, followed by 15
158 min at 85 °C. The concentration of samples was normalized with the SequalPrep
159 Normalization Plate (96 well) Kit (Thermofisher, France). Thereafter, all the samples
160 with different barcode sequences belonging to each run were pooled, and the pooled
161 products were quantified by using the Quant-iT™ PicoGreen (Thermofisher, France).
162 The normalized amplicons were concentrated by using the Wizard SV Gel and PCR Clean
163 up Kit (Promega, France) and amplicons were sequenced one run in an Illumina® MiSeq
164 sequencer using the 2 × 250 bp protocol by FASTERIS SA, Switzerland.

165

166 Sequence analyses

167 Sequence analysis was performed by using a combination of tools, including the
168 USEARCH v9.0.2132 [39] program, Qiime V.1.9.1 [40] and *bash* scripts. Briefly, from
169 demultiplexed fastq files obtained from the sequencing center, forward and reverse reads
170 were merged to create consensus sequences in a single fastq file using *usearch9 -*
171 *fastq_mergepairs*. Assembled reads were quality trimmed by the *usearch9 -fastq_filter*
172 command. Primer sequences were removed from raw amplicon sequences using
173 *strip_primers_exclude.py*. At this point, sequences were dereplicated and denoised using
174 *usearch9 -unoise* (minampsize 8). As a certain number of artifactual short sequences
175 remained, sequences less than 360 bp were removed with a combination of *bash* scripts
176 and the *filter_fasta.py* command from Qiime V.1.9.1. OTU tables were generated by
177 *usearch9 -usearch_global* against the primer-stripped raw sequences with a 97 % identity
178 to OTU representatives ordered by abundance. OTUs were identified using Qiime V.1.9.1
179 *assign_taxonomy.py -m rdp* and a previously described modified version of the
180 Greengenes [41] August 2013 database [9]. The taxonomy assignments were corrected to
181 contain full paths from phylum to species. Final OTU tables were generated using biom-
182 format. Sequences matching “Archaea”, “Eukaryota”, “Unassigned”, “Chloroplasts” and
183 “Mitochondria” were discarded using *filter_taxa_from_otu_table.py*. Details of data
184 analysis, and the analysis environment are described in the supplemental methods.
185 Further details on the bioinformatics methods, as well as the samples characteristics,
186 including the number of reads and of sequences, are in the Electronic Supplementary
187 Material. All the resultant sequences were deposited in the Sequence Read Archive (SRA,
188 <http://www.ncbi.nlm.nih.gov/sra>) (Accession number: PRJNA531247).

189

190 Statistical analyses

191 R 3.5.0 [42] was used to calculate both α -diversity (Shannon diversity index) and β -
192 diversity matrices of distances among samples. Matrices of genera were used since they
193 contained the most detailed information on the total community, because in many cases
194 species assignment was not possible using the rdp classifier and the sequenced region.
195 However, in some of the OTUs, the genus classification was not possible, and in those
196 cases we used the most precise taxonomic level that we could identify (for example the
197 family, or the class). The Shannon diversity index based on OTUs was estimated by the
198 R *vegan* package [43]. Due to the high variation of the index values, it was standardized

199 before subsequent analyses. This technique is very useful and provides simplification,
200 since it modifies the scale and considers the mean as zero (e.g., [44]). This variable was
201 fitted on a normal distribution and met the normality criteria. Differences in Shannon's
202 diversity between fish species and body parts were analysed using one-way ANOVA in
203 Statistica 7.1 [45]. In addition, a General Linear Mixed Model (GLMM) was applied to
204 identify which variables (fish species, body part and sampling date, the last being
205 considered as a random factor) influence bacterial richness.

206 To evaluate the effects that the species, body site, individual fish or sampling date would
207 have in explaining the variability of the bacterial communities' composition in teleosts
208 (i.e., β -diversity), we performed a PERMANOVA analysis. First, a distance matrix on
209 resultant OTUs was constructed with the function *vegdist* in the R package "vegan",
210 which was considered as the dependent variable. Here, bacterial communities of each
211 sample are compared with all the rest, by means of the Bray-Curtis dissimilarity index for
212 pairs of samples [43]. The index values ranging from 0 to 1: a value of 0 (i.e., no distance)
213 indicated that two samples share all the bacterial taxa in the same abundances, and 1 (i.e.,
214 maximum distance) indicated that they did not share any of the detected OTUs. This
215 distance matrix was considered as the dependent variable in further analysis. Then, the
216 *adonis* function of the "vegan" R package was used with all the previously mentioned
217 explanatory factors, considering the sampling date as the stratification variable. Bray-
218 Curtis distances were also represented by means of a non-metric multidimensional scaling
219 (NMDS), implemented in the R package *phyloseq* [46]. Because for some of the groups
220 we have less than 3 replicates (Table 1), we performed the analyses with and without
221 those groups, obtaining virtually the same results. We show here the results with $N > 3$
222 for each category, and in the supplementary material, the results obtained with the whole
223 dataset (Tables S2, S3 and S4).

224 Finally, we performed an analysis to determine indicator species of the community in
225 which the patterns of species distribution were compared among different categories (all
226 body sites within each fish species), by using the *Indicspecies* R package [47, 48]. The
227 results display only bacterial taxa that are significantly ($p < 0.05$) prevalent in a given
228 category while absent in the rest. A target taxon is considered as "indicator" if it is
229 exclusive to a given group (exclusivity) and occurs in all sample units within a group
230 (fidelity). In this way, the analysis is based on the abundance within particular groups
231 (exclusivity), and on the relative frequency of species within groups (fidelity; [31, 49]).

232 However, groups may be composed of a single or multiple categories (such as the case
233 where indicator species are present in all of the samples of both categories but absent
234 from the rest). An indicator value index is assigned between a species and each group,
235 identifying the group with the highest association value. Then, randomization methods
236 (permutation tests) are used to test the statistical significance of values [31, 49].

237

238 **RESULTS**

239 Bacterial taxonomic composition of the fish and water samples

240 We obtained a total of 2,964,227 sequences assigned to bacteria (i.e., filtering out reads
241 belonging to Archaea, Eukarya and unidentified reads). After filtering, the remaining
242 OTUs belonged to 181 genera, spread over 108 families, 67 orders, 37 classes and 13
243 phyla. The most abundant bacterial group in the fish samples was *Proteobacteria*, when
244 considering both body site (Table 2) or host species (Table 3), and this group was also
245 the most abundant in the water column. The second most abundant group in the water
246 samples was *Bacteroidetes* (34.1 %), while in fishes this group was not highly
247 represented, except in *S. salpa* (16.1 %). In fishes, the other abundant bacterial phyla were
248 *Fusobacteria* and *Firmicutes*, that were rare in the surrounding water (0.7 and 0.3 %
249 respectively). Of the most abundant taxa (> 1% of total sequences) *Proteobacteria*
250 showed a relative abundance of around 80 % in the mucus, skin and gills, although its
251 abundance was only 37.6 % in the intestine. The other most abundant taxa in the intestine
252 were *Tenericutes*, *Firmicutes* (25.9 %), and *Fusobacteria* (13.7 %), and these groups
253 were either absent from (*Tenericutes*), or showed lower abundances in the other three
254 body sites.

255 In detail, the majority of the OTUs retrieved from fishes belonged to the family
256 *Vibrionaceae* (*Proteobacteria*, class *Gammaproteobacteria*), and were mainly assigned
257 to the genera *Vibrio* and *Photobacterium*, followed by the genus *Cetobacterium*
258 (*Fusobacteria*; Table 2). The relative abundances of these three genera were similar in
259 mucus, skin and gills, although in the latter tissue, *Cetobacterium* were more abundant
260 than *Photobacterium* (Table 2). In the intestine, the most abundant group was the order
261 *Clostridiales* (*Firmicutes*), and the Genus *Cetobacterium* (*Fusobacteria*). Within the
262 family *Vibrionaceae*, the most abundant genera in the intestine included *Aliivibrio* and
263 *Photobacterium*. *Vibrio* was clearly less abundant in the intestine compared to external

264 surfaces. The proportion of each bacterial group (for groups that represent more than 1%
265 of the total abundance) varied among different species (Table 3), although
266 *Photobacterium* and *Vibrio* were predominant in the majority of the species.

