

The effect of ultrasonic antifouling control on the growth and microbiota of farmed European sea bass (Dicentrarchus labrax)

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1	The effect of ultrasonic antifouling control on the growth and microbiota of farmed European
2	sea bass (Dicentrarchus labrax)
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20 Highlights

21	•	Ultrasound exposure does not reduce growth rate or survival of farmed sea bass.
22	•	Ultrasound exposure increases Vibrio and Pseudomonas in gill tissue.
23	•	Ultrasound exposure increases gut-associated Pelomonas in skin mucus.
24	•	Sea bass gut microbiota remains unaffected by ultrasound exposure.
25		

26 Abstract

27 Biofouling is a serious threat to marine renewable energy structures and marine aquaculture 28 operations alike. As an alternative to toxic surface coatings, ultrasonic antifouling control has 29 been proposed as an environmentally friendly means to reduce biofouling. However, the 30 impact of ultrasound on fish farmed in offshore structures or in marine multi-purpose 31 platforms, combining renewable energy production and aquaculture, has not yet been 32 assessed. Here we study the impact of ultrasound on the growth and microbiota of farmed 33 European sea bass (Dicentrarchus labrax) under laboratory conditions. Whereas growth and 34 survival were not reduced by ultrasound exposure, microbiological analysis using plate counts 35 and 16S rRNA gene based metataxonomics showed a perturbation of the gill and skin 36 microbiota, including an increase in putative pathogenic bacteria. This warrants further 37 research into the long-term effects of ultrasonic antifouling control on the health and 38 wellbeing of farmed fish.

39

40 Keywords: antifouling; ultrasound; aquaculture; microbiome; sea bass; Vibrio

41 1. Introduction

42 Human activities developed within maritime areas are constantly increasing. Among these 43 activities, renewable energies and aquaculture are the most emergent and will grow 44 significantly in the future. The marine environment offers multiple sources to produce 45 renewable energy (wind, wave, tides, currents, temperature and pressure gradients) (Abhinav 46 et al., 2020) and, by 2030, the demand for seafood will exceed the current supply by 40 47 million metric tonnes (FAO, 2018). Consequently, to optimize marine spatial planning, co-48 location opportunities for renewable energies and aquaculture facilities must be exploited to 49 develop multi-purpose platforms (offshore platforms serving the needs of multiple offshore 50 industries; Abhinav et al., 2020) in future decades. Case studies are essential to explore co-51 location options and several recent projects were carried out to study the feasibility to 52 combine offshore energy production and aquaculture (Abhinav et al., 2020; Benassai et al., 53 2014; Dalton et al., 2019; Papandroulakis et al., 2017; Schütz and Slater, 2019; Weiss et al., 54 2018). These studies have demonstrated that co-location reduces operating costs (Weiss et al., 55 2018) and that offshore aquaculture is a viable alternative for increasing the global seafood 56 production while offering several advantages such as ample space for expansion, low 57 exposure to pollution and optimal environmental conditions for a wide variety of marine 58 species (Holm et al., 2017).

59 Nevertheless, offshore activities are associated with high technical risks (e.g., mechanical 60 forces, corrosion, extreme conditions, unreliable moorings and biofouling) and the measures 61 put in place to manage these risks must not harm any of the activities developed around the 62 multi-purpose platforms. Biofouling, which refers to the attachment of undesirable molecules 63 and fouling organisms to the submerged surface, is a major problem facing the marine 64 industry. In the marine renewable energy industry, it initiates or accelerates the corrosion 65 process of metal and concrete structures, compromises device performance and requires

additional maintenance activities (Blackwood et al., 2017; Loxton et al., 2017; Want et al., 66 67 2017). In the aquaculture industry, biofouling hinders nutrient exchange and deforms cages due to extra weight which can lower productivity (Fitridge et al., 2012). Therefore, the use of 68 69 anti-biofouling agents is of high economic importance. In general, to limit biofouling and help 70 to decrease device maintenance requirements and costs associated with the removal of 71 biofouling, the submerged structures are protected with antifouling solutions, such as paints 72 and coatings (Finnie and Williams, 2010; Gu et al., 2019). However, these paints and coatings 73 contain toxic substances, such as trace metals and booster co-biocides (zinc and copper 74 pyrithione, chlorothalonil, ziram, zineb, dichlofluanid, diuron) (Turner, 2010), which can be 75 released into the marine environment and impact non-target marine organisms (Chen and 76 Lam, 2017; Soon et al., 2019; Soroldoni et al., 2020). Thus, it is necessary to develop new 77 environmental-friendly antifouling systems.

78 To date, several environmental-friendly antifouling approaches have been considered to 79 combat marine biofouling, including coatings with dynamic surfaces (Xie et al., 2019), foul 80 release coatings (Tian et al., 2020), bioinspired coatings (Li and Guo, 2019), electrolyzing 81 seawater (Wake et al., 2006) and acoustic methods (Legg et al., 2015). Among acoustic 82 treatments studied, ultrasound has been reported to be effective at inhibiting the formation of 83 biofouling on surfaces suspended in freshwater and seawater (Legg et al., 2015; Park and Lee, 84 2018). The most promising results have been obtained for frequencies between 20 and 85 50 kHz. At these frequencies, ultrasound is, for example, capable of causing mortality of 86 numerous crustacean and bivalve larvae (Haque and Kwon, 2018; Legg et al., 2015), 87 removing fouling organisms (Mazue et al., 2011), inhibiting settlement (Guo et al., 2012), 88 inactivating zooplankton (Holm et al., 2008) and reducing biofilm thickness (Bott, 2000). 89 Consequently, ultrasonic treatment could be an interesting alternative to the use of toxic 90 antifouling molecules in order to protect offshore platforms combining energy production and

91 aquaculture. Nevertheless, ultrasound can be considered as an anthropogenic noise, which 92 could have a potential negative impact on marine life and farmed fish. Currently, only one 93 study has been carried out to evaluate the impact of a long-term ultrasound exposure on a 94 commonly farmed fish species (common carp exposed to a dual-frequency of 23 and 46 kHz 95 from an antibiofilm ultrasound device over a 30-day period; Techer et al., 2017). The results 96 of this study did not provide an indication that ultrasound negatively affects the welfare and 97 physiological homeostasis of carp. However, it is essential to expand our knowledge on the 98 impact that ultrasound could have on different farmed or wild fish species. In addition, recent 99 advances in microbiology show that animal health and welfare are closely linked to its host-100 associated microbiota (Douglas, 2019; Merrifield and Rodiles, 2015). Since ultrasound is 101 known to affect microorganisms, for instance, by impacting their growth, inducing metabolite 102 production or inactivating cells through membrane disruption (Piyasena et al., 2003), it is 103 important to investigate whether ultrasound can impact the microbiome of farmed fish and 104 thereby their growth performance, health or welfare. Indeed, a recent study has demonstrated 105 a correlation between the growth performance of European eel and its associated microbiota 106 (Shi et al., 2020).

The present study was conducted in order to examine the impact of ultrasound on the growth
and microbiota of European sea bass (*Dicentrarchus labrax*), a commercially important
aquaculture species. This work is essential to assess the feasibility and safety of using
ultrasound as an environmental-friendly antifouling agent within offshore platforms
combining energy production and fish farming.

