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Spatial variation in the gastrointestinal microbiome, diet, and nutritional condition of a juvenile flatfish among coastal habitats

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7 **Spatial variation in the gastrointestinal microbiome, diet, and nutritional condition of a juvenile flatfish among**
8 **coastal habitats**

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25 **Keywords:** Essential Fish Habitat; gastrointestinal microbiota; gut microbiome; juvenile fish; nursery habitats; sandy
26 beach; *Pleuronectes platessa*.

27

28 **Short title:** Spatial variation in wild fish gut microbiomes

29 **Abstract**

30 Gut microbiota are important for the health, fitness and development of animal hosts, but little is known
31 about these assemblages in wild populations of fish. Such knowledge is particularly important for juvenile life stages
32 where nutritional intake critically determines early development, growth, and ultimately recruitment. We characterise
33 the microbiome inhabiting the gut of young-of-the-year European plaice ('YOY plaice') on sandy beaches, their key
34 juvenile habitat, and examine how these microbial communities vary spatially in relation to diet and nutritional
35 condition of their plaice hosts. Body size, diet (stomach fullness and eukaryotic 18S ribosomal sequencing), nutritional
36 condition (RNA:DNA) and gut microbiota (16S prokaryotic ribosomal sequencing) were compared in fish at two
37 spatial scales: between beaches separated by 10s of kilometres and between sites at different depths on the same beach,
38 separated by 10s of metres. The main microbial phyla in YOY plaice guts were Proteobacteria, Spirochaetes,
39 Tenericutes and Verrucomicrobiae. Within the Proteobacteria there was an unusual dominance of
40 Alphaproteobacteria. Differences in body size, diet and nutritional condition of YOY plaice between beaches were
41 accompanied by differences in gut microbial assemblage structure. Notably, substantially reduced nutritional
42 condition and size at one of the beaches was associated with lower stomach fullness, reduced consumption of annelids
43 and differences in the abundance and presence of specific microbial taxa. Differences were also detected in microbial
44 assemblages, body size, and diet between depths within the same nursery beach, although stomach fullness and
45 nutritional condition did not vary significantly. The functional links between the environment, gut microbiota, and
46 their hosts are potentially important mediators of the development of young fish through critical life stages. Our study
47 indicates that these links need to be addressed at 10 km and even 10 m scales to capture the variability observed in
48 wild populations of juvenile fish.

49

50 **Introduction**

51 The health, fitness, and development of animals may be influenced by the microbial assemblages inhabiting
52 their gastrointestinal tract (e.g. Bates et al. 2006, Coon et al. 2014). These gut microbiota can aid in host nutrition
53 (Walter & Ley 2011), energy metabolism (Turnbaugh et al. 2006), immune system maturation (Mazmanian et al.
54 2005), and immune responses (Olsson et al. 1992, Macpherson et al. 2000, Round & Mazmanian 2009, Ingerslev et
55 al. 2014b). Alterations in microbiome composition can influence factors such as nutrient acquisition, susceptibility to
56 toxins and behaviour (Sekirov et al. 2010, Mangiola et al. 2016). Furthermore, gut microbial composition varies

57 greatly due to the phylogeny (Anderson et al. 2012, Colman et al. 2012, Phillips et al. 2012) and ontogenetic
58 development (Phillips et al. 2012) of the host as well as extrinsic environmental influences (Phillips et al. 2012, Wong
59 & Rawls 2012, Coon et al. 2014, Dill-McFarland et al. 2014). Establishing the natural variability in the gut microbiota
60 is therefore an important step in appreciating the role of these assemblages in the physiology, behaviour and ecology
61 of animal populations (Talwar et al. 2018).

62 Our knowledge of gut microbial composition and microbiome-host interactions in fishes is developing, but
63 remains limited. Earlier work was based on traditional culture-dependent techniques that detect only a small fraction
64 of the microbial assemblage (Spanggaard et al. 2000, Austin 2006, Romero & Navarrete 2006, Kim et al. 2007,
65 Aguilera et al. 2013) and suffer methodological inconsistencies that make studies incomparable (Romero et al. 2014,
66 Givens et al. 2015). Modern sequencing technologies provide more comprehensive insights into the diversity and
67 structure of microbial assemblages and their functional characteristics in fishes (Austin 2006, Romero & Navarrete
68 2006, Aguilera et al. 2013, Ghanbari et al. 2015). However, most studies to date focus on a few captive-held, cultured
69 species such as rainbow trout (*Oncorhynchus mykiss*) or grass carp (*Cyprinus carpio*), or on developmental model-
70 species such as zebrafish (*Danio rerio*) (Roeselers et al. 2011, Stephens et al. 2016). Far less is known about gut
71 microbiota of wild fish, although previous studies have demonstrated marked differences from captive-held
72 populations (Ramírez & Romero 2017).

73 In particular, the individual and ecological implications of variation in fish gut microbiota need to be
74 established at key life stages. Fishes have complex life histories, across which the composition of gut microbiota can
75 change considerably (Bakke et al. 2015, Burns et al. 2016, Llewellyn et al. 2016). Gut microbiomes in zebrafish, for
76 example, vary considerably during development and display signature characteristics unique to each life stage
77 (Stephens et al. 2016). The diversity and relative abundance of bacterial taxa in a range of other fish species are known
78 to be strongly influenced by developmental events such as first feeding (Hansen et al. 1992, Bergh et al. 1994,
79 Ingerslev et al. 2014a) and settlement (Parris et al. 2016). Younger life stages are a particularly important focus for
80 gut microbiome studies since nutrition is critical for the early developmental processes that underpin successful
81 recruitment into adult fish populations (Sissenwine 1984, Houde 1987, Miller et al. 1988). There is a need for
82 fundamental information about the nature of variation in gut microbiota, accompanying changes in fish diet and
83 nutritional condition, during early life stages when potential influence on population dynamics is large.

84 This study focuses on variation in the gut microbiota, diet and nutritional condition of wild populations of
85 the juvenile life stage of European plaice *Pleuronectes platessa* L., a commercially-important flatfish species
86 distributed across the north-east Atlantic. Following winter spawning and a pelagic larval stage, young-of-the-year
87 European plaice (hereafter ‘YOY plaice’) settle onto moderately exposed sandy shores during the spring and spend
88 their first summer in the shallows (typically < 5 m) feeding on small invertebrates (Gibson 1999). YOY plaice have
89 been an important model for examining causes of variation in physiological performance of young fishes in the wild
90 (reviewed in Ciotti et al. 2014). Despite extensive tidal and diel migrations across the onshore-offshore axis of beaches,
91 juvenile plaice show strong alongshore site fidelity and marked interindividual differences in patterns of habitat use
92 across the depth gradient (Macer 1967, Riley 1973, Burrows et al. 2004, Gibson et al. 2011). Diet and growth rate of
93 juveniles are thought to influence survival to recruitment and therefore population dynamics of plaice, but are highly
94 variable in time and space. In particular, applications of high-throughput, fine-resolution, RNA-based metrics of
95 individual growth have identified substantial, but as yet unexplained variation in YOY plaice growth rate and
96 nutritional condition at spatial scales ranging from 10s of metres to 100s of kilometres on the west coast of Scotland
97 and Irish Sea (Ciotti et al. 2013a, Ciotti et al. 2013b, Ciotti et al. 2013c, Ciotti et al. 2014). This variability could not
98 be explained by factors such as food availability or temperature, prompting calls for a wider consideration of
99 underlying drivers of growth variation (Ciotti et al. 2014). The extent to which gut microbial communities may vary
100 at these scales, and how this may interact with feeding and growth, requires clarification.

101 In a recent metabarcoding study, Heindler et al. (2019) reported relative homogeneity in the diets and gut
102 microbiota of YOY plaice across four geographic regions of open coast in the eastern English Channel and southern
103 North Sea. This study was designed to be both spatially and temporally extensive. Samples from each region were
104 collected from 2-5 sites pooled from offshore and/or beach locations. Distances between regions varied from 10 - 20
105 km to as much as 150 km and samples were collected over the course of several months. Dominant components of the
106 microbiome across these scales were found to be α -, β - and γ -Proteobacteria, Planctomycetia, Acidimicrobiia and
107 chloroplasts. It remains unknown whether this microbial assemblage and its apparent spatial homogeneity is also
108 found in other parts of the species range. Furthermore, given the spatial structuring in other aspects of juvenile plaice
109 feeding, growth and behaviour, over as little as 100 m distances (Ciotti et al. 2013c, Ciotti et al. 2014), the potential
110 for finer-scale spatial variation in gut microbiota needs to be tested through contemporaneous sampling at spatially
111 delimited sampling sites. Understanding variability in the gut microbiome at contrasting spatial scales, in parallel with

112 key aspects of feeding and growth performance, will be critical for moving towards a functional microbiomics
113 approach capable of revealing both the drivers of microbiome variation and their bioenergetic consequences for wild
114 populations.

115 This paper applies a metabarcoding approach to characterise the microbiome in YOY plaice guts at sandy
116 beaches on the west coast of Scotland in relation to the size, diet composition, feeding success and nutritional condition
117 of their hosts. We test how these factors vary at contrasting spatial scales, between fishes living at different water
118 depths on the same beach (separated by 10s of metres) and at different beaches (separated by 10s of kilometres). By
119 revealing the potential for variability in the gut microbiota, we establish factors potentially influencing the
120 physiological performance of juvenile fishes in the wild.