267 Flavobacteriales (Bacteroidetes phylum) were abundant in the seawater samples (30 %)
268 as were the *Rhodobacterales* (family *Rhodobacteraceae*) and *Rickettsiales* (family
269 *Pelagibacteriaceae*) orders of the Alphaproteobacteria class, showing respectively
270 relative abundances of 18.7% and 14.5%. The class *Gammaproteobacteria* constituted
271 7.2 %, with *Vibrio* representing 1.5 % of total reads of this class and the
272 *Synechococcaceae* family (Cyanobacteria) accounted for 4.2 of the total sequences.

273

274 Alpha-diversity of bacterial communities

275 There were significant differences in levels of Shannon α -diversity when comparing all
276 the different fish species and water ($F_{12,174} = 2.16$, $p = 0.015$, Figure 1). However, when
277 removing the water from the analyses, differences among species were no longer
278 significant ($F_{11,171} = 1.54$, $p = 0.12$). This result implied that differences were among the
279 water and the rest of species, with water displaying a higher α -diversity than all the fish
280 species (Fig. 1). When separating by the water and different body sites, we found
281 significant differences among groups, with again water showing the highest α -diversity,
282 and then the mucus ($F_{4,82} = 20.39$, $p < 0.0001$, Fig. 2). Post-hoc tests revealed no
283 significant differences among the water and the mucus samples ($p = 0.28$), while both
284 categories were different from the other three groups (all $p < 0.001$). Therefore, in this
285 case, when removing the water from the analyses, significant differences remained due
286 to differences between the mucus and the rest of body sites ($F_{3,179} = 23.13$, $p < 0.0001$).
287 No differences were found when comparing the skin, gills and intestine (all $p > 0.1$, Fig.
288 2). Results with all the dataset are presented in Table S2.

289 The GLMM results suggest that fish species ($F_{11,165} = 1.82$, $p = 0.05$) and body site ($F_{3,165}$
290 $= 23.84$, $p < 0.0001$), but not the sampling day ($F_{3,165} = 0.85$, $p = 0.47$), influenced the
291 Shannon diversity index. Results were the same with the entire dataset (Table S3). The
292 α -diversity comparisons among the body sites in each species, and in all the species for
293 each body site, are presented in Fig. S1 and S2.

294

295 Beta-diversity

296 The PERMANOVA results based on Bray-Curtis distances (i.e., β -diversity, Fig. S3)
297 determined that both factors, fish species and body site, explained the variation in
298 bacterial community composition among the samples (Table 4). This means that
299 individual fish within a given species have significantly more similar bacterial
300 communities than with fish from other species, which is also the case with the different
301 body sites. However, individuals and sampling date did not have any significant effect on
302 the structure of the microbiota, which means that individuals from the same species share
303 their microbiota, independently of the sampling date (Table 4). This result clearly discards
304 the possibility of any contamination among fishes at the same sampling date. Tests done
305 with the whole dataset were similar (Table S4).

306

307 Indicator bacteria

308 Several indicator bacterial groups were significant (all $p < 0.05$, listed in Supplementary
309 material, Table S5). Results displayed a high variation among different fish species
310 regarding indicator microbial species. *Sarpa salpa* hosts the highest bacterial diversity,
311 and also the highest number of indicator bacteria (95 in total, see Table S5). In addition,
312 only this species hosted the phylum *Bacteroidetes*, which was relatively abundant in the
313 water. No indicator bacterium was found in *Gobius cruentatus*, nor in the water samples.
314 In all fish species except *S. salpa*, the gut samples did not share any indicator bacteria
315 with the other three body sites. For the other body sites, gills and mucus shared indicator
316 bacteria in five host species, the gills with the skin in two species, and the mucus with the
317 skin in seven species. In six fish species, the mucus, skin and gills shared several indicator
318 bacterial taxa (Table S5).

319 The body site with the highest number of indicator bacteria was the mucus (Table S5).
320 However, in the species *S. notata* that lacks external mucus, indicator bacteria from skin
321 were by far the most numerous in this part of the body (18 taxa against nine in *S. salpa*,
322 and one in *G. buccichi*). In the rest of the species, no indicator bacteria were found
323 exclusively in the skin.

324 An overview of indicator bacteria from all fish species showed that mucus had the highest
325 number of exclusive bacterial taxa (30 taxa), followed by skin (15 taxa, Fig. 3). Although
326 there were many bacterial taxa that were shared between the different categories, the

327 highest number was shared by mucus, skin and gills, whereas the gut shared a lower
328 number of indicator bacteria with the other three body sites (Fig. 3).

329

330 **DISCUSSION**

331 Our results confirm that *Proteobacteria* was the predominant phylum in water and teleost
332 fish in the Mediterranean Sea, as it was indicated by previous studies [50]. However, the
333 prevalence of *Bacteroidetes*, *Fusobacteria* and *Firmicutes* differed among the water and
334 fish samples.

335 Both environmental and/or host-associated factors may shape fish microbiota at different
336 levels [9, 51, 52, 53]. Previous studies found that the mucus microbiota may be highly
337 variable and dynamic, depending on the environmental conditions including seasonality
338 [54, 55]. However, other studies suggested that genetic factors have greater effect than
339 environmental factors in shaping mucus bacterial assemblages [56, 57]. In our study, we
340 have found that individuals from the same species shared significantly more bacterial
341 communities than they did with individuals of the other species. This was observed
342 despite being captured in the same place and having overall similar feeding habits, which
343 suggests species-specific assemblages. These results are in accordance with previous
344 works claiming that the autochthonous microbes are not a passive reflection of their
345 habitat communities [11], i.e., fish have their own microbiota. We did not detect any
346 effect of the sampling date, although the variation in abiotic factors was moderate among
347 our four samplings (see Table S1). Therefore, other factors may influence teleosts'
348 microbiota, such as seasonal environmental variation throughout the year (e.g. related to
349 temperature or salinity changes), which should be tested in future studies.

350 Bacterial communities from a given body site were significantly more similar among
351 themselves than with communities from other body sites. Although the majority of
352 bacteria from mucus, skin and gills belong to the Family *Vibrionaceae*, there are
353 differences in the relative prevalence of genera among the gills with respect to mucus and
354 skin. The highest bacterial diversity in the body was found in the outermost external layer,
355 i.e., the mucus, which is in intimate contact with water, thus a continuous exchanging of
356 bacteria among them could be expected. The mucus acts as a protective barrier for fishes,
357 since it may impede the penetration of potentially pathogenic microorganisms inside the
358 body [24]. Our findings that the mucus had a significantly higher diversity than the rest

359 of the body (including the skin), is in accordance with this protection hypothesis,
360 suggesting that environmental bacteria remain trapped here. Moreover, the fact that *S.*
361 *notata*, the only fish species without mucus from our sample, had the highest diversity of
362 indicator species in the skin, also supports this hypothesis since in this case, the skin is
363 the direct interface between the fish and the environment. However, we cannot exclude
364 that since the mucus is rich in polysaccharides (e.g. [58, 59]), it may also constitute a food
365 source that attracts heterotrophic bacteria, which may in turn lead to a more diverse
366 community.

367 Pathogenic bacteria can enter the host fish through the skin, gills or gastrointestinal tract,
368 and the integrity of these physical and immunological barriers determine the outcome of
369 host-pathogen interactions (reviewed in [13]). Balanced and complex interplays within
370 the mucus layer are thus key to disease resistance [60], and are essential for supporting
371 host health and fitness [61].

372 Differences in the relative abundance of bacteria between different sites on the body are
373 more pronounced between the gut and the other three external body parts (mucus, skin
374 and gills, Table 2). For instance, the phylum *Proteobacteria* was found at lower relative
375 prevalence in the intestine than in the other three parts, and relative abundance of
376 *Firmicutes* and *Fusobacteria* also differ. In addition, we found that indicator bacteria can
377 be shared between the gills, mucus and skin (see Table S5); however, almost no indicator
378 bacteria were shared by the gut and those three external body sites. Therefore, our results
379 suggest that, although we found that body site explains a significant fraction of the
380 variance observed in bacterial communities over all the body, it is mostly because the
381 intestine differs markedly from the rest of the body sites. Overall, these results were not
382 unexpected given the very different physico-chemical conditions (oxygen, pH and
383 organic substrate levels) occurring in guts compared to external body parts. Bacterial
384 community composition has been previously shown to vary in the gut depending on the
385 life cycle stage, diet, environment, and region of the gastrointestinal tract, and even varies
386 greatly among individuals within the same species [2, 5, 10, 16, 61, 62]. In our study, we
387 only examined the distal part of the gut, and individuals from the same species were
388 adults, so we did not find any significant within-species effect, while the fish species
389 explained most of the variance in bacterial assemblages.