113 2. Material and methods

114

2.1 Study species and animal maintenance

115 European sea bass, Dicentrarchus labrax L. (Moronidae, Perciformes), used in this 116 experiment were sourced from a seabass aquaculture broodstock at the Ifremer aquaculture 117 station (Palavas-les-Flots, France). A batch of 569 individuals was transferred to the 118 experimental station at the CNAM Intechmer (Tourlaville, France) at 240 days-post-hatching 119 (dph). After a 56-day acclimation period, all individuals were tagged with passive integrated 120 transponder (PIT tag) at 296 dph to track individual fish weight. This operation was 121 performed under benzocaine anaesthesia (200 ppm). A sub-sample of 360 individuals was 122 randomly split into six 600 litre tanks (N = 60 individuals per tank) supplied with filtered seawater in a recirculating system (flow rate: 0.4 m³h⁻¹ and 100% water renewal per day). 123 124 Mean water temperature, oxygen concentration and salinity were measured throughout the experiment with average values being 20.4 \pm 1.7 °C, 7.21 \pm 2.8 mg L⁻¹, and 35 \pm 0 g L⁻¹, for 125 126 each parameter respectively. The light was switched on for 14 h and off for 10 h of each 24-h 127 cycle (light switched on at 07:00 U.T. + 1), with twilight transition periods of 30 min. The 128 individuals were hand-fed twice a day (8:30 a.m. and 4:00 p.m.), with commercial food (Le 129 Gouessant Aquaculture). The size of the pellets and the rations were adjusted after each 130 weight measurement according to the manufacturer's recommendations.

131

132 2.2 Ultrasound equipment and experiment set-up

133 Ultrasonic frequencies were generated using ultrasound transducers (20 - 80 kHz, Model 2

134 Harsonic for Propellers, Harsonic) connected to a frequency generator (Virtual Bench,

135 VB8012, National instruments). The LabVIEW (NXG 2017, National instrument) software

136 was programmed to generate randomly alternated frequency sequence loops between 20 and

137 80 kHz. At 462 dph, an ultrasound transducer was placed at the centre-bottom of each tank to

138 avoid any biases due to the presence of the device in the tank. However, only the transducers 139 in three tanks were activated (Ultrasound exposure group, US) while the three others were 140 kept inactive (Control group). The ultrasound devices remained active for 71 days and were 141 turned off at 533 dph (see Figure 1). The frequencies generated by the transducers in the three 142 tanks were measured at 512, 514 and 517 dph using a wideband hydrophone (Hydrophone 143 HTI-99-HF, Scorpions Oceanics). The 999 first frequencies generated by the transducers were 144 recorded (See Supplemental Figure S1) giving average frequencies of 26.2 ± 49.7 kHz, $26.7 \pm$ 145 45.0 kHz and 26.0 ± 17.5 kHz in each of the tanks with active devices, respectively.

146



147

148 *Figure 1: Overview of experimental timeline, weight measurements and sample collection.*

149

150 2.4 Growth performance

151 Individual fish weight was assessed four times during the experiment (Figure 1). Before each

152 measurement, the fish were fasted for 24 hours then anesthetized with Benzocaine (Sigma

153 Aldrich, 200 ppm). Individual growth performance was assessed by calculating the specific

154	growth rate (SGR, % increase in body weight per day) for the periods before (386 – 462 dph,
155	76 days), during (462 – 533 dph, 71 days) and after (533 – 589 dph, 56 days) ultrasound
156	exposure. Individual fish weight and SGR are presented as averages with standard deviations.
157	

158 2.5 Cultivation-based microbiological analyses

159 2.5.1 Sample preparation

160 Samples for cultivation-based microbiological analysis were collected before ultrasound 161 exposure (T0), after 55 days of ultrasound exposure (T1) and 55 days after cessation of 162 ultrasound exposure (T2) (see Figure 1). At each occasion, two sea bass and 100 mL seawater 163 were sampled from each experimental tank. Sea bass were caught one at a time, euthanized 164 using a lethal dose of 300 ppm benzocaine placed individually in sterile stomacher bags (400 165 mL, 180×300 mm, Stomacher, Seward) and immediately transported to the laboratory in a 166 cooler with ice packs. Cooler and ice packs were previously cleaned with 70% ethanol. 167 For the gill samples, whole gills were weighed and placed in a sterile stomacher bag (80 mL, 168 100×150 mm, Stomacher, Seward) with 20 mL sterile seawater. They were then mashed for 3 169 min using a stomacher (Stomacher, Lab-Blender 400, Seward), in order to obtain the initial 170 suspension. Each initial suspension was then serially diluted up to 10^2 -fold in sterile seawater. 171 At times T0 and T2, the gills of the two sea bass from the same tank were mixed to form a 172 single sample, while at time T1, the gills of the two sea bass from the same tank were 173 analysed separately. Therefore, in total, six microbiological analyses were performed at T0, 174 twelve microbiological analyses were performed at T1 (six analyses for control condition and 175 six analyses for ultrasound condition) and six microbiological analyses were performed at T2 176 (three analyses for control condition and three analyses for ultrasound condition). 177 For the skin mucus samples, 1 g of skin mucus was collected at the lateral line using a sterile 178 Teflon spatula and suspended in a tube containing 9 mL of sterile seawater. This suspension

180	serially diluted up to 10 ¹ -fold in sterile seawater. Sample pooling was performed as for the
181	gill samples resulting in the same number of samples at T0 ($n = 6$), T1 ($n = 12$) and T2 ($n = 12$)
182	6), respectively.
183	For the tank seawater, water samples from each tank were collected using a 250 mL sterile
184	vial and serially diluted up to 10^2 -fold in sterile seawater.
185	
186	2.5.2 Enumeration of cultivable bacteria by plate count method
187	100 μ L of all initial suspensions and dilutions were plated in triplicates onto marine agar
188	plates (Difco) for heterotrophic plate counts (HPC) (Lyons et al., 2007); onto Pseudomonas
189	agar base supplemented with cephalothin, fucidin, cetrimide supplement (Merck) and
190	modified according to Stanbridge and Board (1994) (CFC agar modified) to enumerate
191	Pseudomonas spp.; and onto thiosulfate citrate bile salts sucrose agar plates (TCBS agar;
192	Merck) to enumerate Vibrio spp. (Bolinches et al., 1988). All plates were incubated for 48 h
193	to 72 h at 22 °C. After incubation, plates showing 30-300 colonies on marine agar and 15-150
194	colonies on CFC agar and TCBS agar were used to calculate the Colony Forming Units
195	(CFU) per g of gill, per g of skin mucus and per mL of seawater. Counts of CFU are presented
196	as logarithmic values and calculated as $log10(x + 1)$ to account for counts of 0.
197	
198	2.6 Metataxonomic analysis
199	2.6.1 Sample preparation
200	Samples for metataxonomic analysis were collected at times T1 and T2 (see Figure 1) to
201	analyse differences between the ultrasound exposure and control group during and after
202	treatment. Prior to sample collection, the fish were euthanised as described above. Skin mucus
203	of two fish per tank ($N = 12$) was collected from the whole body of the fish using a sterile

was homogenized using a vortex mixer (initial suspension). Each initial suspension was then

179

Teflon spatula. For gut samples, eight fish per tank (N = 48) were first immerged successively in three baths of sterile distilled water to remove loosely attached microorganisms on the skin. Then the mid- and hindgut were collected using sterile dissection tools. Seawater samples (1 L) were collected from each tank (N = 6) and filtered through a 0.2 μ m pore size polycarbonate membrane (Whatman). All samples were immediately stored at -80°C in sterile vials until DNA extraction for metataxonomic analysis.