121

122 **Material and methods**

123 Variation in gut microbiota, diet, and nutritional condition of YOY plaice was examined at two spatial scales.
124 The large scale (10s of kilometers) compared fish caught at 0.5 m depth below waterline from two nursery beaches
125 on the west coast of Scotland: Tralee Beach ('Tralee Shallow'; 56°29'N, 05°25'W) and Caolisport Beach ('Caolisport
126 Shallow'; 55°55'N, 05°36'W). The small scale (10s of metres within a single beach) compared fish caught at Tralee
127 Shallow with fish from 2 m below waterline at the same beach ('Tralee Deep'). YOY plaice were collected within 2 h
128 of low water on 19th and 20th July 2016 using a 1.5 m beam trawl (6 mm mesh) towed parallel to the shore, either by
129 hand (0.5 m depth) or by boat (2 m depth). Seventy five YOY plaice (n = 20 for Caolisport Shallow and Tralee Deep,
130 n = 35 for Tralee Shallow) were immediately flash-frozen and stored below -70°C until processing. Bottom water
131 temperatures and salinities measured at the time of sampling indicated that Tralee Shallow (temperature = 15.0°C ,
132 salinity = 25.3) was cooler and fresher than Caolisport Shallow (temperature = 17.4°C, salinity = 27.8). Tralee Deep
133 was cooler and more saline (temperature = 14.1°C, salinity = 26.0) than Tralee Shallow.

134 ***Size, nutritional condition and feeding success***

135 Size, nutritional condition and feeding success were inferred from the length and mass, RNA:DNA (Ciotti et
136 al. 2010) and stomach fullness, respectively, in a haphazardly selected subsample of fish frozen from each site (n =
137 15 for Caolisport Shallow and Tralee Deep, n = 30 for Tralee Shallow). RNA:DNA is a robust and well established
138 metric of nutritional condition which also correlates strongly with individual growth rate in early life stage fishes
139 (Bulow 1970, Ferron & Leggett 1994, Bergeron 1997, Buckley et al. 1999, Ciotti et al. 2010). This metric has

140 previously been applied to reveal growth dynamics of YOY plaice on the west coast of Scotland (Ciotti et al. 2010,
141 Ciotti et al. 2013b, Ciotti et al. 2013a, Ciotti et al. 2013c) and in other areas (De Raedemaeker et al. 2012). Total
142 length was measured from photos of fish taken upon capture (prior to freezing) using the image processing software
143 ImageJ (Abràmoff et al. 2004). In the laboratory, frozen fish were weighed and two 0.01 g subsamples of white muscle
144 tissue were dissected from the epaxial eyed side for RNA:DNA measurement. Stomach contents were then removed,
145 blotted and weighed fresh.

146 Nucleic acids were quantified using a one-dye, two-enzyme fluorometric assay modified from Caldarone et
147 al. (2001). Unless otherwise stated, reagents and enzymes for measurements of nucleic acids were obtained from
148 Sigma-Aldrich (Hampshire, UK) and prepared in Tris-EDTA buffer (TE, 5 mM Tris-HCl, 0.5 mM EDTA, adjusted
149 to pH 7.5 with NaOH). To avoid contamination with nucleases, reagents were made using diethylpyrocarbonate
150 (DEPC)-treated ultrapure water in baked glassware.

151 Nucleic acids were extracted from fresh tissue in 0.23 ml 2% n-lauroylsarcosine sodium salt solution with
152 rapid shaking at 4°C (30 min at 1,500 rpm; Eppendorf Thermomixer C, Stevenage, UK), sonication (four 4 s 20 kHz
153 pulses; Sonics and Materials Vibra-Cell VCX130PB, Leicestershire, UK) followed by a second 30 min shake. Once
154 digested, subsamples were diluted with 1.610 ml TE buffer, then centrifuged at 14,000 g for 15 min to precipitate cell
155 debris. Supernatants were removed and diluted 272-fold to produce working preparations within the limits of detection
156 at a final n-lauroylsarcosine concentration of 0.1%.

157 Nucleic acid concentrations in diluted supernatants were quantified by the subtraction of fluorescence
158 attributed to RNA and DNA upon sequential digestion with RNase then DNase. Duplicate 0.075 ml aliquots of diluted
159 supernatants were loaded into non-binding surface 96 well microplates (Corning 3650; Flintshire, UK) alongside
160 nucleic acid standards (16S + 23S ribosomal RNA from *E. coli* MRE600, 10206938001; DNA from calf thymus,
161 D4764) and stained with 0.075 ml 2000-fold diluted Quant-iT™ RiboGreen® (Invitrogen, Paisley, UK). Total
162 fluorescence at 485 nm excitation and 520 nm emission wavelengths was then measured in a fluorometer (BMG
163 Labtech FLUOstar Omega; Buckinghamshire, UK). Plates were incubated at 30°C inside the fluorometer for 15 min
164 prior to all fluorescence measurements in order to stabilize temperatures between and within readings. RNA was
165 measured as the reduction in fluorescence after digestion with RNase A (0.010 ml 0.5 U ml⁻¹ from bovine pancreas,
166 R6513). DNA fluorescence was separated from background fluorescence by digestion with DNase 1 (0.010 ml 0.075
167 U ml⁻¹ from bovine pancreas, D4263) and MgCl₂ and CaCl₂ cofactors for 1 h at 37°C. RNA and DNA concentrations

168 were calculated based on standard curves, expressed as a ratio and averaged across both duplicates and both tissue
169 subsamples for analysis. The mean \pm SD ratio of DNA to RNA standard curve slopes was 2.21 ± 0.38 .

170 The mass of the stomach contents was expressed as a proportion of the total fish mass (including stomach
171 contents; hereafter 'stomach fullness'). Normality and homoscedasticity of residuals were assessed visually:
172 RNA:DNA did not require transformation, but fish mass and stomach fullness were log₁₀ and arcsine square root
173 transformed, respectively. Differences in fish mass and stomach fullness between beaches or depths were tested with
174 t-tests. Tests of differences in RNA:DNA between beaches or depths also included fish mass as a covariate in a 2-way
175 general linear model. Interaction terms were tested by comparing the two-way, fully-crossed model with the additive
176 model (containing main effects only). If the interaction term could be removed, main effects were tested by dropping
177 them from the additive model. Models were fitted by ordinary least squares in R (R Core Team 2013). Comparisons
178 were based on partial F-tests (Quinn & Keough 2002) and changes in Akaike's Information Criterion (with correction
179 for small sample sizes, AICc) (Burnham & Anderson 2002). Terms were retained if removal led to a significant drop
180 in variance explained ($\alpha = 0.05$) or an increase in AICc by more than 2.

181 *16S and 18S rRNA sequencing*

182 Gut microbiome and prey types were examined using high throughput sequencing in the remaining five fish
183 collected from each site. Fish gastrointestinal tracts were removed whole in sterile conditions using autoclave-
184 sterilised tools. Gut content was separated from the gut wall with the exception of three Caolisport Shallow fish which
185 were too small. DNA extractions were performed on the gut wall (for 16S sequencing) or gut contents (for 18S
186 sequencing) using the DNeasy™ Blood & Tissue Kit (QIAGEN, West Sussex, UK) according to the manufacturer's
187 recommendations. In the case of the three smaller Caolisport fish, DNA was extracted from whole gut samples (wall
188 + gut) on which both the 16S and 18S sequencing was performed. No sample pooling was performed.

189 Prokaryotic 16S rRNA V3-V4 regions were amplified using fusion primers 341F (5'-
190 [TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]CCTACGGGNGGCWGCAG-3') and 805R (5'-
191 [GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]GACTACHVGGGTATCTAATCC-3') (Klindworth et
192 al. 2013), which consisted of the locus-specific primer sequence ligated to Illumina adapter consensus sequence
193 (indicated in square brackets). The eukaryotic 18S rRNA V4 region was amplified with the fusion primers F-574 (5'-
194 -[TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]GCGGTAATCCAGCTCCAA-3') and R-952 (5'-
195 [TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]TTGGCAAATGCTTTCGC-3') (Hadziavdic et al. 2014).

196 The PCR reactions were carried out in 25 µl volumes consisting of 12.5 µl KAPA HiFi HotStart Mastermix (Roche
197 Diagnostics), 12.5 ng genomic DNA and 1 µl forward and reverse primers (10 µM). Amplification was carried out on
198 a Veriti 96-well thermocycler (Thermo Fisher Scientific) using the following conditions: 95°C for 3 min, followed by
199 25 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, with a final extension of 7 min at 72°C. Following
200 clean-up of the resulting amplicons with 0.8x volume AMPure XP beads (Beckman Coulter Ltd), libraries were dual
201 indexed using a Nextera XT v2 Index Kit (Illumina) for a further 8 PCR cycles. Tagged amplicon libraries were
202 sequenced on an Illumina™ MiSeq using a MiSeq v3 Reagent Kit (Illumina) in the Environmental Genomics Facility
203 at the University of Southampton, UK.

204 Sequencing reads were de-multiplexed on-instrument by the MiSeq Control Software (version 2.6) and
205 checked for quality using FastQC version 0.11.8 (www.bioinformatics.babraham.ac.uk/projects/fastqc/). Nextera XT
206 sequencing adaptors and low quality 3' bases (quality threshold: 15) were trimmed using CutAdapt version 2.3 (Martin
207 2011). Reads shorter than 250 bp after trimming and quality filtering were discarded.