390 There are some groups that predominate in the gut of marine fish across different studies,
391 such as *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*. Also,

392 *Fusobacteria*, *Clostridia*, *Bacilli* and *Verrucomicrobia* have been identified (reviewed in
393 [2, 10]), as well as *Mycoplasma* [5], a group that we found exclusively in the gut samples
394 of teleosts. There is some congruence in the identity of bacterial taxa we found in the gut
395 (including *Vibrio*, *Clostridium* and *Mycoplasma*) with other marine fish belonging to
396 different species, including some from aquaculture (e.g. Atlantic salmon, [5]). The
397 presence of similar bacterial taxa in the gut-associated microbiota across different fish
398 species, populations or geographic locations, suggests that these microbes are important
399 contributors to host gut functions, such as digestion, nutrient absorption and immune
400 response [63]. In general, although the intestine ecosystem is expected to harbour a dense
401 population of microbes, sequence-based analyses have demonstrated that it comprises the
402 lowest phylogenetic diversity compared to external parts of the body [2]. This was also
403 observed in the present study when comparing α -diversity (Shannon diversity index) to
404 the external mucus layer. Previous works also found a higher bacterial diversity in
405 external organs such as skin or gills than in the gut [56, 60], which could be due to a
406 reflection of environmental diversity in the exterior of the body, whereas the gut may
407 offer more stable habitats leading to a more specialized microbial communities [60].

408 There was no evidence that sampling day had an effect on the microbiota, which is in
409 accordance with the difference between fish and water microbiota. That is, the identity of
410 the fish species was more important in the structuring of bacterial communities than
411 environmental conditions at the moment of their capture. This observation suggests that
412 our results are not biased by the sampling date, and that fishes display their characteristic
413 microbiota independently of the external conditions, at least between these two time
414 points. Different samplings in the same location and with the same species could be
415 performed throughout the year to verify an effect of seasonality on the bacterial diversity
416 of our fish community as described in other aquatic systems (e.g. [54]).

417 In conclusion, we found that the thirteen Mediterranean teleost species studied here,
418 living in the same environment and sharing most ecological traits, differed in their
419 bacterial microbiota composition. This suggests that host taxonomic status mainly shapes
420 fish microbiota. In addition, we found a characteristic microbial community in different
421 parts of the body, indicating that microbiota are also influenced by local characteristics
422 of their animal-associated microhabitats. The absence of a significant effect of individuals
423 on the bacterial communities that they host suggests that the level of intra-specific
424 variation is significantly lower than the level of inter-tissue and inter-species variations

425 in shaping fish microbiota. Interestingly, the mucus cover showed the highest bacterial
426 diversity, which supports the hypothesis that it is a barrier between the fish and its
427 environment. That is, bacteria may be retained in this layer but do not reach the skin, and
428 therefore do not penetrate into the body. This physical barrier may thus help to impede
429 pathogenic infections.

430 Microbial communities associated with fishes are key factors in host physiology, ecology
431 and evolution [1, 64]. Therefore, the knowledge of the factors shaping microbiota may
432 help predict how changes in abiotic or biotic conditions affect bacterial assemblages and
433 their functions. Our results help to clarify these factors in wild fish, highlighting the
434 importance of fish species and body sites. Further studies should investigate the possible
435 functions of bacterial taxa on their hosts, and also investigate environment-induced
436 variations through time-series samplings.

437

438 **Acknowledgements**

439 This work was funded by Sorbonne Université, programme Emergence 2016 (project SU-
440 16-R-EMR-22-MICROFISH), which included a post-doctoral contract for MRR. Fish
441 sampling was performed by the “Service des Moyens à la Mer (FR3724) de
442 l’Observatoire Océanologique de Banyuls/Mer (OOB)”. We are grateful to the
443 BIO2MAR platform (<http://bio2mar.obs-banyuls.fr>) for providing technical help and
444 support and access to instrumentation.

445

446 **Ethics statement**

447 All applicable international, national, and/or institutional guidelines for the use of animals
448 were followed. The Observatoire Océanologique de Banyuls sur Mer holds the
449 authorization from the “Direction interrégionale de la Mer Méditerranée” for fishing and
450 handling wild Mediterranean teleosts. Wild fish were caught (see above for details) by
451 competent persons on the research vessel “Nereis II” and in accordance with the European
452 Union Regulations concerning the protection and welfare of experimental animals
453 (European directive 91/492/CCE).

454

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630 **Table 1.** Total number of DNA samples sequenced, by category: fish species, body site,
 631 and water. Note that *Spicara maena* is considered as belonging to the family Sparidae
 632 based on phylogenetic evidence [32].

Fish Family	Fish species	No. of individuals	Mucus	Skin	Gills	Gut
Gobiidae	<i>Gobius bucchichi</i>	5	3	5	5	3
	<i>Gobius cruentatus</i>	2	2	2	2	2
	<i>Gobius niger</i>	3	2	3	3	3
Labridae	<i>Symphodus tinca</i>	5	5	5	5	3
Scorpaenidae	<i>Scorpaena notata</i>	5	-	5	5	4
Serranidae	<i>Serranus scriba</i>	5	5	5	5	3
Sparidae	<i>Diplodus annularis</i>	5	5	5	5	3
	<i>Diplodus vulgaris</i>	5	2	5	5	2
	<i>Oblada melanura</i>	5	4	4	5	3
	<i>Pagellus bogaraveo</i>	4	3	4	4	2
	<i>Pagellus erythrinus</i>	5	5	5	5	5
	<i>Sarpa salpa</i>	5	5	5	5	3
	<i>Spicara maena</i>	5	5	5	5	2
Total of each category		59	46	58	59	38
Water samples (1 per sampling)		4				
Total samples			205			

633

634 **Table 2.** Percentage of the most abundant bacterial taxa (abundance higher than 1 % in the total community) found in the Mediterranean teleost
 635 fish captured in the present work, in total and in each body site.

Taxonomy					Relative abundance (% of reads)					
Phylum	Class	Order	Family	Genera	Total	Mucus	Skin	Gills	Intestine	Water
Firmicutes	Clostridia	Clostridiales	Lachospiraceae	<i>Clostridium</i>	2.2	-	1.5	-	8.6	-
			Peptostreptococcaceae		3.4	1.7	2.3	-	12.8	-
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	<i>Cetobacterium</i>	10.5	5.7	6.2	18.2	13.2	0.68
				<i>Propionigenium</i>	1.5	2.7	1.4	1.5	-	0.08
Proteobacteria	Betaproteobacteria				2.3	-	-	8.6	-	1.06
	Gammaproteobacteria	Alteromonadales	Shewanellaceae	<i>Shewanella</i>	5.5	6.3	6.5	7.3	-	-
		Oceanospirillales	Endozoicomonaceae	<i>Endozoicomonas</i>	2.1	-	1	6.4	-	-
		Vibrionales	Vibrionaceae	<i>Aliivibrio</i>	3.2	3.1	5.8	2.2	12.4	0.03
				<i>Enterovibrio</i>	1.4	1.5	1.7	-	2.2	0.1
				<i>Photobacterium</i>	15.3	21.9	20.4	15.5	9.7	0.35
<i>Vibrio</i>	29.5			42.5	38	25.1	1.5	6.2		
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	3.1	-	-	-	15.4	-	

636

637 **Table 3.** Percentage of the most abundant bacterial taxa (abundance higher than 1 % in the total community) in each fish species. Legend: DA:
638 *Diplodus annularis*; DV: *Diplodus vulgaris*; GB: *Gobius bucchichi*; GC: *Gobius cruentatus*; GN: *Gobius niger*; OM: *Oblada melanura*; PB:
639 *Pagellus bogaraveo*; PE: *Pagellus erythrinus*; SM: *Spicara maena*; SN: *Scorpaena notata*; SSa: *Sarpa salpa*; SSc: *Serranus scriba*; ST: *Simphodus*
640 *tinca*.