- 210
- 211

2.7.2 DNA extraction, PCR and sequencing

212 DNA from all samples was extracted using the MasterPure Complete DNA Purification Kit 213 (Epicentre) according to the manufacturer's instructions for total DNA extraction. The gut 214 samples were pre-processed as described in Benhaïm et al. (2020) to reduce PCR inhibition. 215 The water sample residues were resuspended by adding 300µl of Tissue and Cell Lysis 216 Solution to each filter and scraping the surface gently with a sterile scalpel before continuing 217 with the manufacturer's protocol. DNA from each sample was subject to PCR using the 218 universal prokaryotic primer pair S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') 219 and S-D-Bact-0785-a-A-21(5'- GACTACHVGGGTATCTAATCC-3') (Klindworth et al., 220 2013) covering the V3-V4 region of the 16S rRNA gene and yielding an amplicon of 221 approximately 465 base pairs. The PCR reactions were performed with Phusion High-Fidelity 222 DNA polymerase (NEB) according to the manufacturer's recommendations using 10 ng of 223 template DNA in a 25 µl reaction volume. The thermocycler conditions were set to an initial 224 denaturation step at 98 °C for 30 s followed by 30 cycles of denaturation at 98 °C for 10 s, 225 annealing at 52 °C for 30 s and extension at 72 °C for 30 s. Final extension was set to 72 °C 226 for 5 min. The libraries were then multiplexed with Nextera XT v2 indices (Illumina), 227 normalised using Sequel-Prep Normalisation Plates (ThermoFisher Scientific) and sequenced 228 on a MiSeq system (Illumina) using v3 chemistry and 2 x 300 cycles.

229

230

2.7.3 Bioinformatic analysis

231 Bioinformatic analysis was performed in R version 4.0.2 (R Core Team, 2020) implemented 232 in RStudio version 1.2.5019 (RStudio Team, 2016). Raw sequence reads were demultiplexed 233 and amplicon sequence variants (ASVs) inferred with the R package DADA2 version 1.16 234 (Callahan et al., 2016). Briefly, raw reads were filtered with the *filterAndTrim* command set to 235 truncLen = c(260,240), maxEE = 3, trimLeft =21, truncQ = 2. Error rates were learned with the *learnError* command performed on a subset of 10⁸ bases. Taxonomic assignment of ASVs 236 237 was performed using the assignTaxonomy command against a training set of the SILVA 238 database version 138 (Quast et al., 2013). Taxa classified as *Eukarya*, chloroplasts or 239 mitochondria were removed from the dataset. In addition, ASVs that were 20 base pairs 240 shorter or larger the expected amplicon size were removed, as well as one ASV classified as 241 Escherichia sp. which was detected in all samples at a similar relative abundance and hence 242 likely a contaminant. Microbial community analysis was performed with packages phyloseq 243 (McMurdie and Holmes, 2013) and vegan (Oksanen et al., 2013). For further taxonomic 244 analysis, ASVs were compared against the NCBI 16S rRNA sequence database using the 245 blast web application (Johnson et al., 2008). Plots were generated with package ggplot2 246 (Wickham, 2009).

247

248 2.8 Statistics analysis

Differences in the SGR was analysed using generalized linear mixed-effects models
(GLMM). The explanatory variables included in the full model were "Treatment" (control vs
ultrasound) in interaction with "Period" (before, during and after ultrasound treatment). In
addition to these fixed effects, the random effects were "Tank nested within treatment" and
"individual fish ID". The full model was reduced by backward selection based on the Akaike

254 Information Criterion (AIC) (Zuur et al., 2009). Diagnostics based on residuals of the model 255 were performed to assess the adequacy of the reduced model and compliance with the 256 underlying assumptions. Finally, the effects of the independent variables were estimated from 257 the reduced models and their significance was tested by likelihood ratio tests (LRT) between 258 models respecting marginality of the effects that are supposed to follow a χ^2 distribution 259 under the null hypothesis (type II tests; (Fox and Weisberg, 2011)). This analysis was 260 followed by a post-hoc multiple comparison test (Hothorn et al., 2008) to assess pairwise 261 differences.

262 Plate counts (HPC, Pseudomonas spp. and Vibrio spp.) were subject to Student's Two Sample 263 t-test at time T1 (control: n= 6; US: n = 6) and Welch's Two Sample t-test at time T2 (control: 264 n=3; US: n=3). Results were considered significant when the p-value was below 0.05. 265 For metataxonomic analysis, differential abundance of taxa between groups was compared 266 with DESeq2 (Love et al., 2014) implemented in the R package animalcules version 1.4.0 267 (Zhao et al., 2020) at genus level taxonomic assignment with default parameters and a 268 minimum count cut-off of 10. Results were considered significant when the adjusted p-value 269 was below 0.05. Significance of alpha-diversity indices (number of observed ASVs and 270 Shannon diversity index) between groups was determined using Student's Two Sample t-test. 271 Beta-diversity was plotted using Non-metric Multi-dimensional Scaling (NMDS) of Bray-272 Curtis dissimilarities and weighted UniFrac distances and significance of results between groups was determined using PERMANOVA implemented in the vegan package with 999 273 274 permutations.

276 3. Results

277 3.1 Growth performance and survival 278 The average individual body weight of the fish increased from 13.47 ± 3.73 g in the control group and 13.36 ± 3.77 g in the ultrasound exposure group at the beginning of the experiment 279 280 (386 dph) to 97.77 ± 23.57 g and 102.03 ± 25.33 g, in each of the group respectively, at the 281 end of the experiment (589 dph). The average SGR in the control group was 1.13 ± 0.15 , 0.77 \pm 0.1 and 0.61 \pm 0.11 % bodyweight d⁻¹ before, during and after ultrasound exposure period, 282 283 respectively (Figure 2). In the ultrasound exposed group, the average SGR was 1.17 ± 0.16 , 0.78 ± 0.1 and 0.61 ± 0.1 % bodyweight d⁻¹ before, during and after ultrasound exposure, 284 285 respectively. No significant difference was detected between the SGR of the control and 286 ultrasound exposed group over the entire duration of the experiment ($\chi 2 = 2.5$, df = 1, p = 287 0.11) using GLMM. However, the post hoc test showed that the SGR was significantly higher 288 in the ultrasound exposed group before activation of the ultrasound transducer (Z = 2.8, p =289 0.025). No significant differences between the groups were observed by the post hoc test 290 during (Z = 0.33, p = 0.99) and after (Z = 0.06, p = 1) activation of the ultrasound transducer. 291 No mortality was recorded in either group over the course of the experiment. 292



Figure 2: Specific growth rate (SGR) of sea bass in the control group (Control, N = 180) and ultrasound exposure group (US, N = 180) for the periods before (386 – 462 dph), during (462 - 533 dph) and after (533 – 589 dph) ultrasound (US) exposure.