208 *16S and 18S metabarcoding analysis*

209 Analysis of 16S and 18S amplicon sequences was carried out with QIIME2 (Bolyen et al. 2019). Forward
210 and reverse reads were initially trimmed with Cutadapt (Martin 2011) and merged using PandaSeq (Masella et al.
211 2012) with default parameters. Merged sequences were imported into QIIME2, quality filtered with “qiime quality-
212 filter q-score-joined” and denoised with either “qiime deblur denoise-16S” or “qiime deblur denoise-other” for 16S
213 and 18S, respectively. Phylogenetic trees were constructed from the resulting representative sequences using MAFFT
214 for multiple sequence alignment (Kato & Standley 2013) and FastTree (Price et al. 2010) for tree construction. To
215 assign taxonomy to representative sequences, target regions of the 16S and 18S genes were extracted from the SILVA
216 database (v132, ref) at 99% redundancy, and a Naïve Bayes Classifier was trained on the extracted sequences using
217 the “feature-classifier” plugin (Bokulich et al. 2018). Host sequences and putative contaminants were removed from
218 the 18S dataset by excluding sequences assigned to “Teleostei” and “Mammalia” using the “qiime taxa filter-table”
219 command with “-p-exclude” option. This created a first 18S dataset called “18S_all” which also included the putative
220 parasitic groups Apicomplexa (protozoans) and Ascaridida (Nematodes) reported in a separate section. Subsequently,
221 to focus on fish diet, non-metazoan sequences were removed from the 18S dataset by keeping sequences assigned to
222 “Metazoa”, and also excluding parasitic sequences assigned to “Ascaridida”. This generated a 18S dataset called
223 “18S_metazoan” which included only non-parasitic metazoan sequences that was used to assess differences in diet

224 among fish. The 16S dataset was similarly filtered to exclude sequences classified as “Chloroplast” as these likely
225 resulted from the diet and were deemed not to be functional components of the microbiome.

226 Bar plots were generated with the command “qiime taxa barplot” and the resulting data were imported into
227 R and plotted using phyloseq (McMurdie & Holmes 2013) and ggplot2 (Wickham 2016) packages. PCoA ordination
228 plots were generated using the phyloseq function “qiime diversity core-metrics-phylogenetic” and then plotted using
229 ggplot2. Variation in beta diversity among beaches and depths was assessed by means of PERMANOVA (default
230 options) implemented in the “qiime diversity beta-group-significance” command. For beta-diversity analyses, the 16S
231 feature table was rarefied to 579 sequences per sample, while the 18S feature table was rarefied to 2823 sequences per
232 sample, following inspection of the “interactive sample detail” plots from the feature-table summaries. Analysis of
233 microbiome composition (ANCOM; Mandal et al. 2015), implemented in the “qiime composition ancom” command
234 with default parameters and $\alpha = 0.05$, was then applied to identify features that differed in relative abundance between
235 beaches at the same depth (Tralee Shallow vs. Caolisport Shallow) and between depths at Tralee (Tralee Shallow vs.
236 Tralee Deep).

237

238 Results

239 *Size, nutritional condition and feeding success*

240 The size and stomach fullness (estimated feeding success) of YOY plaice differed between Tralee and
241 Caolisport and size also differed between the two depths at Tralee. Fish at Tralee Shallow were 75% longer ($t = 13$,
242 $df = 43$, $p < 0.0001$), over five times heavier ($t = 16$, $df = 43$, $p < 0.0001$) and had much fuller stomachs ($t = 5.7$, $df =$
243 38 , $p < 0.0001$) than those at Caolisport Shallow (Table 1; Table 2). The fish from Tralee Deep were 9% longer ($t =$
244 2.1 , $df = 43$, $p < 0.046$) and 37% heavier ($t = 2.4$, $df = 43$, $p = 0.02$) but had similar stomach fullness ($t = 0.41$, $df =$
245 38 , $p > 0.05$) relative to those at Tralee Shallow (Table 1; Table 2).

246

247 **Table 1:** Mean \pm SD size (total length (TL) and mass), feeding success (stomach fullness), and nutritional condition
248 (RNA:DNA) of young-of-the-year European plaice *Pleuronectes platessa* at two beaches on the west coast of Scotland
249 in July 2016. Fish at Tralee were sampled from both 0.5 (Shallow) and 2 m (Deep) depth below waterline ($n = 15$ for
250 Caolisport Shallow and Tralee Deep, $n = 30$ for Tralee Shallow).

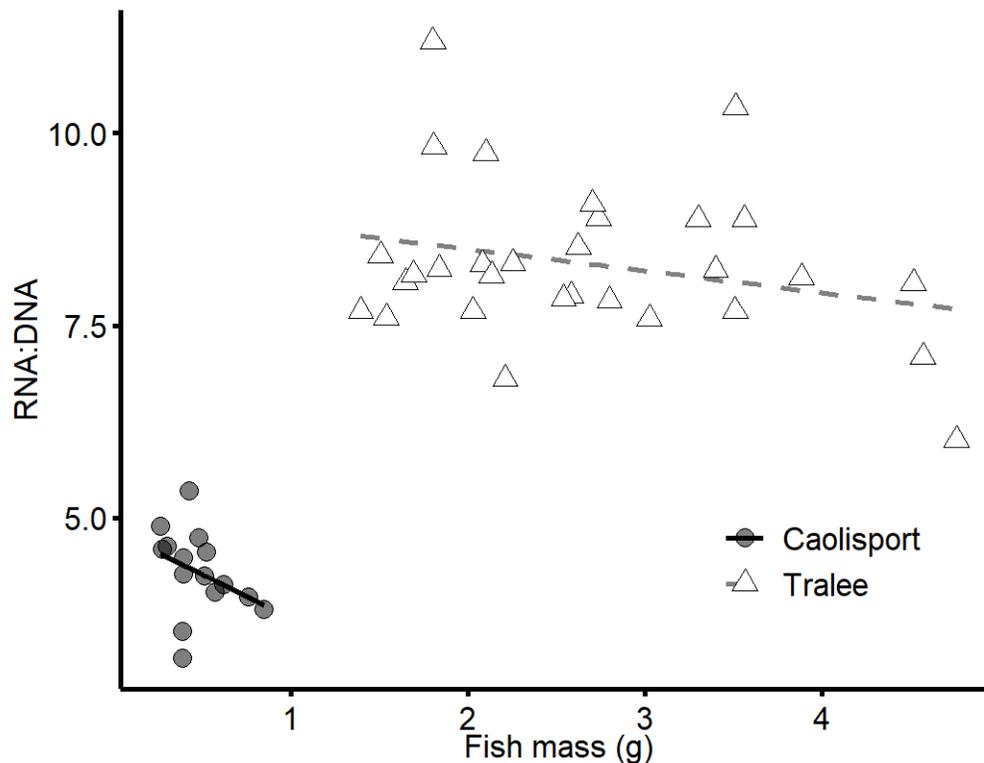
	Caolisport Shallow	Tralee Shallow	Tralee Deep
Body length (mm TL)	35.8 \pm 4.60	62.6 \pm 7.6	68.5 \pm 11.6
Body mass (g)	0.47 \pm 0.17	2.7 \pm 0.95	3.7 \pm 1.6
Stomach fullness	0.0036 \pm 0.0046	0.022 \pm 0.015	0.025 \pm 0.025

251

252 Analysis of 2-way linear models identified a difference in RNA:DNA (nutritional condition) between Tralee
253 and Caolisport Beaches but not between the two depths at Tralee Beach. In models comparing beaches, RNA:DNA
254 of fish at Tralee Shallow was almost twice that at Caolisport Shallow (Figure 1; Table 1; $F_{(1,42)} = 101$, $p < 0.0001$;
255 $\Delta AICc = 53$), but did not vary with body mass, either as a main effect ($F_{(1,42)} = 3.04$, $p > 0.05$; $\Delta AICc = 0.74$) or as an
256 interaction with beach ($F_{(1,41)} = 0.37$, $p > 0.05$; $\Delta AICc = -2.1$; Table 2). In models comparing depths at Tralee,
257 RNA:DNA did not vary with body mass ($F_{(1,42)} = 2.3$, $p > 0.05$; $\Delta AICc = -0.050$), depth ($F_{(1,42)} = 0.23$, $p > 0.05$; $\Delta AICc$
258 $= -2.2$) or their interaction ($F_{(1,41)} = 0.45$, $p > 0.05$; $\Delta AICc = -1.9$; Table 2).