641

Taxonomy					Relative abundance (% of reads)													
Phylum	Class	Order	Family	Genera	Total	DA	DV	GB	GC	GN	OM	PB	PE	SM	SN	SSa	SSc	ST
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	<i>Clostridium</i>	2.2	0.36	0.83	0.16	0.24	3.43	0.39	0.37	4.08	0.6	21.87	1.65	5.72	0.25
			Peptostreptococcaceae		3.4	1.81	10.68	11.81	0.52	7.82	0.52	2.72	12.34	0.23	1.58	0.32	0.26	3.93
Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	<i>Cetobacterium</i>	10.5	5.34	8.71	5.03	16.21	2.81	10.27	24.86	19.25	23.98	3.93	8.84	15.78	10.41
				<i>Propionigenium</i>	1.5	3.02	1.11	3.67	0.37	1.79	0.49	2	0.12	0.82	0.02	0.72	0.36	7.77
Proteobacteria	Betaproteobacteria				2.3	0.64	4.52	4.6	0.03	9.04	0.01	6.71	0.02	0	0.42	18.38	-	-
	Gammaproteobacteria	Alteromonadales	Shewanellaceae	<i>Shewanella</i>	5.5	6.64	3.93	4.43	2.08	1.88	8.97	6.56	10.91	7.69	1.84	2.42	1.11	18.72
		Oceanospirillales	Endozoicimonaceae	<i>Endozoicomonas</i>	2.1	0.01	0.01	11.41	1.71	7.51	0.14	-	-	-	25.07	-	0.01	0.01
		Vibrionales	Vibrionaceae	<i>Aliivibrio</i>	3.2	0.73	13.64	0.74	1.12	0.48	7.94	1.16	1.16	6.34	2.36	8.17	5.18	2.2
				<i>Enterovibrio</i>	1.4	1.12	1.77	0.21	0.3	12.19	4.21	0.43	0.66	0.46	2.67	0.75	0.83	0.57
				<i>Photobacterium</i>	15.3	16.09	11.52	9	10.87	5.76	16.71	19.88	33.16	24.41	19.94	26.78	25.89	8.84
				<i>Vibrio</i>	29.5	56.94	42.83	25.5	48.14	27.76	46.55	32.82	19.3	34.77	18.82	31.38	43.77	35.62
Vibrionaceae			1.7	7.3	0.44	0.23	0.1	0.19	3.54	2.46	0.11	0.7	0.71	0.55	0.97	6.24		
Tenericutes	Mollicutes	Mycoplasmatales	Mycoplasmataceae	3.1	0.01	0.01	23.21	18.31	19.34	0.26	0.05	-	-	0.75	0.07	0.12	5.42	

642

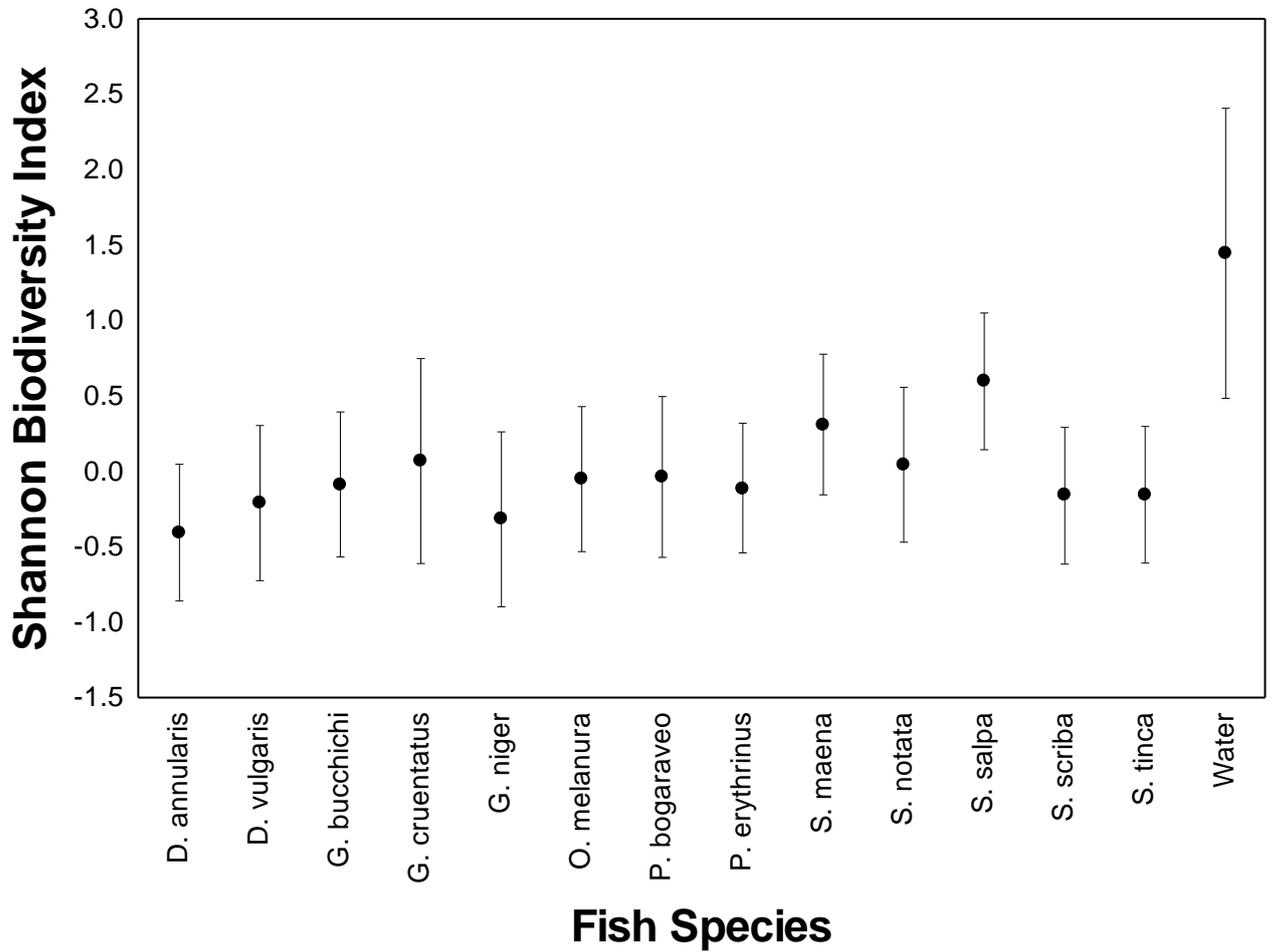
643 **Table 4.** Results of PERMANOVA on factors explaining bacterial communities
644 composition (β -diversity). Significant results are in bold.

	F	D. f.	p-value
Species	2.74	12,169	0.001
Body site	4.68	3,169	0.001
Individual	1.63	1,169	0.173
Sampling event	1.72	1,169	0.117

645

646 **Figure 1.** Standardized Shannon diversity index of species and water. Vertical bars
647 denote 0.95 confidence intervals.

648



649

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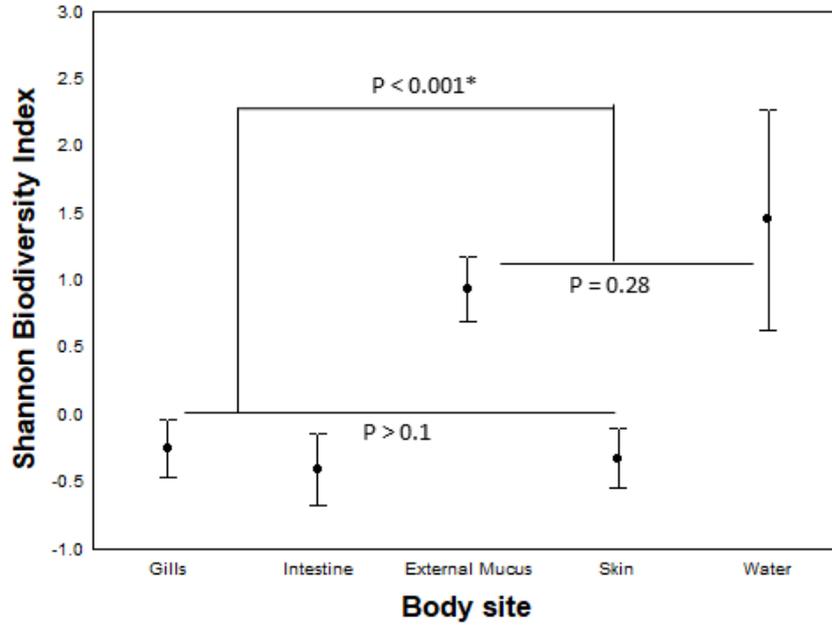
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652

653 **Figure 2.** Standardized Shannon diversity index of different body parts and water.