297

298 3.3 Cultivation-based microbiological analyses

299 Bacterial counts of gills, skin mucus and tank seawater at times T0, T1 and T2 are presented 300 in Figure 3 and Table 1. Ultrasound exposure had an impact on the cultivable bacterial flora 301 on the gills at time T1, with the counts of HPC, Vibrio spp. and Pseudomonas spp. being 302 significantly higher for ultrasound exposed fish than for the control fish. The mean increase was log 0.45 CFU g⁻¹, log 0.62 CFU g⁻¹ and log 0.55 CFU g⁻¹ for HPC, *Pseudomonas* spp. 303 304 and Vibrio spp., respectively. At time T2, the mean counts remained higher for fish in the 305 ultrasound exposure group than for the control fish, although the differences between the two 306 groups were not significant anymore. For fish in the control group, the mean counts of 307 *Pseudomonas* spp. and *Vibrio* spp. on the gills remained constant throughout the experiment

(about log 3.7 CFU g⁻¹ and log 3.8 CFU g⁻¹, respectively), while the mean concentration of
HPC was lower at time T2 (log 4.8 CFU g⁻¹) than at times T0 and T1 (log 5.3 CFU g⁻¹).

The counts of cultivable bacteria on the skin mucus was not significantly impacted by ultrasound exposure (Figure 3B, Table 1). HPC increased from T0 to T1 but remained relatively stable between T1 and T2 as well as between the ultrasound exposed and control group. At T1, *Vibrio* spp. and *Pseudomonas* spp. were detected in 3 and 5 out of 12 skin mucus samples, respectively, and at T2 both taxa were detected in 4 out of 6 samples each. During ultrasound exposure (time T1) no counts of *Vibrio* spp. were detected in the control group compared to a mean count of log 2.8 CFU g⁻¹ in the ultrasound exposed group.

318





- 321 at times T0, T1 and T2 in sea bass gills (A), skin mucus (B) and seawater (C) of the control
- 322 group (Control) and ultrasound exposed group (US), presented as log CFU per g for gills and

323 skin mucus samples and per ml for seawater samples.

325 Counts of cultivable bacteria in the tank seawater were not significantly impacted by

326 ultrasound exposure (Figure 3C, Table 1). The concentration of HPC and *Vibrio* spp.

327 remained constant and similar between the two groups (US and Control), throughout the study

328 (about log 4.8 CFU mL⁻¹ and log 2.3 CFU mL⁻¹ for HPC and *Vibrio* spp., respectively). The

329 mean counts of *Pseudomonas* spp. remained the same at all three times for the control group,

330 while it increased by log 0.8 CFU mL⁻¹ between T1 and T2 for the ultrasound exposed group.

331

332 Table 1: Statistical analysis of CFU counts between the control group (Control) and the

333 ultrasound exposure group (US). Counts are presented as log CFU per g for gills and skin

334 mucus samples and per ml for seawater samples. Values in bold indicate significant

335 differences (p < 0.05) between groups for each timepoint.

	T0 (N = 6)		T1 (N = 12)			T2 (N = 6)						
	Mean log CFU	Mean log CFU (Control)	Mean log CFU (US)	t-value ¹	p-value ¹	Mean log CFU (Control)	Mean log CFU (US)	t-value ²	p-value ²			
Gills												
HPC	5.31	5.35	5.79	-2.46	0.03*	4.78	5.80	-1.4 1	0.29			
Pseudomonas spp.	3.62	3.88	4.49	-2.75	0.02*	3.76	4.33	-2.26	0.10			
Vibrio spp.	3.92	3.79	4.34	-2.57	0.03*	3.66	4.52	-1.14	0.37			
Skin mucus												
HPC	3.60	4.90	5.00	-0.50	0.63	4.86	4.77	-0.52	0.63			
Pseudomonas spp.	1.34	2.86	2.94	-0.21	0.84	2.46	3.06	-2.12	0.10			
Vibrio spp.	0.85	< DL	2.76	-2.00	0.10	3.54	3.06	1.22	0.33			
Seawater												
HPC	4.50	5.07	5.04	0.41	0.70	4.84	4.83	0.03	0.98			
Pseudomonas spp.	1.45	1.65	1.43	0.61	0.57	1.53	2.28	-1.00	0.42			
Vibrio spp.	2.48	2.27	2.05	1.23	0.25	2.36	2.39	-0.15	0.90			

¹ Student's Two Sample t-test ² Welch's Two Sample t-test

*p < 0.05

T0: Before US exposure

T1: During US exposure

T2: After US exposure DL: Detection level

336

338 3.4 Metataxonomic analysis

339 The sea bass gut microbial community was dominated by the classes Gammaproteobacteria 340 and Alphaproteobacteria, representing 77.1% and 8.9% of the mean relative abundance 341 respectively across the control gut samples from T1 and T2 (Figure 4). One ASV, assigned to 342 the genus Pelomonas of the Gammaproteobacteria class, was the dominant taxon with a mean 343 relative abundance of 57.8% and the only ASV present in all gut samples (Figure 5). 344 Alignment of its partial 16S rRNA gene sequence showed closest sequence similarity to 345 Pelomonas aquatica (99.7% sequence identity to GenBank accession NR_042614.1). Apart 346 from *Pelomonas*, the variability of genera between samples was high with only six additional 347 genera (Acinetobacter, Brevundimonas, Curvibacter, Acidibacter and Sphingomonas) 348 detected in over half of the control gut samples, together accounting for 14.1% of the mean 349 relative abundance (Figure 5). At time T1, none of the genera had a significantly differential 350 abundance in the gut microbial community between the ultrasound exposed and the control 351 group. However, at time T2, the genus Brevinema was significantly decreased and 352 Photobacterium significantly increased in the ultrasound exposed group compared to the 353 control (adjusted p-value ≤ 0.001). No significant differences were detected between the 354 number of observed ASVs or the Shannon diversity index in the control and ultrasound 355 exposed group at T1 and T2, nor between Bray-Curtis dissimilarities and weighted UniFrac 356 distances. (Figure 4 and Supplemental Figure S2).

357

The skin microbial community of the sea bass differed significantly from the gut environment ($R^2 = 0.38$, p-value < 0.001, PERMANOVA on Bray-Curtis distances, Figure 4) and shared more taxa with the seawater in the rearing tanks, with the exceptions of the genera *Pelomonas* and *Acinetobacter* which were abundant both the gut and skin, but largely absent from the seawater (Figure 5). *Gammaproteobacteria*, *Bacteroidia*, *Verrucomicrobiae* and

363 Alphaproteobacteria were the most abundant classes of the skin microbiome with a mean 364 relative abundance of 43.3%, 26.2%, 14.4% and 9.6% respectively (Figure 4). At time T1, the 365 genus *Pelomonas* was significantly increased (adjusted p-value < 0.05) in the skin 366 microbiome of the ultrasound exposed fish compared to the control group. Whereas a 367 separation of their respective clusters in the NMDS plot based on Bray-Curtis distances was 368 apparent (Figure 4), possibly due to the differential abundance of *Pelomonas* between groups, 369 there was no significant difference between communities based on PERMANOVA. At time 370 T2, no significant difference in the relative abundance of *Pelomonas* was detected between 371 the ultrasound exposed and control group anymore. The average number of observed ASVs in 372 the control group was significantly higher than in the ultrasound exposed group at T1 (p <373 0.01) (Supplemental Figure S2). Conversely, at T2, the average number of observed ASVs in 374 the ultrasound exposed group was higher than in the control, however, these results were not 375 statistically significant. The seawater microbial community consisted mainly of Bacteroidia, 376 Alphaproteobacteria, Gammaproteobacteria and Verrucomicrobiae which together represent 377 93.2% of the relative abundance in the control samples at time T1 and T2 (Figure 4). No 378 genera were significantly differentially abundant between the ultrasound exposed and control 379 group and no significant differences were detected in the number of ASVs or the Shannon 380 diversity index between both groups (Supplemental Figure S2).