259 *18S: YOY Plaice Diet*

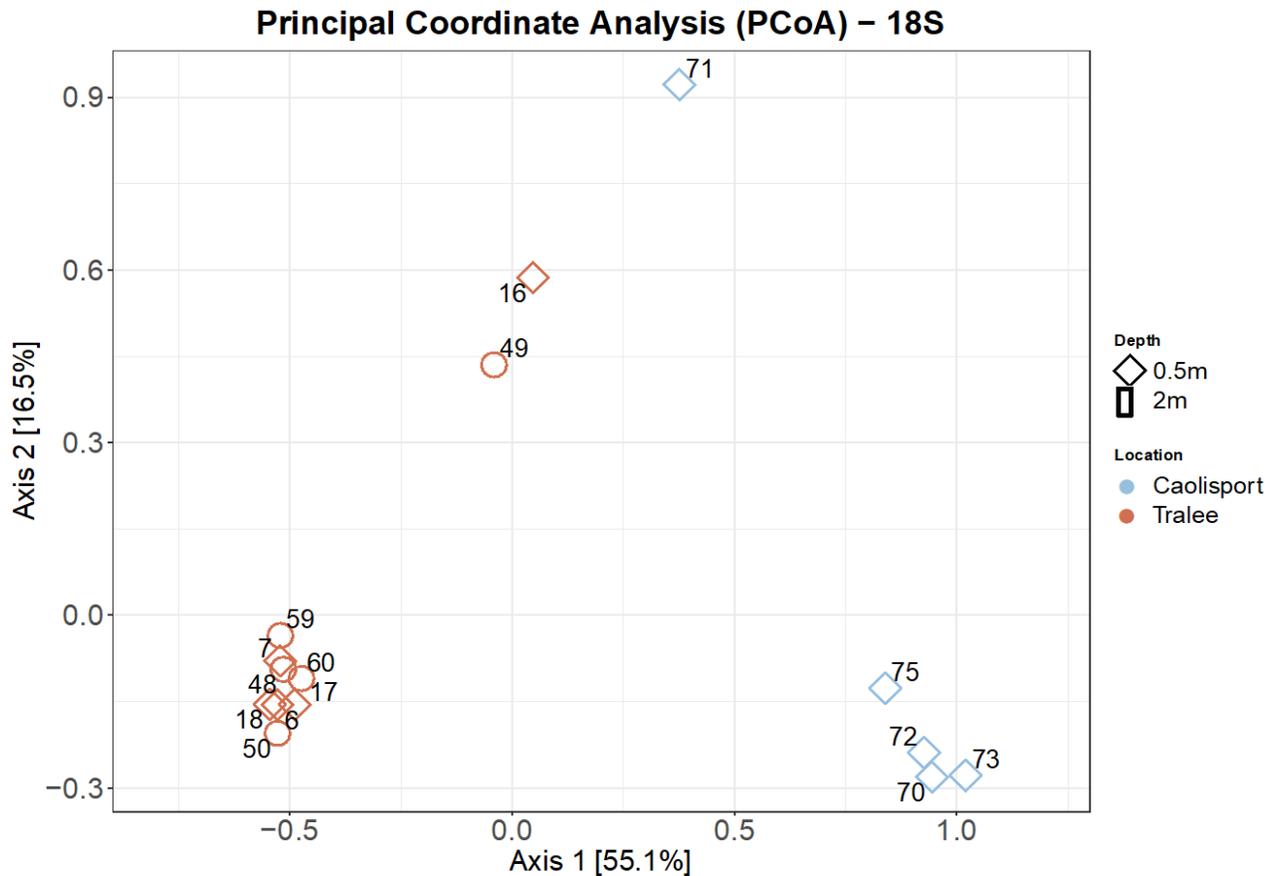
260 Sequencing of the 18S V4 region from YOY plaice gut contents yielded a total of 521,569 sequences for the
261 “18S_metazoan” database. Beta-diversity analysis revealed a significant difference in diet assemblages between
262 Caolisport and Tralee Beaches at 0.5 m depth (Figure 2; Table 2; PERMANOVA pseudo-F 2.2, 999 permutations, q-
263 value = 0.016) but no difference between depths at Tralee (Figure 2, Table 2; PERMANOVA pseudo-F 0.95, 999
264 permutations, q-value = 0.487).



265

266 **Figure 1:** Relationship between RNA:DNA of white muscle tissue and mass of individual young-of-the-year
267 European plaice *Pleuronectes platessa* at two beaches on the west coast of Scotland in July 2016. Fish were sampled
268 from 0.5 m depth below waterline at both locations. Lines represent fit of 2-way general linear model.

269
270 In the “18S_metazoan” dataset, the predominant phyla were Annelida and Arthropoda comprising 55.8% and
271 42.8% of total sequences respectively (Figure 3). Approximately 99.5% of the annelid sequences were assigned to
272 *Lanice conchilega*, but *Clymenura clypeata* accounted for 41.5% of sequences in one Caolisport fish (fish 71).
273 Arthropod sequences were mostly copepods (99.7%) followed by podocopa (found in two Caolisport Shallow fish,
274 fish 72 and 73, 0.2% of total sequences). Copepods were mostly assigned to order Harpacticoida (43.8%) but the
275 majority (56.1%) could not be classified below the subclass level. Non-parasitic Nematode sequences were also
276 observed (0.76% of the total sequences), but were concentrated in two Tralee samples (fish 16: 8.1%, fish 49: 89.4%
277 of sequences for each fish). These were assigned to Chromadorida in the family Chromadoridae, a family of free-
278 living worms commonly associated with freshwater and marine sediments. The relative abundances of known prey
279 other than polychaetes, crustaceans and nematodes were very low, and there was a notable scarcity of molluscs (<
280 0.1% of total sequences).



281

282 **Figure 2** Ordination of Principal Coordinate Analysis (PCoA), representing Bray-Curtis distances among prey
 283 assemblages in stomachs of individual young-of-the-year European plaice *Pleuronectes platessa* at two beaches on
 284 the west coast of Scotland in July 2016, based on the sequencing of the 18S V4 region (n = 5 from each site) using the
 285 “18S_metazoan” dataset (see Materials and Methods). Numbers associated with individual points refer to fish ID:
 286 associated body sizes are provided in Figures 3-5.

287

288 Diets of YOY plaice differed between the two beaches and two depths compared. Gut contents were dominated by

289 crustaceans at Caolisport Shallow, annelids at Tralee Shallow and nematodes or annelids at Tralee Deep (Figure 3).

290 ANCOM analysis indicated that phylum Annelida was significantly more abundant in Tralee Shallow fish compared

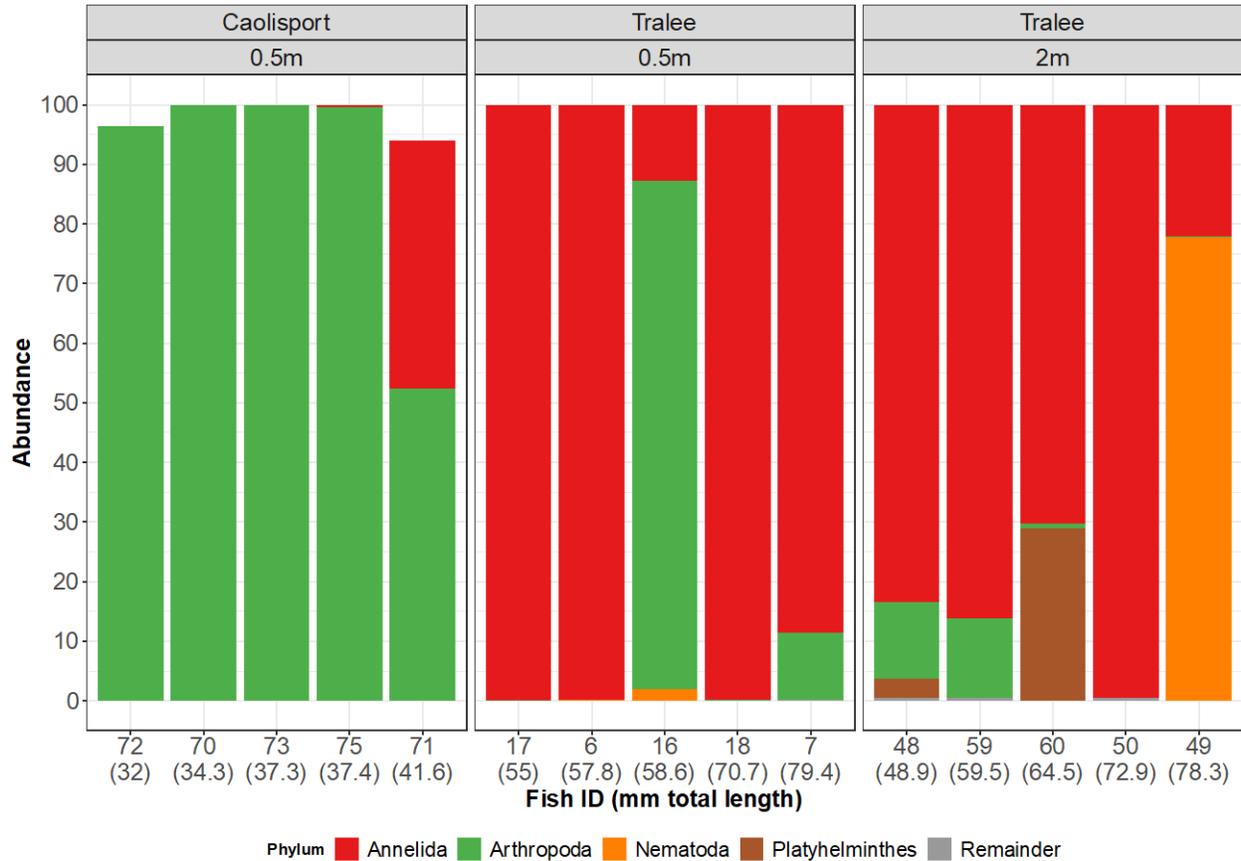
291 to Caolisport Shallow (ANCOM W = 7); whereas no statistical difference was found for the phylum Arthropoda (W

292 = 3; Table 2). Annelida were also significantly more abundant in fish from Tralee Shallow than in fish from Tralee