654 Vertical bars denote 0.95 confidence intervals.

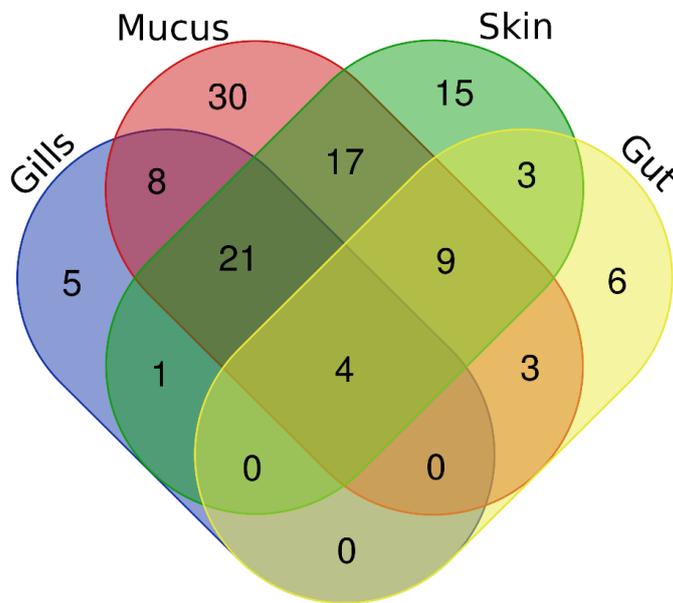
655



656

657 **Figure 3.** Venn diagram representing how many indicator bacterial taxa are exclusive or
658 shared among the different body sites across all the fish species. More information on the
659 specific indicator bacteria is given in Table S5.