381

The genera *Pseudomonas* and *Vibrio*, which were quantified by cultivation, were only minor taxa in the analysed microbial communities. *Pseudomonas* represented 0.5% and 0.4% of the relative abundance in the gut and skin mucus samples, respectively, and was absent in the seawater. *Vibrio* represented 0.5% and 0.1% of the relative abundance in the skin mucus and seawater samples, respectively, and was absent in the gut (data not shown). No significant

- 387 differences in the relative abundance of these genera were detected between the ultrasound
- 388 exposed and control group at time T1.
- 389



Figure 4: Microbial community profiles at class level assignment (left) and non-metric
multidimensional scaling (NMDS) plot of Bray-Curtis distances (right) of gut, skin mucus and
seawater microbiota at time T1 (A) and T2 (B) from fish in the control group (Control) or
ultrasound exposed group (US). NMDS ordination is based on normalised data and stress
values < 0.1.



Figure 5: Heatmap showing the relative abundance of the 50 most abundance genera across
gut, skin mucus and seawater samples with logarithmic colour scale. Genera which showed

- 400 significantly differential abundance between the control group (Control) and ultrasound
- *exposed group (US) in one of the sample types are highlighted in bold with an asterisk.*

402 4. Discussion

403 Overall, sea bass growth performances were in the range of those observed in previous studies 404 at a similar age and under comparable conditions (Benhaïm et al., 2011). A faster SGR in the 405 ultrasound exposure group led to a higher average weight at the end of the experiment 406 compared to the control group. However, this could not be attributed to ultrasound exposure, 407 as the increase in SGR occurred before activation of the ultrasound transducers. A previous 408 study on the toxicity of ultrasound towards farmed carp (Cyprinus carpio), showed that fish 409 welfare and physiological homeostasis was unaffected by ultrasound exposure over a 30-day 410 period (Techer et al., 2017). The present study assessed the impact of ultrasound on the 411 growth performance and survival of a marine fish species over a 71-day period. Here, we 412 confirm that ultrasound exposure does not have a negative impact on fish growth and survival. 413 An additional 56-day period with the transducers kept inactive confirmed no long-term effect 414 of the ultrasound treatment on the growth or survival of sea bass.

415

416 Whereas growth and survival were unaffected, our results show that ultrasound exposure can 417 impact the fish microbiota. Concerning the cultivable microbiota, CFU analysis showed that 418 the gills harboured an increased number of heterotrophic bacteria as well as potentially 419 pathogenic bacteria, such as *Pseudomonas* spp. and *Vibrio* spp. after ultrasound exposure. 420 Higher levels of these bacteria, even after inactivating the transducers, indicate that ultrasound 421 could have a long-term impact on the gill microbiota. Several Pseudomonas and Vibrio 422 species are known to cause serious diseases in farmed fish and lead to significant economic 423 losses (Novriadi, 2016; Toranzo et al., 2005). Among Pseudomonas, the species 424 P. anguilliseptica is considered the most significant pathogen for farmed fish (Toranzo et al., 425 2005). Within the genus *Vibrio*, many species have been described as fish pathogens, 426 including V. anguillarum, V. ordalii, V. salmonicida, V. vulnificus, V. parahaemolyticus, V.

427 alginolyticus, V. harveyi (Novriadi, 2016), V. toranzoniae (Lasa et al., 2015) and V. tapetis 428 (Vercauteren et al., 2018), and vibriosis is a well-known cause of mortality in aquaculture 429 (Novriadi, 2016). Fish gills perform vital functions (e.g., respiration, osmoregulation, pH 430 balance, ammonia excretion, hormone regulation, detoxification) and their mucus harbours a 431 complex community of commensal microorganisms that play a major part in maintaining 432 homeostasis and protection against pathogens (Reverter et al., 2017). Although pathogenic 433 bacteria can form a part of the mucus microbiome in healthy farmed fish (Rosado et al., 434 2019), they can also cause disease if there is a shift in their abundance (Hess et al., 2015; 435 Rosado et al., 2019). Consequently, an increase in the abundance of *Pseudomonas* spp. and 436 Vibrio spp. within the gills could potentially increase the risk of pathologies. However, it is 437 well established that cultivable bacteria represent only a negligible part ($\leq 2\%$) of complex 438 bacterial communities associated with various environmental and animal habitats (Vartoukian 439 et al., 2010). Their enumeration therefore does not provide a functional implication of the 440 whole gill microbiome (Legrand et al., 2020), but rather warrants further research into 441 putative pathogenicity of the bacteria propagated by ultrasound exposure.

442

443 At time T1, ultrasound exposure also seemed to facilitate the colonization of the skin mucus 444 by Vibrio spp. as shown by cultivation-based analysis. As Vibrio only constituted a minor 445 taxon in the skin microbial community and was not detected in all samples, these results could 446 not be confirmed by metataxonomic analysis. However, a significant increase in the relative 447 abundance of *Pelomonas* in the skin microbial community during ultrasound exposure further 448 highlights the potential disruption of the skin microbiota by ultrasound. Pelomonas has 449 previously been detected in the gut of farmed sea bass (Nikouli et al., 2018) and is a dominant 450 member of the gut microbiome of other fish species (Nikouli et al., 2020; Rasheeda et al., 451 2017; R. Zhao et al., 2020). Pelomonas has also been identified on the skin of brook charr

452 (Boutin et al., 2013) and the gills of rainbow trout (Lowrey et al., 2015), however at low 453 relative abundances. As the gut and skin environment in the current study shared the same 454 Pelomonas-associated ASV, it appears that this gut-associated bacterium is a low-abundant 455 opportunist on the skin. The near absence of Pelomonas in the seawater further demonstrates 456 that it is not a transient bacterium in the farming environment. A previous study has shown 457 that stress may induce changes in skin mucus microbiota, such as a decrease in the abundance 458 of probiotic-like bacteria and an increase in opportunistic pathogenic bacteria (Boutin et al., 459 2013). As such, these results could demonstrate a state of stress in fish subjected to 460 ultrasound.

461

462 The sea bass gut microbial profiles detected in this study showed similarities to previous 463 reports on farmed sea bass and sea bream, with a dominance of the phylum Proteobacteria 464 (Kokou et al., 2020; Kormas et al., 2014; Nikouli et al., 2018; Rimoldi et al., 2020). High 465 inter-individual variability of the gut microbiome has also been reported, possibly being 466 linked to life history and genetic background (Gatesoupe et al., 2016), or pointing towards 467 insufficient replicate sampling (Panteli et al., 2020). Compared to the gills and skin mucus, 468 the absence of differentially abundant taxa between the ultrasound exposed and control group 469 in the present study indicate that ultrasound exposure does not directly impact the fish gut 470 microbial community. Differences in the relative abundance of Brevinema and 471 Photobacterium eight weeks after the end of the ultrasound exposure may be attributed to a 472 belated or long-term effect of ultrasound exposure, especially as members of *Photobacterium* 473 are known fish pathogens (Bakopoulos et al., 2003; Terceti et al., 2016). However, this could 474 also be an artefact of the high inter-individual variability in the gut microbiota and to support 475 this observation it would be necessary to define a temporally stable core gut microbiota in 476 farmed sea bass.