293 Deep (W = 2; Table 2). ANCOM at a feature level suggested that *Lanice conchilega* were more abundant in fish from

294 Tralee Shallow than Caolisport Shallow (W =) but no differences among depths were detected.

295



296

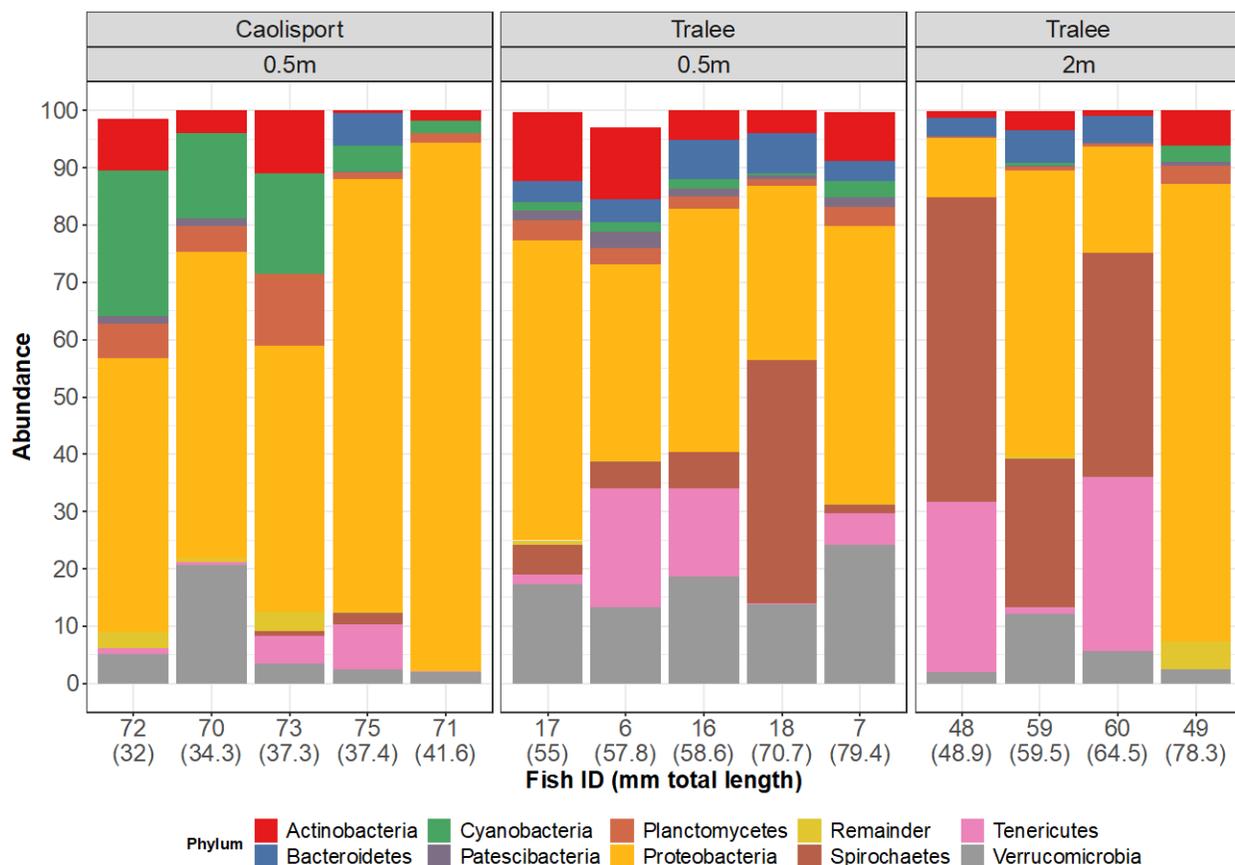
297 **Figure 3:** Composition at phylum level of 18S sequences (using the “18S_metazoan” dataset; see Material and
 298 methods) from guts of young-of-the-year European plaice *Pleuronectes platessa* at two beaches on the west coast of
 299 Scotland in July 2016, based on sequencing of the 18S V4 region. Fish were sampled from 0.5 m depth below
 300 waterline at both locations and also from 2 m below waterline at Tralee (n = 5 from each site). Abundance is
 301 expressed as a percentage of the total sequence library for individual fish. Only macrofaunal phyla present at above
 302 1% mean overall relative abundance are plotted. The excluded phyla were combined into a ‘Remainder’ category.
 303 Some bars do not reach 100% since they are missing the sequences that could not be identified to phylum level. Fish
 304 within sites are arranged in order of increasing size. Numbers below bars refer to fish ID with fish size (mm total
 305 length) in parentheses.
 306

307 **18S: Parasite Sequences**

308 Alongside diet-related taxa, the 18S libraries also contained sequences from putative parasitic groups. Within
 309 the “18S_all” dataset, Apicomplexa was the most common of these phyla and while usually present at low relative
 310 abundances of < 5% of individual fish libraries, they reached burdens > 50% in three Tralee Deep fish. Nearly all
 311 (99%) apicomplexan sequences were assigned to *Coccidia* (genus), a parasitic group known to infect a range of marine
 312 vertebrate and invertebrate hosts, including flatfishes and polychaetes. Putative parasitic Nematodes belonging to the
 313 family Ascaridida were also found. They were mostly present in one Tralee deep fish (sample 48, > 99% of the
 314 sequences) and in one Caolisport fish (sample 72). ANCOM analysis revealed that *Coccidia* sequence relative

315 abundances did not differ between locations (Tralee Shallow vs. Caolisport Shallow, W = 0) or depths (Tralee Shallow
 316 vs. Tralee Deep, W = 0).

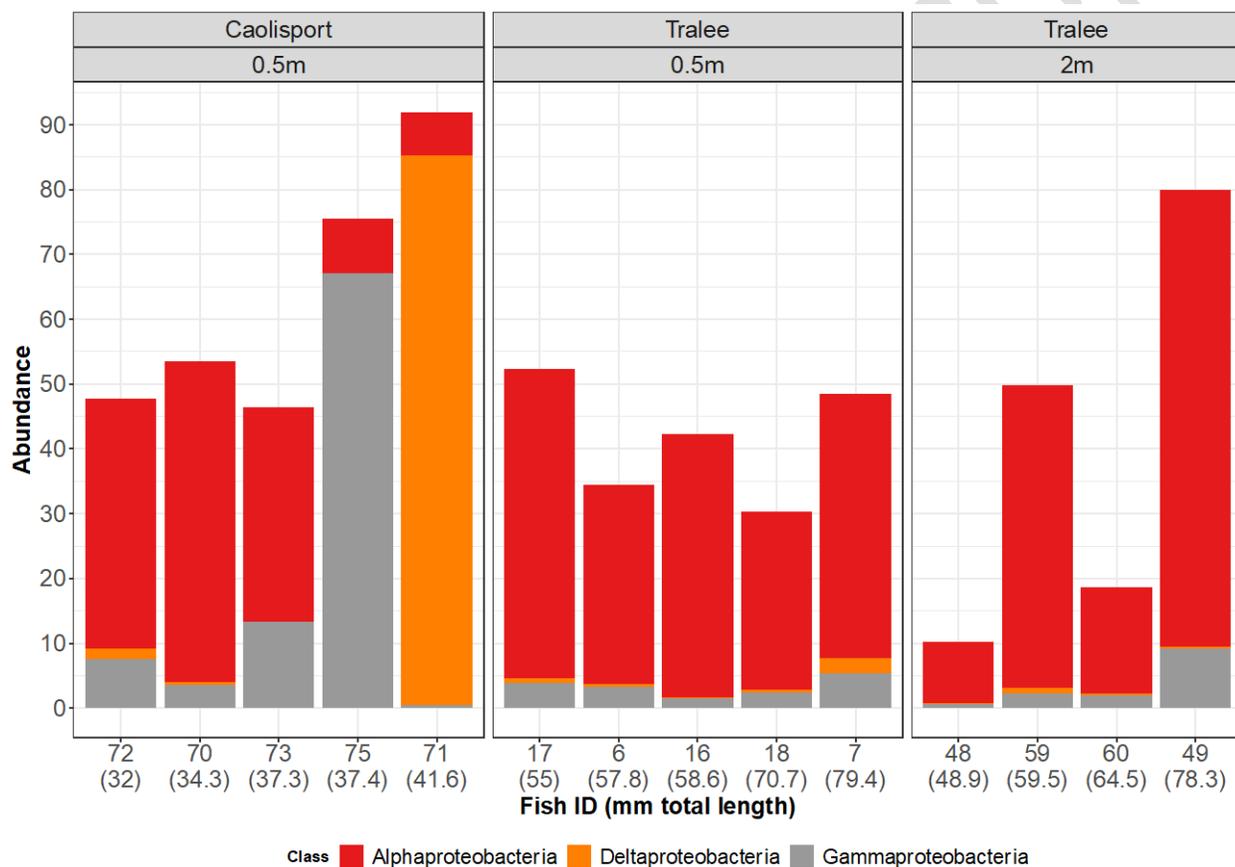
317 **16S: YOY Plaice Gut Microbiome**



318
 319 **Figure 4:** Composition at phylum level of the gut microbiota of young-of-the-year European plaice *Pleuronectes*
 320 *platessa* at two beaches on the west coast of Scotland in July 2016, based on the sequencing of the 16S V3-V4
 321 regions (after excluding sequences that were assigned to “Chloroplast”). Plaice were sampled from 0.5 m depth
 322 below waterline at both locations and also from 2 m below waterline at Tralee (n = 5 from both shallow sites and n =
 323 4 from Tralee Deep). Abundance is expressed as percentage of the total sequence library for individual fish. Only
 324 phyla present at above 0.5% mean overall relative abundance were plotted. The excluded phyla collectively
 325 represented less than 0.5% of total sequences and were combined into a ‘Remainder’ category. Some bars do not
 326 reach 100% since they are missing the sequences that could not be identified to phylum level. Fish within sites are
 327 arranged in order of increasing size. Numbers below bars refer to fish ID with fish size (mm total length) in
 328 parentheses.
 329