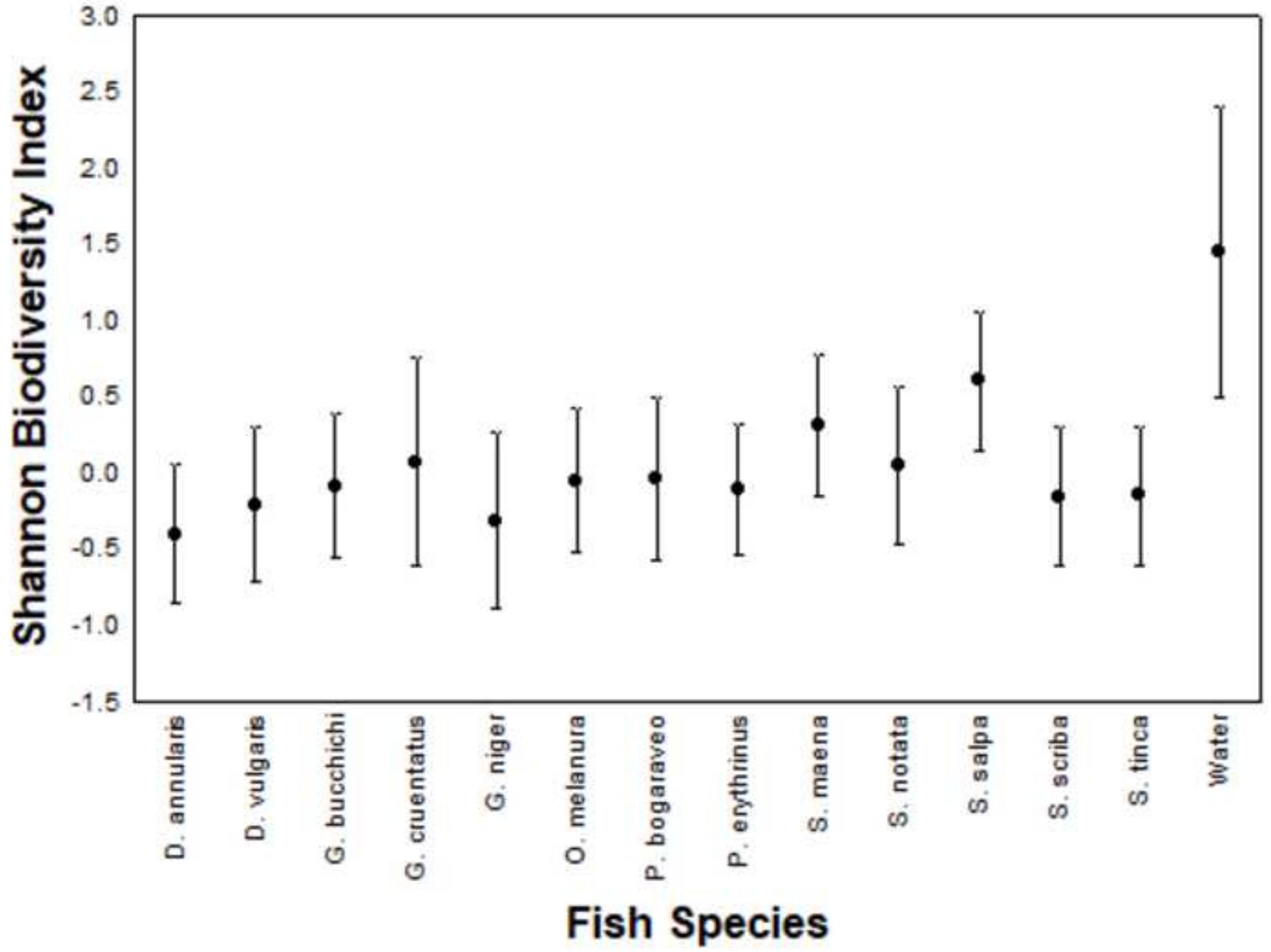
660

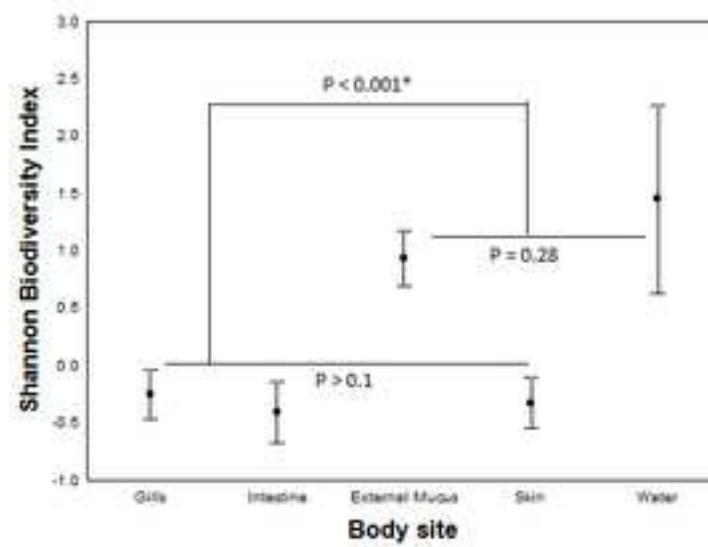


661

662

663





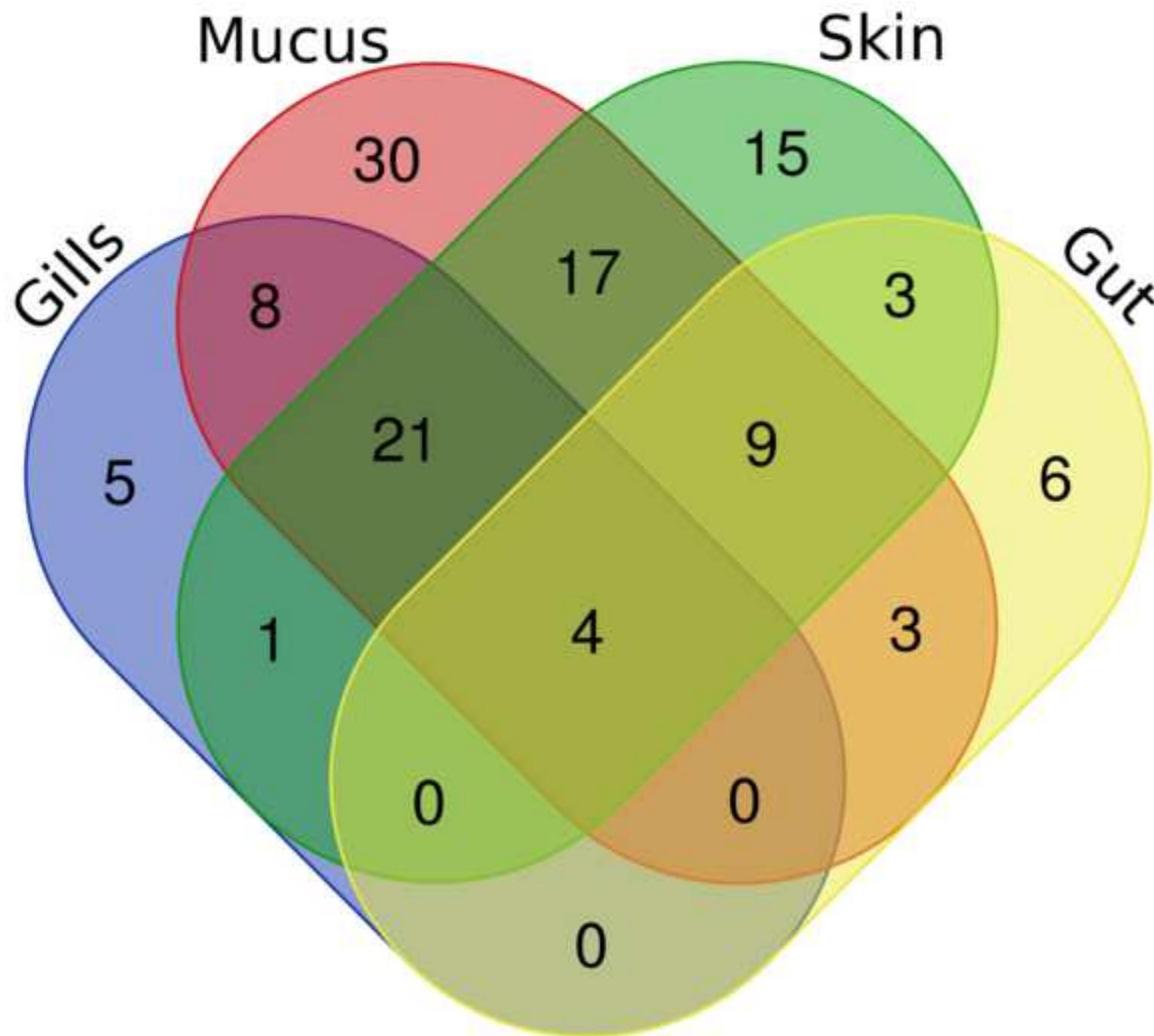


Table S1. Abiotic descriptors for each sampling day obtained from the station SOLA (http://sooob.obs-banyuls.fr/fr/les_sites_d_observation/le_site_sola.html), located in the Bay of Banyuls-sur-Mer, close to the fish sampling sites. All captures were performed in 2017.

	June 21		June 27		July 18		October 4	
	Surface	Seabed	Surface	Seabed	Surface	Seabed	Surface	Seabed
Temperature °C	20.92	16.319	27.97	15.986	22.235	14.5	19.378	18.015
Salinity (PSU)	37.896	38.223	37.798	38.012	37.952	38.457	38.098	38.228
Oxygen (ml/l)	5.29	5.64	5.07	5.78	4.84	5.33	5.24	5.27
pH	8.14	8.14	8.11	8.14	8.07	8.08	8.03	8.07
NH₄ (μmol/l)	0	0.01	0.09	0.04	0.052	0.034	0.014	0.019
NO₃ (μmol/l)	0.08	0.04	0.18	0.21	0.08	0.54	0.11	0.02
NO₂ (μmol/l)	0.01	0.01	0.01	0.01	0.02	0.09	0.02	0.01
PO₄ (μmol/l)	0.01	0.01	0.01	0.01	0.01	0.06	0.01	0.01
SiOH₄ (μmol/l)	0.6	0.86	0.5	0.6	0.55	1.52	0.76	0.8

Table S2. Results of ANOVA testing on differences in α -diversity (Shannon diversity index) among different species, body sites and water, including all the samples, i.e., with categories in which $N < 3$.

	F	df	p		
Species with water	1.82	13, 191	0.04		
Species without water	1.21	12, 188	0.28	Fisher LSD Post-hocs	
Body sites with water	23.44	4, 200	< 0.0001	Water vs. mucus	p = 0.24
				Water vs. Mucus	Gills Skin Gut p < 0.001
Body sites without water	18.07	3, 197	< 0.0001	Mucus vs. Gills	p < 0.001
				Skin vs. Gills vs. Gut	All p > 0.36

Table S3. Results of the GLMM analysis considering all the dataset (i.e., including those categories with $N < 3$).

	F	df	p
Fish species	1.83	12,185	0.04
Body site	29.17	3,185	<0.0001
Sampling day	0.75	3,185	0.50

Table S4. Results of the PERMANOVA analysis considering all the dataset (i.e., including those categories with $N < 3$).

	F	D. f.	p-value
Species	2.50	13,169	0.001
Body site	5.06	3,169	0.001
Individual	1.57	1,169	0.193
Sampling day	1.91	1,169	0.054

Table S5. Distribution of indicator bacterial groups among all the body sites, in each fish species. Legends: G: Gills, I: Intestine, M: Mucus, S: Skin

Fish species	No. bacterial taxa	No. indicator bacterial taxa	Distribution in the body	Bacterial taxa
<i>Diplodus annularis</i>	59	12	G	<i>Unidentified Betaproteobacteria, Neorickettsia</i>
			I	<i>Unidentified Peptostreptococcaceae</i>
			M + S	<i>Pseudoalteromonas</i>
			G + M	<i>Bacillus, Unidentified Saprospiraceae, Tenacibaculum, Hyphomonadaceae, Croceitalea, Verrucomicrobium, Winogradskyella,</i>
			G + M + S	<i>Clostridium</i>
<i>Diplodus vulgaris</i>	66	3	G	<i>Bacillales</i>
			M	<i>Unidentified Deltaproteobacteria</i>
			M + S	<i>Unidentified Ruminococcaceae</i>
<i>Gobius bucchichi</i>	43	17	G	<i>Polynucleobacter, Unidentified Betaproteobacteria</i>
			I	<i>Unidentified Rhodobacteraceae, Actibacter, Unidentified Planctomycetes, Loktanella, Lutimonas, Unidentified Desulfobulbaceae, Thiohalorhabdales, Aliivibrio, Lawsonia, Unidentified Desulfovibrionaceae</i>
			M	<i>Flexibacter</i>
			S	<i>Spironema</i>
			M + S	<i>Bacillus</i>
			G + M	<i>Francisella</i>
			G + M + S	<i>Shewanella</i>
<i>Gobius cruentatus</i>	63	0		
<i>Gobius niger</i>	38	3	G	<i>Unidentified Betaproteobacteria</i>
			I	<i>Roseobacter, Unidentified Phyllobacteriaceae</i>
<i>Oblada melanura</i>	69	6	I	<i>Unidentified Mycoplasmataceae</i>
			M	<i>Winogradskyella, Actibacter, Hyphomonadaceae, Polaribacter, Tenacibaculum</i>
<i>Pagellus bogaraveo</i>	30	6	M	<i>Kiloniellales, Unidentified Betaproteobacteria, Croceitalea</i>
			M + S	<i>Unidentified Flavobacteriaceae, Unidentified Bacillales, Saprospiraceae</i>
<i>Pagellus erythrinus</i>	24	7	G	<i>Unidentified Betaproteobacteria</i>
			M + S	<i>Bacillus, Planomicrobium</i>