477

478 In conclusion, our study shows that close-proximity and continuous ultrasound exposure can 479 impact the surface microbial community of farmed sea bass. The skin and gill mucus layers 480 act as a protective barrier against the external environment and are essential in protecting the 481 fish from pathogenic organisms (Merrifield and Rodiles, 2015). In addition, commensal 482 microorganisms can prevent the colonisation of the skin by pathogens (Pérez-Sánchez et al., 483 2011). Hence, even though an adverse effect of ultrasound exposure was not evident on the 484 growth performance and survival of sea bass, alterations to the microbiome could lead to a 485 higher incidence of disease and mortality in the long-term and therefore warrants further 486 investigation. As these results were generated under controlled laboratory conditions and the 487 fish were kept in close proximity to the ultrasound source, further studies should be conducted 488 to determine the impact of ultrasonic antifouling control on the fish microbiota under *in situ* 489 conditions, while assessing the potential benefits of this technology compared to traditional 490 antifouling control strategies.

491

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495

496 **Competing interest statement**

497 The authors declare that there are no competing interests.

498 **References**

- 499 Abhinav, K.A., Collu, M., Benjamins, S., Cai, H., Hughes, A., Jiang, B., Jude, S., Leithead,
- 500 W., Lin, C., Liu, H., Recalde-Camacho, L., Serpetti, N., Sun, K., Wilson, B., Yue, H.,
- 501 Zhou, B.Z., 2020. Offshore multi-purpose platforms for a Blue Growth: A technological,
- 502 environmental and socio-economic review. Sci. Total Environ. 734, 138256.
- 503 https://doi.org/10.1016/j.scitotenv.2020.138256
- 504 Bakopoulos, V., Volpatti, D., Gusmani, L., Galeotti, M., Adams, A., Dimitriadis, G.J., 2003.
- 505 Vaccination trials of sea bass, Dicentrarchus labrax (L.), against Photobacterium damsela
- 506 subsp. piscicida, using novel vaccine mixtures. J. Fish Dis. 26, 77–90.
- 507 https://doi.org/10.1046/j.1365-2761.2003.00438.x
- 508 Benassai, G., Mariani, P., Stenberg, C., Christoffersen, M., 2014. A Sustainability Index of
- 509 potential co-location of offshore wind farms and open water aquaculture. Ocean Coast.

510 Manag. 95, 213–218. https://doi.org/10.1016/j.ocecoaman.2014.04.007

- 511 Benhaïm, D., Leblanc, C.A.L., Horri, K., Mannion, K., Galloway, M., Leeper, A., Knobloch,
- 512 S., Sigurgeirsson, Ó., Thorarensen, H., 2020. The effect of triploidy on the performance,
- 513 gut microbiome and behaviour of juvenile Atlantic salmon (Salmo salar) raised at low
- 514 temperature. Appl. Anim. Behav. Sci. 229, 105031.
- 515 https://doi.org/10.1016/j.applanim.2020.105031
- 516 Benhaïm, D., Péan, S., Brisset, B., Leguay, D., Bégout, M.L., Chatain, B., 2011. Effect of size
- 517 grading on sea bass (Dicentrarchus labrax) juvenile self-feeding behaviour, social
- 518 structure and culture performance. Aquat. Living Resour. 24, 391–402.
- 519 https://doi.org/10.1051/alr/2011140
- 520 Blackwood, D.J., Lim, C.S., Teo, S.L.M., Hu, X., Pang, J., 2017. Macrofouling induced
- 521 localized corrosion of stainless steel in Singapore seawater. Corros. Sci. 129, 152–160.
- 522 https://doi.org/10.1016/j.corsci.2017.10.008

- 523 Bolinches, J., Romalde, J.L., Toranzo, A.E., 1988. Evaluation of selective media for isolation
- and enumeration of vibrios from estuarine waters. J. Microbiol. Methods 8, 151–160.

525 https://doi.org/10.1016/0167-7012(88)90016-4

- 526 Bott, T.R., 2000. Biofouling control with ultrasound. Heat Transf. Eng. 21, 43–49.
- 527 https://doi.org/10.1080/014576300270898
- 528 Boutin, S., Bernatchez, L., Audet, C., Derôme, N., 2013. Network Analysis Highlights
- 529 Complex Interactions between Pathogen, Host and Commensal Microbiota. PLoS One 8,
 530 e84772. https://doi.org/10.1371/journal.pone.0084772
- 531 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.
- 532 DADA2: High-resolution sample inference from Illumina amplicon data. Nat Meth 13,
- 533 581–583. https://doi.org/10.1038/nmeth.3869
- Chen, L., Lam, J.C.W., 2017. SeaNine 211 as antifouling biocide: A coastal pollutant of
 emerging concern. J. Environ. Sci. 61, 68–79. https://doi.org/10.1016/j.jes.2017.03.040
- 536 Dalton, G., Bardócz, T., Blanch, M., Campbell, D., Johnson, K., Lawrence, G., Lilas, T.,
- 537 Friis-Madsen, E., Neumann, F., Nikitas, N., Ortega, S.T., Pletsas, D., Simal, P.D.,
- 538 Sørensen, H.C., Stefanakou, A., Masters, I., 2019. Feasibility of investment in Blue
- 539 Growth multiple-use of space and multi-use platform projects; results of a novel
- 540 assessment approach and case studies. Renew. Sustain. Energy Rev. 107, 338–359.
- 541 https://doi.org/10.1016/j.rser.2019.01.060
- 542 Douglas, A.E., 2019. Simple animal models for microbiome research. Nat. Rev. Microbiol.
- 543 https://doi.org/10.1038/s41579-019-0242-1
- 544 FAO, 2018. The State of World Fisheries and Aquaculture 2018-Meeting the sustainable
 545 development goals. FAO Rome, Italy.
- 546 Finnie, A.A., Williams, D.N., 2010. Paint and Coatings Technology for the Control of Marine
- 547 Fouling, in: Biofouling. Wiley-Blackwell, Oxford, UK, pp. 185–206.

- 548 https://doi.org/10.1002/9781444315462.ch13
- 549 Fitridge, I., Dempster, T., Guenther, J., de Nys, R., 2012. The impact and control of
- 550 biofouling in marine aquaculture: a review. Biofouling 28, 649–669.
- 551 https://doi.org/10.1080/08927014.2012.700478
- 552 Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, 2nd editio. ed. SAGE
- 553 Publications, Los Angeles, CA.
- 554 Gatesoupe, F.J., Huelvan, C., Le Bayon, N., Le Delliou, H., Madec, L., Mouchel, O.,
- 555 Quazuguel, P., Mazurais, D., Zambonino-Infante, J.L., 2016. The highly variable
- 556 microbiota associated to intestinal mucosa correlates with growth and hypoxia resistance
- 557 of sea bass, Dicentrarchus labrax, submitted to different nutritional histories. BMC
- 558 Microbiol. 16, 1–13. https://doi.org/10.1186/s12866-016-0885-2
- 559 Gu, L., Xie, M.-Y., Jin, Y., He, M., Xing, X.-Y., Yu, Y., Wu, Q.-Y., 2019. Construction of
- 560 Antifouling Membrane Surfaces through Layer-by-Layer Self-Assembly of
- 561 Lignosulfonate and Polyethyleneimine. Polymers (Basel). 11, 1782.
- 562 https://doi.org/10.3390/polym11111782
- 563 Guo, S., Lee, H.P., Teo, S.L.M., Khoo, B.C., 2012. Inhibition of barnacle cyprid settlement
- using low frequency and intensity ultrasound. Biofouling 28, 131–141.
- 565 https://doi.org/10.1080/08927014.2012.658511
- 566 Haque, M.N., Kwon, S., 2018. Effect of ultra-sonication and its use with sodium hypochlorite
- as antifouling method against Mytilus edulis larvae and mussel. Environ. Geochem.
- 568 Health 40, 209–215. https://doi.org/10.1007/s10653-016-9894-1
- 569 Hess, S., Wenger, A.S., Ainsworth, T.D., Rummer, J.L., 2015. Exposure of clownfish larvae
- 570 to suspended sediment levels found on the Great Barrier Reef: Impacts on gill structure
- 571 and microbiome. Sci. Rep. 5, 1–8. https://doi.org/10.1038/srep10561
- 572 Holm, E.R., Stamper, D.M., Brizzolara, R.A., Barnes, L., Deamer, N., Burkholder, J.A.M.,