330 Sequencing of the 16S V3-V4 regions from YOY plaice gut contents yielded a total of 179,769 sequences
 331 after quality filtering and denoising for a total of 1243 Operational Taxonomic Units (OTUs). One fish from Tralee
 332 Deep (fish 50) yielded a low quality 16S library (< 1000 sequences) and was removed from the analysis. Following
 333 removal of chloroplast sequences, which originally made up 96% of the cyanobacterial fraction, nine bacterial phyla,

334 each represented at > 0.5% of total sequences accounted for 98.9% of the total sequences: Actinobacteria,
 335 Bacteroidetes, Cyanobacteria, Patescibacteria, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, and
 336 Verrucomicrobia. Proteobacteria was the dominant phylum in the dataset, representing 45.5% of the total sequences
 337 (Figure 4). The next most abundant phyla were Spirochaetes (17.5% of total), Tenericutes (11.5% of total) and
 338 Verrucomicrobia (10.7% of total, Figure 4). All other phyla were present at < 10% of the total sequences (Figure 4).
 339 At the phylum level, ANCOM analysis revealed higher relative abundances of Cyanobacteria ($W = 7$) but lower
 340 abundances of Actinobacteria and Spirochaetes ($W = 4$ and $W = 3$, respectively) at Caolisport Shallow compared to
 341 Tralee Shallow (Table 2). No difference was found between the two depths at Tralee (Table 2).



342
 343 **Figure 5:** Class composition within phylum Proteobacteria in gut microbiota of young-of-the-year European plaice
 344 *Pleuronectes platessa* at two beaches on the west coast of Scotland in July 2016, based on the sequencing of the 16S
 345 V3-V4 regions. Plaice were sampled from 0.5 m depth below waterline at both locations and also from 2 m below
 346 waterline at Tralee (n = 5 from both shallow sites and n = 4 from Tralee Deep). Abundance is expressed as percentage
 347 of the total sequence library for individual fish. Fish within sites are arranged in order of increasing size. Numbers
 348 below bars refer to fish ID with fish size (mm total length) in parentheses.
 349

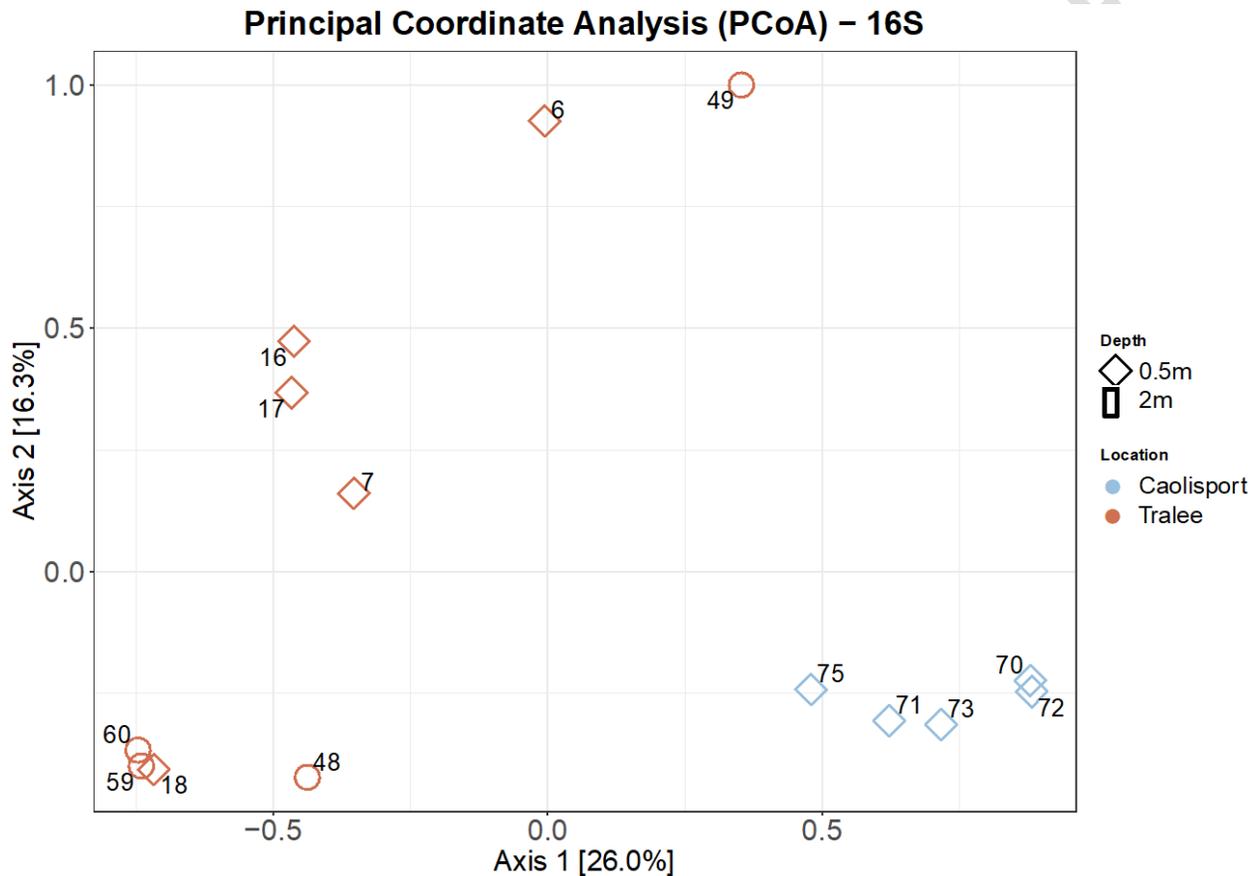
350 Dominant classes within the phylum Proteobacteria were Alphaproteobacteria, Gammaproteobacteria, and
351 Deltaproteobacteria (Figure 5). Alphaproteobacteria accounted for 59.7% of proteobacterial sequences overall and
352 represented between 71.3% and 96.0% of those in individual fish libraries (median 88.6%), except in two Caolisport
353 Shallow samples where they represented only 7.2% and 11.0% (Figure 5). The next most abundant proteobacterial
354 class was Gammaproteobacteria (21.2% of proteobacterial sequences), which represented between 0.5% and 28.6%
355 of proteobacterial sequences in individual fish, with the exception of one Caolisport sample in which it composed
356 approximately 88.8% of proteobacterial sequences (Figure 5). Similarly, Deltaproteobacteria accounted for 92.1% of
357 proteobacterial sequences in another Caolisport sample, but represented a much lower median relative abundance of
358 1.2% overall (Figure 5). Betaproteobacteria and Epsilonproteobacteria were not recorded.

359 The Alphaproteobacteria were mainly composed of members of the family Rhodobacteraceae that could not
360 be classified to genus level (50.8% of total alphaproteobacterial sequences). The majority (69.0%) of
361 Gammaproteobacteria sequences were assigned to *Photobacterium*, but this was largely due to high levels of
362 Gammaproteobacteria in one sample (fish 75). In other specimens, gammaproteobacterial taxa were more varied and
363 included Vibrionales, Cellvibrionales and Thiotrichales. Other common marine genera such as *Vibrio* and
364 *Pseudomonas* were unusually rare (less than 0.05 % of total sequences). Deltaproteobacteria was predominantly
365 represented by an uncultured bacterial genus from the SAR324 clade (Marine group B), which was almost exclusively
366 responsible for the unusually high relative abundance (84% of all sequences in that library) of Deltaproteobacteria in
367 one Caolisport sample.

368 Genera within the other bacterial phyla accounted for only a small proportion of the bacterial assemblage.
369 Within the spirochaetes the most abundant genera were *Spirochaeta* (13% total sequences) and *Brevinema* (3.6% total
370 sequences). Within the Verrucomicrobia, the most abundant family was Rubritaleaceae, with *Rubritalea*,
371 *Luteolibacter*, *Roseibacillus*, and *Haloferula*, as well as two uncultured genera from the DEV007 family accounting
372 for > 1% total sequences in at least one library. Tenericutes mostly consisted of *Mycoplasma*.

373 PCoA ordination of Bray-Curtis distances suggested that 16S microbiome compositions clustered based on
374 sampling location (Figure 6). There was clear separation between Tralee Shallow and Caolisport Shallow samples
375 (Figure 6; Table 2; PERMANOVA, 999 permutations, pseudo-F = 4.99, q-value = 0.009). At the feature level,
376 ANCOM revealed that three features were differentially abundant: two Alphaproteobacteria, one of the family
377 Rhizobiaceae (W = 499) and one Rhodobacteraceae (W = 607), which were both absent from Caolisport Shallow and

378 present in all Tralee Shallow samples; and a Cyanobacteria of the family Chanobiaceae (W = 607) which was absent
 379 from Tralee Shallow but present in Caolisport Shallow (Table 2). In addition, separation between Tralee stations at
 380 different depths was observed along Axis 2 (Figure 6; PERMANOVA, 999 permutations, pseudo-F = 1.72, q-value =
 381 0.009), although ANCOM did not detect specific features that differed in relative abundance between depths (Table
 382 2).