			G + M	<i>Moritella</i>
			G + S	<i>Propionigenium</i>
			G + M + S	<i>Shewanella, Psychrilyobacter</i>
<i>Spicara maena</i>	31	5	M	<i>Eudoraea, Planomicrobium, Unidentified Flavobacteriaceae, Unidentified Planococcaceae</i>
			M + S	<i>Unidentified Bacillales</i>
<i>Scorpaena notata</i>	66	24	S	<i>Unidentified Acidimicrobiales, Halomiconema, Unidentified Trueperaceae, Unidentified Chromatiales, Unidentified Saprospiraceae, Unidentified Phyllobacteriaceae, Unidentified Rhodobacteraceae, Kiloniellales, Eudoraea, Lutimonas, Amaricoccus, Sulfitobacter, Unidentified Anaerolineae, Unidentified Pseudanabaenaceae, Unidentified Alphaproteobacteria, Unidentified Unidentified Sphingomonadales, Thiothrix, Unidentified Acidimicrobiales (2)</i>
			G + S	<i>Unidentified Flavobacteriaceae, Unidentified Planococcaceae, Unidentified Alphaproteobacteria, Endozoicomonas, Psychroserpens, Pseudoalteromonas</i>
<i>Sarpa salpa</i>	189	95	G	<i>Unidentified Chlamydia, Unidentified Burkholderiales</i>
			I	<i>Unidentified Rickettsiales</i>
			M	<i>Agrococcus, Pseudonocardia, Fulvivirga, Unidentified Chloroflexi, Rivularia, Unidentified Sphingomonadaceae, Unidentified Bacteriovoracaceae, Unidentified Verrucomicrobiaceae, Unidentified Rhizobiales, Unidentified Spirochaetaceae, Unidentified Kiloniellales, Unidentified Chroococcales, Unidentified Actinomycetales, Unidentified Myxococcales, Unidentified Nocardiodaceae, Enterococcus, Unidentified Trueperaceae, Unidentified Chromatiales, Unidentified Gaiellales, Devosia, Unidentified Acidimicrobiales, Lutibacterium, Fluviicola, Thiohalorhabdals, Halorhodospira, Loktanella, Lysobacter, Leptolyngbya, Unidentified Bacteroides, Planctomyces, Turicibacter, Alcanivorax, Cytophaga</i>
			S	<i>Salegentibacter, Coccinimonas, Roseivirga, Odoribacter, Anaerofilum, Unidentified Mollicutes, Unidentified Desulfobulbaceae, Coraliomargarita, Paludibacter</i>
			M + S	<i>Piscirickettsiaceae, Polaribacter, Unidentified Porphyromonadaceae, Unidentified Flavobacteriales, Desulfovibrio, Winogradskyella, Eudoraea, Unidentified Bacillales, Bacillus, Clostridium, Croceitalea, Planctomycete, Unidentified Flammeovirgaceae, Unidentified Spirobacillales, Grimontia, Unidentified Saprospiraceae, Marixanthomonas, Unidentified Deferribacteraceae, Anaerotruncus, Dinoroseobacter, Unidentified Desulfarculaceae</i>
G + M	<i>Unidentified Hyphomonadaceae, Unidentified Verrucomicrobiaceae, Unidentified Anaplasmataceae</i>			

			G + M + S	<i>Unidentified Flammeovirgaceae, Aliivibrio, Unidentified Flavobacteriaceae, Unidentified Alteromonadales, Tenacibaculum, Unidentified Pirellulaceae, Unidentified Rhodobacteraceae, Unidentified Planctomycete, Unidentified Hyphomicrobiacea, Enterovibrio</i>
			I + M + S	<i>Unidentified Macellibacteroides, Unidentified Cerasiococcaceae, Unidentified Opitutae, Unidentified Bacteroidales, Unidentified Rikenellaceae, Unidentified Ruminococcaceae, Unidentified Alphaproteobacteria, Unidentified Mollicutes, Subdoligranulum, Unidentified Victivallaceae</i>
<i>Serranus scriba</i>	30	7	G	<i>Pseudoalteromonas, Arcobacter</i>
			M	<i>Psychrobacter, Methylobacterium</i>
			G + M + S	<i>Propionigenium, Shewarella, Moritella</i>
<i>Symphodus tinca</i>	29	6	M	<i>Endozoicomonas</i>
			G + M	<i>Octadecabacter, Unidentified Bacillales</i>
			G + M + S	<i>Moritella, Propionigenium, Unidentified Vibrionaceae</i>

```

# Sequences were previously demultiplexed into forward and reverse reads
# Example :
# 171222_SN1126_A_L001_JIC-2-171_R2.fastq.gz and 171220_SN234_A_L001_JIC-1-
193_AdapterTrimmed_R2.fastq.gz
# In the mapping file fields 16 and 17 contains prefixes and suffixes given by the sequencing center
first field is the sample code

# Unzip files

ls *.gz | awk '{print "gzip -df \"$1\" | /bin/sh}'

# Rename files to have new sample name. Forward first

cat map.txt | sed '1d' | awk '{print "mv \"$16\"_\"$17\"_R1.fastq \"$1\"_R1.fastq"}' | /bin/sh
cat map.txt | sed '1d' | awk '{print "mv \"$16\"_\"$17\"_R2.fastq \"$1\"_R2.fastq"}' | /bin/sh

# Usearch 9 merges reverse and forward from all reads, renames samples based on file name

usearch9 -fastq_mergepairs *R1*.fastq -fastq_maxdiffs 0 -fastqout merged.fastq -relabel @ 2>
fastqmergepairs.log &

# Usearch 9 filters bad quality reads

usearch9 -fastq_filter merged.fastq -fastq_maxee 1.0 -fastaout merged.fasta -fasta_cols 0 &

# T. Walters https://gist.github.com/walterst/2fce207ff38ad04c0bcbb2e8531ac230 script to remove
primers

strip_primers_exclude.py map.txt merged.fasta merged1.fasta log

# Denoise with Usearch 9,
# 1 dereplicate

usearch9 -fastx_uniques merged1.fasta -fastaout uniques.fasta -sizeout

# 2 denoise

usearch9 -unoise uniques.fasta -tabbedout uniques_unoise.txt -fastaout uniques_denoised.fasta

# Remove sequences shorter than 360 bp
# Make single line fasta

mv uniques_denoised.fasta uniques_denoised

cat uniques_denoised | awk '{if ($1 ~ />.*\/){printf ("%s", "\n");printf ("%s", $0);printf ("%s", "\n")}else
{printf ("%s", $0)}}' | sed '1d' > uniques_denoised.fasta"

# Identify reads shorter than 360 bp

cat uniques_denoised.fasta | gawk -F "" '{if($1~/>\/){printf("%s ", $0)}else{printf("%s\n", NF)}}' | awk
'if(NF<360)print $1' >less360

```

```

# Qiime1 removes reads less than 360 bp identified above

filter_fasta.py -n -s less360 -f uniques_denoised.fasta -o uniques_denoised_plus360.fasta

# Make OTU table

usearch9 -usearch_global merged1.fasta -db uniques_denoised_plus360.fasta -strand plus -id 0.97 -
otutabout otu_table.txt

# Qiime1 assigns taxonomy with rdp classifier and a modified green genes 13_8 database (Reverter
et al., 2017)

assign_taxonomy.py -m rdp -i uniques_denoised_plus360.fasta -t
/Volumes/data/greengenesrdp/gg_13_8_otus/taxonomy/99_otu_taxonomy_species.txt -r
/Volumes/data/greengenesrdp/gg_13_8_otus/rep_set/99_otus.fasta -o
gg_13_8Assigned_tax_species --rdp_max_memory=4000 &

# Fix names in taxonomy table since it is different from the OTU table (i.e. contains read count)

cat gg_13_8Assigned_tax_species/uniques_denoised_plus360_tax_assignments.txt | awk
'{{sub(/;.*;/,"",$1)};print $1"\t"$2"\t"$3}' > tax_assignments

# Add missing phylogenetic levels to taxonomy

cat tax_assignments | gawk -F "\t"
'{{if($2~/p_/){printf("%s\t",$1);printf("%s;p__c__f__g__s__\t",$2);printf("%s\n",$3)}else{print
$0}}' | gawk -F "\t"
'{{if($2~/c_/){printf("%s\t",$1);printf("%s;c__f__g__s__\t",$2);printf("%s\n",$3)}else{print $0}}' |
gawk -F "\t" '{{if($2~/f_/){printf("%s\t",$1);printf("%s;f__g__s__\t",$2);printf("%s\n",$3)}else{print
$0}}' | gawk -F "\t"
'{{if($2~/g_/){printf("%s\t",$1);printf("%s;g__s__\t",$2);printf("%s\n",$3)}else{print $0}}' | gawk -F
"\t" '{{if($2~/s_/){printf("%s\t",$1);printf("%s;s__\t",$2);printf("%s\n",$3)}else{print
$0}}}'>tax_assignments_fixed

# biom http://biom-format.org/ , convert table to biom format

biom convert --table-type="OTU table" -i otu_table.txt -o otu_table.biom --to-json &

# biom, add taxonomy

biom add-metadata --sc-separated taxonomy --observation-header OTUID,taxonomy --observation-
metadata-fp tax_assignments_fixed -i otu_table.biom -o otu_table_final.biom
filter taxa

# Qiime remove chloroplasts, mitochondria, archaea and unclassified taxa from table

filter_taxa_from_otu_table.py -i otu_table_final.biom -o otu_table_final_f.biom -n
c__Chloroplast,f__mitochondria,k__Archaea,Unclassified

# Summarize table

biom summarize-table -i otu_table_final_f.biom -o summary.txt; less summary.txt