- 573 2008. Sonication of bacteria, phytoplankton and zooplankton: Application to treatment
- of ballast water. Mar. Pollut. Bull. 56, 1201–1208.
- 575 https://doi.org/10.1016/j.marpolbul.2008.02.007
- 576 Holm, P., Buck, B.H., Langan, R., 2017. Introduction: New Approaches to Sustainable
- 577 Offshore Food Production and the Development of Offshore Platforms, in: Buck, B.H.,
- 578 Langan, R. (Eds.), Aquaculture Perspective of Multi-Use Sites in the Open Ocean: The
- 579 Untapped Potential for Marine Resources in the Anthropocene. Springer International

580 Publishing, Cham, pp. 1–20. https://doi.org/10.1007/978-3-319-51159-7_1

- 581 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric
- 582 models. Biometrical J. 50, 346–363. https://doi.org/10.1002/bimj.200810425
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., Madden, T.L., 2008.
- 584 NCBI BLAST: a better web interface. Nucleic Acids Res. 36.
- 585 https://doi.org/10.1093/nar/gkn201
- 586 Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O.,
- 587 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and
- 588 next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1–e1.
- 589 https://doi.org/10.1093/nar/gks808
- 590 Kokou, F., Sasson, G., Mizrahi, I., Cnaani, A., 2020. Antibiotic effect and microbiome
- 591 persistence vary along the European seabass gut. Sci. Rep. 10, 10003.
- 592 https://doi.org/10.1038/s41598-020-66622-5
- 593 Kormas, K.A., Meziti, A., Mente, E., Frentzos, A., 2014. Dietary differences are reflected on
- 594 the gut prokaryotic community structure of wild and commercially reared sea bream
- 595 (Sparus aurata). Microbiologyopen 3, 718–728. https://doi.org/10.1002/mbo3.202
- 596 Lasa, A., Avendaño-Herrera, R., Estrada, J.M., Romalde, J.L., 2015. Isolation and
- 597 identification of Vibrio toranzoniae associated with diseased red conger eel (Genypterus

- 598 chilensis) farmed in Chile. Vet. Microbiol. 179, 327–331.
- 599 https://doi.org/10.1016/j.vetmic.2015.06.003
- 600 Legg, M., Yücel, M.K., Garcia De Carellan, I., Kappatos, V., Selcuk, C., Gan, T.H., 2015.
- 601 Acoustic methods for biofouling control: A review. Ocean Eng.
- 602 https://doi.org/10.1016/j.oceaneng.2015.04.070
- 603 Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., Oxley, A.P.A., 2020. A microbial sea of
- 604 possibilities: current knowledge and prospects for an improved understanding of the fish
- 605 microbiome. Rev. Aquac. 12, 1101–1134. https://doi.org/10.1111/raq.12375
- 606 Li, Z., Guo, Z., 2019. Bioinspired surfaces with wettability for antifouling application.
- 607 Nanoscale. https://doi.org/10.1039/c9nr05870b
- 608 Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion
- 609 for RNA-seq data with DESeq2. Genome Biol. 15, 550. https://doi.org/10.1186/s13059610 014-0550-8
- 611 Lowrey, L., Woodhams, D.C., Tacchi, L., Salinas, I., 2015. Topographical mapping of the
- 612 rainbow trout (Oncorhynchus mykiss) microbiome reveals a diverse bacterial community
- 613 with antifungal properties in the skin. Appl. Environ. Microbiol. 81, 6915–6925.
- 614 https://doi.org/10.1128/AEM.01826-15
- 615 Loxton, J., Macleod, A.K., Nall, C.R., McCollin, T., Machado, I., Simas, T., Vance, T.,
- 616 Kenny, C., Want, A., Miller, R.G., 2017. Setting an agenda for biofouling research for
- 617 the marine renewable energy industry. Int. J. Mar. Energy 19, 292–303.
- 618 https://doi.org/10.1016/j.ijome.2017.08.006
- 619 Lyons, M.M., Lau, Y.T., Carden, W.E., Ward, J.E., Roberts, S.B., Smolowitz, R., Vallino, J.,
- 620 Allam, B., 2007. Characteristics of marine aggregates in shallow-water ecosystems:
- 621 Implications for disease ecology. Ecohealth 4, 406–420. https://doi.org/10.1007/s10393-
- 622 007-0134-0

- Mazue, G., Viennet, R., Hihn, J.Y., Carpentier, L., Devidal, P., Albaïna, I., 2011. Large-scale
- 624 ultrasonic cleaning system: Design of a multi-transducer device for boat cleaning (20

625 kHz), in: Ultrasonics Sonochemistry. Elsevier B.V., pp. 895–900.

- 626 https://doi.org/10.1016/j.ultsonch.2010.11.021
- 627 McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive
- 628 Analysis and Graphics of Microbiome Census Data. PLoS One 8, e61217.
- 629 Merrifield, D.L., Rodiles, A., 2015. The fish microbiome and its interactions with mucosal
- 630 tissues, in: Mucosal Health in Aquaculture. Elsevier Inc., pp. 273–295.
- 631 https://doi.org/10.1016/B978-0-12-417186-2.00010-8
- 632 Nikouli, E., Meziti, A., Antonopoulou, E., Mente, E., Kormas, K., 2018. Gut Bacterial
- 633 Communities in Geographically Distant Populations of Farmed Sea Bream (Sparus
- aurata) and Sea Bass (Dicentrarchus labrax). Microorganisms 6, 92.
- 635 https://doi.org/10.3390/microorganisms6030092
- 636 Nikouli, E., Meziti, A., Smeti, E., Antonopoulou, E., Mente, E., Kormas, K.A., 2020. Gut
- 637 Microbiota of Five Sympatrically Farmed Marine Fish Species in the Aegean Sea.
- 638 Microb. Ecol. 1–11. https://doi.org/10.1007/s00248-020-01580-z
- 639 Novriadi, R., 2016. Vibriosis in aquaculture. Omni-Akuatika 12.
- 640 https://doi.org/10.20884/1.oa.2016.12.1.24
- 641 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson,
- 642 G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community
 643 Ecol. Packag. version 2.
- 644 Panteli, N., Mastoraki, M., Nikouli, E., Lazarina, M., Antonopoulou, E., Kormas, K.A., 2020.
- 645 Imprinting statistically sound conclusions for gut microbiota in comparative animal
- 646 studies: A case study with diet and teleost fishes. Comp. Biochem. Physiol. Part D
- 647 Genomics Proteomics 36, 100738. https://doi.org/10.1016/j.cbd.2020.100738