383
 384 **Figure 6** Ordination of Principal Coordinate Analysis (PCoA), representing Bray-Curtis distances among gut
 385 microbiota assemblages of individual young-of-the-year European plaice *Pleuronectes platessa* at two beaches on the
 386 west coast of Scotland in July 2016, based on the sequencing of the 16S V3-V4 regions (n = 5 from both shallow sites
 387 and n = 4 from Tralee Deep). The 16S dataset was filtered to exclude sequences classified as “Chloroplast”. Numbers
 388 associated with individual points refer to fish ID: associated body sizes are provided in Figures 3-5.

389 **Table 2:** Spatial scales over which differences in size, diet, nutritional condition and microbiome were detected in young-of-the-year European plaice
 390 *Pleuronectes platessa* at two beaches on the west coast of Scotland in July 2016. No difference indicated by '-'.
 391

Spatial scale	Biological parameters								
	Body length and mass (Table 1)	Diet				RNA:DNA (nutritional condition) (Figure 1)	Microbiome		
		Stomach fullness (feeding success) (Table 1)	β diversity of prey (PERMANOVA) (Figure 2)	Relative abundance of phyla (ANCOM) (Figure 3)	Relative abundance of features (ANCOM)		β diversity of microbiota (PERMANOVA) (Figure 6)	Relative abundance of phyla (ANCOM) (Figure 4)	Relative abundance of features (ANCOM) (Figure 5)
Beaches (10s of km): Tralee Shallow relative to Caolisport Shallow	1.7 x longer 5.7 x heavier	6.1 x fuller	Different prey assemblage	More annelids	More <i>Lanice conchilega</i>	1.9 x higher	Different microbial assemblage	Less Cyanobacteria. More Actinobacteria and Spirochaetes.	Presence of Rhizoniaceae and Rhodobacteriaceae. Absence of Chanobiaceae
Depths (10s of m): Tralee Deep (2m) relative to Tralee Shallow (0.5m)	1.1 x longer 1.4 x heavier	-	-	Less annelids	-	-	Different microbial assemblage	-	-

392

393 **Discussion**

394 Although *Pleuronectes platessa* is of considerable commercial importance and an extensively studied model
395 species in fish ecology, factors driving variation in growth and condition of juveniles are not well resolved (Ciotti et
396 al. 2014). In this study, we used high-throughput sequencing to characterise the diet and gut microbiome of YOY
397 plaice at beaches on the west coast of Scotland, and to test how these vary at contrasting spatial scales in relation to
398 the physiological status of the host. We found substantial differences in the body size, stomach fullness, nutritional
399 condition, diet, and gut microbiome of YOY plaice between beaches separated by 10s of kilometers. Surprisingly, we
400 also found smaller, but notable differences in the body size, diet and gut microbiome between sampling depths at the
401 same beach, even though these were only separated by a few 10s of metres. Therefore, our study shows that there can
402 be considerable differences in key metrics of feeding and growth at fine spatial scales along complex coastlines typical
403 of much YOY plaice habitat (Ciotti et al. 2013c).

404 Our results contrast with the relative homogeneity at *ca.* 10 - 100 km spatial scales in the diets and gut
405 microbiota of YOY plaice found by Heindler et al. (2019) in the eastern English Channel and southern North Sea.
406 This may be due to the fact that the area studied by Heindler et al. (2019), in contrast to the west coast of Scotland, is
407 a relatively open stretch of coast, perhaps offering more uniform environmental conditions and prey assemblages. An
408 alternative explanation is that samples used for regional comparisons by Heindler et al. (2019) were pooled from
409 several sites, themselves separated by tens of kilometres, and across a time window spanning several months: pooling
410 may have smoothed out differences expected based on known small-scale (100s of metres, daily) spatiotemporal
411 heterogeneity in the diet and growth of YOY plaice.

412 Diets of YOY plaice in the current study were dominated by polychaetes and crustaceans, consistent with
413 results from studies using visual examination of gut contents from the west coast of Scotland (Edwards & Steele 1968,
414 Steele et al. 1970, Poxton et al. 1983) and other areas (Macer 1967, Amara et al. 2001, Freitas et al. 2010, De
415 Raedemaecker et al. 2011, Jones et al. 2020). In contrast to these previous studies, however, molluscs were
416 conspicuously absent. Polychaete prey was almost entirely *Lanice conchilega*, a species known to form an important
417 component of the diet at Tralee Beach (M. T. Burrows, unpublished data) but not necessarily nearby sites (Edwards
418 & Steele 1968, Steele et al. 1970, Poxton et al. 1983). Besides resolving key prey items, 18S sequencing also identified
419 putative parasitic taxa. The influence of parasitic infection on wild YOY plaice is unknown and would benefit from
420 further investigation.

421 Diets differed considerably between beaches, with fish at Tralee having fuller stomachs and feeding almost
422 exclusively on polychaetes and those at Caolisport preying predominantly on crustaceans. The metabarcoding study
423 of YOY plaice by Heindler et al. (2019) found that diets along a > 100 km stretch of open coastline in the eastern
424 English Channel and southern North Sea were remarkably stable and dominated by the crustacean *Crangon crangon*
425 (72%) and the polychaete *Owenia fusiformis* (9%). They reported that diets changed little between spring and summer
426 although there were small differences with body size and among years. Our results align with a growing recognition
427 that YOY plaice are opportunistic, generalist feeders and that diets along the complex coastlines of northern Britain
428 are highly variable at very small temporal (hours or days) and spatial (100s of metres) scales (M. T. Burrows,
429 unpublished data; Poxton et al. 1983, Ansell & Gibson 1990, Ciotti et al. 2013c).

430 Our understanding of the role of the environment and prey availability on driving differences in diet and
431 microbiota between Caolisport and Tralee is somewhat confounded by fish size as YOY plaice at Caolisport were
432 smaller than those from Tralee. Smaller, slower growing fish at Caolisport is a consistent but as yet unexplained
433 difference between these two sites (Ciotti et al. 2010, Ciotti et al. 2013b, Ciotti et al. 2013a, Fox et al. 2014). In this
434 study, Caolisport fish fed primarily on copepods, while Tralee fish fed on the polychaete *Lanice conchilega*. However,
435 there is no evidence that within each site fish were transitioning from a copepod-based to an annelid-based diet with
436 increased size (Figure 3; fish arranged in size order). In fact, annelids (either parts or whole animals) have been
437 previously shown to dominate the diet of the full size range of YOY plaice from Tralee (M. T. Burrows, unpublished
438 data). Given that YOY plaice are known to exhibit considerable spatiotemporal variation in diet (Poxton et al. 1983,
439 Ansell & Gibson 1990, Gibson 1999), we suggest that spatial differences are the cause of the diet differences, which,
440 along with local environmental conditions and associated microbial community, are primary determinants of the gut
441 microbiota in fishes (Talwar et al. 2018).

442 While this study did not examine consequences of variation in diet quality and quantity for the nutritional
443 condition and size of juveniles at Caolisport vs. Tralee, a link seems plausible and would benefit from further
444 investigation. Spatial variation in YOY plaice growth on the west coast of Scotland is not related to temperature,
445 interspecific competitor density or beach productivity but is weakly and negatively related to conspecific density
446 (Ciotti et al. 2013b, Ciotti et al. 2013a). Physical characteristics of beaches, including wave exposure and tidal range,
447 explain growth variation well, but the mechanisms underlying this relationship are unresolved (Ciotti et al. 2013b).
448 Meanwhile, little is known about how variation in the quality of prey may influence growth rates. In the current study,

449 we found that YOY plaice from Caolisport, the site with small fish in low nutritional condition and empty stomachs,
450 consumed small crustaceans. While such prey are often abundant (Edwards & Steele 1968), they may be too small
451 (Bregnballe 1961) or expose feeding plaice to unacceptably high levels of predation risk (Steele et al. 1970) to sustain
452 high rates of food intake.

453 YOY plaice at Tralee were larger at the deeper site: this was accompanied by reduced relative abundance of
454 annelids in their diet, but the overall diet composition, feeding success and nutritional condition did not vary. Despite
455 some exceptions (Ciotti et al. 2013c), previous studies at Tralee (Gibson 1973, Gibson & Robb 1996, Gibson et al.
456 2002) and a range of other locations (Bregnballe 1961, Macer 1967, Edwards & Steele 1968, Poxton et al. 1983, Teal
457 et al. 2008) have found a similar pattern of size difference with depth, due either to size-related differences in depth
458 selection behaviour or to depth-related differences in growth conditions. Our study suggests that growth conditions,
459 as reflected in stomach fullness and nutritional condition, were similar across depths. Prey quality may have differed,
460 however, since the abundance of annelids was lower at the deeper site. We found high proportions of nematodes or
461 platyhelminthes in stomachs of some individuals from the deeper site, although it is important to note that 18S
462 sequencing provided relative abundances such that absolute quantities may be small if stomachs were not full.
463 Nematodes and platyhelminthes have not previously been reported as YOY plaice prey at beaches on the west coast
464 of Scotland (Edwards & Steele 1968, Steele et al. 1970, Poxton et al. 1983) but nematodes have been encountered in
465 other areas (Amara et al. 2001, Heindler et al. 2019).