```

System configuration

```
uname -a > version
system_profiler SPSoftwareDataType >> version
pip freeze >> version
conda list >> version
```

Version :

```
Darwin woese.obs-banyuls.fr 15.6.0 Darwin Kernel Version 15.6.0: Tue Apr 11 16:00:51 PDT 2017;
root:xnu-3248.60.11.5.3~1/RELEASE_X86_64 x86_64
Software:
```

System Software Overview:

```
System Version: OS X 10.11.6 (15G1510)
Kernel Version: Darwin 15.6.0
Boot Volume: woese
Boot Mode: Normal
Computer Name: woese
User Name: Marcelino Suzuki (suzuki)
Secure Virtual Memory: Enabled
System Integrity Protection: Enabled
Time since boot: 111 days 6:45
```

```
appnope==0.1.0
backports.shutil-get-terminal-size==1.0.0
biom-format==2.1.5
biopython==1.68
bitarray==0.8.1
burrito==0.9.1
burrito-fillings==0.1.1
bz2file==0.98
checkm-genome==1.0.5
click==6.6
cogent==1.5.3
decorator==4.0.11
DendroPy==4.1.0
EMIRGE==0.61.1
emperor==0.9.51
enum34==1.1.6
funcsigs==1.0.2
future==0.16.0
gdata==2.0.18
h5py==2.6.0
ipython==5.1.0
ipython-genutils==0.1.0
joblib==0.9.3
khmer==2.0
matplotlib==1.4.3
mmtf-python==1.0.5
mock==2.0.0
```

```

msgpack-python==0.4.8
natsort==3.5.0
nose==1.3.7
numpy==1.10.4
pandas==0.18.1
pathlib2==2.2.0
pbr==1.10.0
pexpect==4.2.1
pickleshare==0.7.4
PICRUSt==1.1.0
Pillow==4.0.0
poretools==0.6.0
prompt-toolkit==1.0.9
ptyprocess==0.5.1
Pygments==2.1.3
pygobject==3.10.0
pynast==1.2.2
pyparsing==2.0.3
pyqi==0.3.2
pysam==0.10.0
python-dateutil==2.3
pytz==2016.10
qcli==0.1.1
qiime==1.9.1
qiime-default-reference==0.1.3
quast==4.4
reportlab==3.3.0
ruffus==2.6.3
scandir==1.4
scikit-bio==0.2.3
scipy==0.17.1
ScreamingBackpack==0.2.333
screed==0.9
seaborn==0.7.1
simplegeneric==0.8.1
simplejson==3.8.1
six==1.10.0
traitar==1.1.2
traitlets==4.3.1
wcwidth==0.1.7
# packages in environment at /usr/local/miniconda3/envs/bioinfo2:
#

```

appnope	0.1.0	py27_0	
backports	1.0	py27_0	
bamtools	2.4.0	3	bioconda
bcftools	1.6	1	bioconda
biom-format	2.1.5	py27_1	bioconda
biopython	1.68	py27_0	bioconda
bitarray	0.8.1	py27_0	

boost	1.60.0	py27_0	
bowtie	1.2.0	py27_0	bioconda
burrito	0.9.1	py27_0	bioconda
burrito-fillings	0.1.1	py27_0	bioconda
bz2file	0.98	py27_0	
bzip2	1.0.6	hd86a083_4	
checkm-genome	1.0.5	py27_0	bioconda
click	6.6	py27_0	bioconda
cogent	1.5.3	py27_0	bioconda
curl	7.45.0	2	bioconda
decorator	4.0.11	py27_0	
dendropy	4.1.0	py27_0	bioconda
emirge	0.61.1	py27_0	bioconda
emperor	0.9.51	py27_0	bioconda
enum34	1.1.6	py27_0	
freetype	2.5.5	1	
funcsigs	1.0.2	py27_0	
future	0.16.0	py27_1	
gdata	2.0.18	py27_0	
get_terminal_size	1.0.0	py27_0	
google-sparsehash	2.0.3	1	bioconda
h5py	2.6.0	np110py27_7	conda-forge
hdf5	1.8.17	2	
hmmer	3.1b2	2	bioconda
htslib	1.3.2	0	bioconda
icu	54.1	0	
infernai	1.1.2	0	bioconda
ipython	5.1.0	py27_1	
ipython_genutils	0.1.0	py27_0	
java-jdk	8.0.92	1	bioconda
jbig	2.1	0	
jemalloc	3.6.0	1	bioconda
joblib	0.9.3	py27_0	bioconda
jpeg	9b	0	bioconda
khmer	2.0	py27_1	bioconda
libgcc	4.8.5	1	
libpng	1.6.17	0	
libtiff	4.0.6	3	
lordec	0.8	0	atgc-montpellier
matplotlib	1.4.3	np110py27_3	
mkl	11.3.3	0	
mmtf-python	1.0.5	py27_0	bioconda
mock	2.0.0	py27_0	
msgpack-python	0.4.8	py27_0	
natsort	3.5.0	py27_0	
nose	1.3.7	py27_1	

numpy	1.10.4	py27_2	
openssl	1.0.2k	0	
pandas	0.18.1	np110py27_0	
path.py	10.0	py27_0	
pathlib2	2.2.0	py27_0	
pbr	1.10.0	py27_0	
pear	0.9.6	3	bioconda
perl	5.22.0.1	0	conda-forge
perl-threaded	5.22.0	pl5.22.0_12	bioconda
pexpect	4.2.1	py27_0	
pickleshare	0.7.4	py27_0	
picrust	1.1.0	py27_0	bioconda
pillow	4.0.0	py27_0	
pip	9.0.1	py27_1	
poretools	0.6.1a1	py27_5	bioconda
prompt_toolkit	1.0.9	py27_0	
ptyprocess	0.5.1	py27_0	
pygments	2.1.3	py27_0	
pygobject	3.10.0	py27_1	vgauthier
pygtk	2.24.0	py27_1	vgauthier
pynast	1.2.2	py27_0	bioconda
yparsing	2.0.3	py27_0	
pyqi	0.3.2	py27_0	bioconda
pyqt	4.11.4	py27_4	
pysam	0.10.0	py27_1	bioconda
python	2.7.13	0	
python-dateutil	2.3	py27_0	bioconda
pytz	2016.10	py27_0	
qcli	0.1.1	py27_0	bioconda
qiime	1.9.1	np110py27_0	bioconda
qiime-default-reference	0.1.3	py27_0	bioconda
qt	4.8.7	4	
quast	4.4	boost1.60_1	bioconda
readline	6.2	2	
reportlab	3.3.0	py27_0	
ruffus	2.6.3	py27_0	bioconda
samtools	1.3.1	5	bioconda
scandir	1.4	py27_0	
scikit-bio	0.2.3	np110py27_0	bioconda
scipy	0.17.1	np110py27_1	
screamingbackpack	0.2.333	py27_0	bioconda
screed	0.9	py27_0	bioconda
seaborn	0.7.1	py27_0	
seqtk	1.2	0	bioconda
setuptools	27.2.0	py27_0	

simplegeneric	0.8.1	py27_1	
simplejson	3.8.1	py27_0	bioconda
sip	4.18	py27_0	
six	1.10.0	py27_0	
sqlite	3.13.0	0	
tbb	4.4_20150728	0	bioconda
tk	8.5.18	0	
traitar	1.1.2	<pip>	
traitlets	4.3.1	py27_0	
trimmomatic	0.36	3	bioconda
vsearch	2.4.0	0	bioconda
wcwidth	0.1.7	py27_0	
wheel	0.29.0	py27_0	
xz	5.2.2	1	
zlib	1.2.11	hf3cbc9b_2	