466 Overall, the gut microbiome of YOY plaice was principally composed of Proteobacteria, with a lower relative
467 abundance of Spirochaetes, Tenericutes and Verrucomicrobiae along with minor contributions of Actinobacteria,
468 Bacteroidetes, Cyanobacteria, Patescibacteria, and Planctomycetes. These phyla may play key roles in the nutrition,
469 metabolism, immunity, and development of their fish hosts. Proteobacteria also dominated gut microbiota in YOY
470 plaice from the eastern English Channel and southern North Sea (Heindler et al. 2019), but in this case the next most
471 dominant phyla were Planctomycetes and Actinobacteria and there were also differences in the composition of minor
472 phyla. Neither study separated gut microbiota from that found in the water, sediment or prey, and although sequencing
473 largely focused on gut wall samples, some sequences would have been allochthonous, transient members of the gut
474 microbial assemblage derived from prey. Despite this, we did not find marked anomalies in samples where both gut
475 wall and contents were sequenced together or where diets differed from others at the same site (fish 16, 48, 49 and 71;
476 Figures 3 and 4). Therefore, there is no strong evidence that the YOY plaice gut microbiome we describe derived from

477 the prey. Indeed, Heindler et al. (2019) concluded that habitat, rather than prey composition, was the main driver of
478 variation in YOY plaice gut microbiomes in their study.

479 Proteobacteria, the most common microbial phylum we encountered in YOY plaice, is an abundant and
480 dominant member of gut microbiota in both freshwater and marine fishes (Nayak 2010, Clements et al. 2014, Givens
481 et al. 2015). This phylum has been observed in the gut of numerous freshwater fish such as salmonids (Wong et al.
482 2013, Al-Hisnawi et al. 2015, Llewellyn et al. 2016) and cyprinids (Wu et al. 2010, Wu et al. 2012, Wu et al. 2013,
483 Larsen et al. 2014), where it is a dominant element of both the transient and autochthonous microbial communities.
484 Similarly, Givens et al. (2015) found similar results for 12 brackish water and marine bony fish species, including the
485 flatfishes hogchoker (*Trinectes maculatus*) and southern flounder (*Paralichthys lethostigma*). High relative
486 abundances of Proteobacteria have also been observed in the gut of other flatfishes including farmed turbot
487 *Scophthalmus maximus* (79%; Xing et al. 2013) and wild fine flounder *Paralichthys adspersus* (68%; Ramírez &
488 Romero 2017).

489 Our study of YOY plaice adds to growing evidence that the dominance of Proteobacteria is not restricted to
490 adult fish, and also extends to earlier life stages. Bates et al. (2006) and Lan and Love (2012) observed that
491 Proteobacteria were the most prominent taxa and numerically abundant at all stages in zebrafish juveniles. Likewise,
492 Dulski et al. (2018) found that the core intestinal microbiome of juvenile pikeperch (*Sander lucioperca*) was
493 predominantly Proteobacteria (92-95%), and Parris et al. (2016) reported similar results in larval and juvenile reef
494 damselfish and cardinalfish. The relative abundance of Proteobacteria for YOY plaice in this study (29.1%) and in
495 Heindler et al. (2019; range = 40.1 - 71.6% across regions) is lower than estimates for other fishes, although Ramírez
496 and Romero (2017) found lower relative abundances of Proteobacteria (30% ± 24%) in aquacultured fine flounder.

497 The more striking difference in the gut microbiome of YOY plaice from other known fish gut microbial
498 assemblages is in the taxonomic composition within the phylum Proteobacteria. Indeed, the majority of fish-gut
499 microbiota studies agree that Gammaproteobacteria is the most numerically abundant protobacterial class (Desai et
500 al. 2012, Parris et al. 2016, Dulski et al. 2018), especially in marine fishes (Ingerslev et al. 2014a). Families such as
501 Enterobacteriaceae, Vibrionaceae, and in particular the *Vibrio* and *Photobacterium* genera, Shewanellaceae, and
502 Alteromonadaceae usually represent major fractions of the microbial assemblages in marine fish guts, largely due to
503 their ubiquitous distribution in marine environments (Ingerslev et al. 2014a, Givens et al. 2015). Sullam et al. (2015),
504 for example, found a prevalence of *Vibrionales* and *Aeromonadales* in Trinidadian guppies, and Xing et al. (2013)

505 reported that *Vibrionales*, *Alteromonadales*, and *Enterobacteriales* were the three most abundant bacterial groups in
506 the gut microbiome of farmed adult turbot, collectively constituting nearly 89% of bacterial sequences recovered. In
507 the present study however, Gammaproteobacteria was dominant in only a single Caolisport individual, where
508 *Photobacterium* represented 64% of total sequences. All the other YOY plaice gut microbiomes were instead
509 characterised by high relative abundances of Alphaproteobacteria or, in the case of one individual,
510 Deltaproteobacteria. Heindler et al. (2019) also found that YOY plaice gut microbiota contained relatively low
511 Gammaproteobacteria abundances, but in their study both Alpha- and Betaproteobacteria dominated. Despite being
512 reported in the gut microbiota of surgeonfish (Miyake et al. 2015) and in larval and juvenile reef fishes (Parris et al.
513 2016), Alphaproteobacteria have not commonly been encountered in the microbiome of fish. Both these previous
514 studies suggested that Alphaproteobacteria were transient members of the gut microbiota accidentally ingested along
515 with debris. However, despite only sequencing gut contents, Alphaproteobacteria abundances were lower than in the
516 current study suggesting that the unusual Alphaproteobacteria dominance in YOY plaice may not be solely derived
517 from their prey.

518 In the current study, most Alphaproteobacteria sequences were assigned to Rhodobacteraceae: a common
519 family and widely distributed in marine environments (Wagner-Döbler & Biebl 2006). The functional role of
520 Rhodobacteraceae in the gut is as of yet unclear, although some members of the family have been proposed as possible
521 probiotics for the aquaculture industry (Hjelm et al. 2004, Planas et al. 2006, Balcázar et al. 2007) owing to their
522 production of antibacterial compounds (Hjelm et al. 2004, Wagner-Döbler & Biebl 2006, Bruhn et al. 2007). They are
523 present in most marine environments, such as seawater or sediments, are key members of biofilms (Bruhn et al. 2007,
524 Elifantz et al. 2013) and can occur in algae-associated microbiota (Friedrich 2012, Martin et al. 2015).

525 Verrucomicrobia is a commonly reported phylum in gut microbiome studies of marine adult and juvenile
526 fishes, but is usually rare and present at much lower relative abundances than in the present study (Ingerslev et al.
527 2014a, Ghanbari et al. 2015, Givens et al. 2015). It is not a dominant taxon in YOY plaice in the eastern English
528 Channel and southern North Sea (Heindler et al. 2019) nor in reports on other marine flatfishes, such as fine flounder
529 (Ramírez & Romero 2017).

530 We found that the phylum Tenericutes was represented primarily by the genus *Mycoplasma* in YOY plaice.
531 *Mycoplasma* are abundant members of gut microbiota in numerous vertebrate hosts (Whitcomb & Tully 1989, Giebel
532 et al. 1990, Razin et al. 1998, Clemente et al. 2012) and have been found to dominate in the gut of Atlantic salmon

533 (*Salmo salar*; Holben et al. 2002), farmed rainbow trout (*Oncorhynchus mykiss*; Lyons et al. 2017), Trinidadian
534 guppies (*Poecilia reticulata*; Sullam et al. 2015), and the long-jawed mudsucker (*Gillichthys mirabilis*; Bano et al.
535 2007). *Tenericutes* has been reported at lower relative abundances in the guts of several other fishes (Moran et al.
536 2005, Kim et al. 2007, Sukhanova et al. 2014), but was not an important component in the previous study of YOY
537 plaice (Heindler et al. 2019). Most known species of *Mycoplasma* are pathogens or parasites (Razin et al. 1998,
538 Dandekar et al. 2002), although other species seem to have no harmful effects on their hosts and *Mycoplasma* may be
539 normal members of the gut microbiota in some fish (Giebel et al. 1990, Bano et al. 2007).

540 The physiological performance of wild fish is determined by interactions between the external environment,
541 the individual and its gastrointestinal microbiota (Talwar et al. 2018). Characterising geographic variation within and
542 among wild animal populations can be an informative step in the challenging task of building a functional
543 understanding of the influence that the gut microbiome has on physiological performance. Our paper demonstrates
544 that the gut microbiome in YOY plaice along the west coast of Scotland varies at 10 km scales, that differences can
545 also be distinguished at 10 m scales and furthermore that this variability is accompanied by variations in key aspects
546 of fish feeding and bioenergetics at similar geographic scales (Table 2). The processes driving variation in gut
547 microbiomes of coastal fishes, and potentially the influence of the gut microbiome on physiological performance,
548 therefore appear to operate, and should be studied, at as little as 10 m scales. Although we did not examine the
549 correlation among factors directly, substantially reduced nutritional condition and size at one of the beaches
550 (Caolisport Shallow) was associated with lower stomach fullness, reduced consumption of annelids and differences
551 in the abundance and presence of specific microbial taxa: Cyanobacteria, Actinobacteria, Spirochaetes, Rhizobiaceae,
552 Rhodobacteraceae and Chanobiaceae.

553 Our conclusion that processes determining the diet, microbiota, and the associated nutritional condition of
554 juvenile fish operate at fine spatial scales forms an important departure point for future studies to understand drivers
555 of variation in physiological performance of young fish *in situ*. The next step could be a much larger study involving
556 extensive sampling to better partition the variation across different spatial scales. There is also a need for targeted lab
557 and field manipulations as well as mensurative experiments to establish the relative contribution of the environment,
558 host and diet in driving variations in the microbiome and to quantify the influences of these variations on YOY plaice
559 physiology. Ultimately, by resolving how environmental, host-specific and feeding-related factors interact to shape

560 gut microbial assemblages and identifying resulting consequences for host performance, we stand to elucidate
561 fundamental processes underpinning the dynamics of wild fish populations (Talwar et al. 2018).

562

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567

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