- 648 Papandroulakis, N., Thomsen, C., Mintenbeck, K., Mayorga, P., Hernández-Brito, J.J., 2017.
- 649 The EU-Project ``TROPOS'', in: Buck, B.H., Langan, R. (Eds.), Aquaculture
- 650 Perspective of Multi-Use Sites in the Open Ocean: The Untapped Potential for Marine
- 651 Resources in the Anthropocene. Springer International Publishing, Cham, pp. 355–374.
- 652 https://doi.org/10.1007/978-3-319-51159-7_12
- 653 Park, J.S., Lee, J.H., 2018. Sea-trial verification of ultrasonic antifouling control. Biofouling
- 654 34, 98–110. https://doi.org/10.1080/08927014.2017.1409347
- 655 Pérez-Sánchez, T., Balcázar, J.L., García, Y., Halaihel, N., Vendrell, D., de Blas, I.,
- 656 Merrifield, D.L., Ruiz-Zarzuela, I., 2011. Identification and characterization of lactic
- 657 acid bacteria isolated from rainbow trout, Oncorhynchus mykiss (Walbaum), with
- 658 inhibitory activity against Lactococcus garvieae. J. Fish Dis. 34, 499–507.
- 659 https://doi.org/10.1111/j.1365-2761.2011.01260.x
- 660 Piyasena, P., Mohareb, E., McKellar, R.C., 2003. Inactivation of microbes using ultrasound:
- A review. Int. J. Food Microbiol. 87, 207–216. https://doi.org/10.1016/S01681605(03)00075-8
- 663 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner,
- 664 F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing
- and web-based tools. Nucleic Acids Res. 41, D590–D596.
- R Core Team, 2020. R: A language and environment for statistical computing. R Foundation
 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
- 668 Rasheeda, M.K., Rangamaran, V.R., Srinivasan, S., Ramaiah, S.K., Gunasekaran, R., Jaypal,
- 669 S., Gopal, D., Ramalingam, K., 2017. Comparative profiling of microbial community of
- 670 three economically important fishes reared in sea cages under tropical offshore
- 671 environment. Mar. Genomics 34, 57–65. https://doi.org/10.1016/j.margen.2017.04.003
- 672 Reverter, M., Sasal, P., Tapissier-Bontemps, N., Lecchini, D., Suzuki, M., 2017.

- 673 Characterisation of the gill mucosal bacterial communities of four butterflyfish species: a
- 674 reservoir of bacterial diversity in coral reef ecosystems. FEMS Microbiol. Ecol. 93, 51.

675 https://doi.org/10.1093/femsec/fix051

- 676 Rimoldi, S., Torrecillas, S., Montero, D., Gini, E., Makol, A., Victoria Valdenegro, V.,
- 677 Izquierdo, M., Terova, G., 2020. Assessment of dietary supplementation with
- 678 galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass
- 679 (Dicentrarchus Labrax) fed low fishmeal and fish oil based diet. PLoS One 15,
- 680 e0231494. https://doi.org/10.1371/journal.pone.0231494
- 681 Rosado, D., Xavier, R., Severino, R., Tavares, F., Cable, J., Pérez-Losada, M., 2019. Effects
- of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed
- seabass (Dicentrarchus labrax). Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-01955314-4
- RStudio Team, 2016. Rstudio: Integrated development environment for r [Computer software
 manual]. Boston, MA.
- 687 Schütz, S.E., Slater, A.M., 2019. From strategic marine planning to project licences Striking
- a balance between predictability and adaptability in the management of aquaculture and

offshore wind farms. Mar. Policy 110, 103556.

- 690 https://doi.org/10.1016/j.marpol.2019.103556
- 691 Shi, Y., Ma, D.-Y., Zhai, S.-W., 2020. Revealing the difference of intestinal microbiota
- 692 composition of cultured European eels (Anguilla anguilla) with different growth rates.693 Isr. J. Aquac. 72.
- 694 Soon, Z.Y., Jung, J.H., Jang, M., Kang, J.H., Jang, M.C., Lee, J.S., Kim, M., 2019. Zinc
- 695 Pyrithione (ZnPT) as an Antifouling Biocide in the Marine Environment—a Literature
- 696 Review of Its Toxicity, Environmental Fates, and Analytical Methods. Water. Air. Soil
- 697 Pollut. 230, 1–18. https://doi.org/10.1007/s11270-019-4361-0

- 698 Soroldoni, S., Vieira da Silva, S., Castro, Í.B., de Martinez Gaspar Martins, C., Leães Pinho,
- 699 G.L., 2020. Antifouling paint particles cause toxicity to benthic organisms: Effects on

two species with different feeding modes. Chemosphere 238, 124610.

- 701 https://doi.org/10.1016/j.chemosphere.2019.124610
- 702 Stanbridge, L.H., Board, R.G., 1994. A modification of the Pseudomonas selective medium,
- 703 CFC, that allows differentiation between meat pseudomonads and Enterobacteriaceae.
- 704 Lett. Appl. Microbiol. 18, 327–328. https://doi.org/10.1111/j.1472-765X.1994.tb00880.x
- 705 Techer, D., Milla, S., Banas, D., 2017. Sublethal effect assessment of a low-power and dual-
- 706 frequency anti-cyanobacterial ultrasound device on the common carp (Cyprinus carpio):
- 707 a field study. Environ. Sci. Pollut. Res. 24, 5669–5678. https://doi.org/10.1007/s11356-
- 708 016-8305-6
- Terceti, M.S., Ogut, H., Osorio, C.R., 2016. Photobacterium damselae subsp. Damselae, an
 emerging fish pathogen in the Black Sea: Evidence of a multiclonal origin. Appl.
- 711 Environ. Microbiol. 82, 3736–3745. https://doi.org/10.1128/AEM.00781-16
- Tian, L., Yin, Y., Jin, H., Bing, W., Jin, E., Zhao, J., Ren, L., 2020. Novel marine antifouling
- coatings inspired by corals. Mater. Today Chem. 17, 100294.
- 714 https://doi.org/10.1016/j.mtchem.2020.100294
- 715 Toranzo, A.E., Magariños, B., Romalde, J.L., 2005. A review of the main bacterial fish
- 716 diseases in mariculture systems. Aquaculture 246, 37–61.
- 717 https://doi.org/10.1016/j.aquaculture.2005.01.002
- 718 Turner, A., 2010. Marine pollution from antifouling paint particles. Mar. Pollut. Bull. 60,
- 719 159–171. https://doi.org/10.1016/j.marpolbul.2009.12.004
- 720 Vartoukian, S.R., Palmer, R.M., Wade, W.G., 2010. Strategies for culture of 'unculturable'
- 721 bacteria. FEMS Microbiol. Lett. 309, no-no. https://doi.org/10.1111/j.1574-
- 722 6968.2010.02000.x

723	Vercauteren,	М.,	De Swaef.	E.,	Declercq	A.,	Bosseler,	L.,	Gulla, S	5.,	Balboa,	S.,	Romalde.
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- J.L., Devriese, L., Polet, H., Boyen, F., Chiers, K., Decostere, A., 2018. First isolation of
- 725 *Vibrio tapetis* and an atypical strain of *Aeromonas salmonicida* from skin ulcerations in
- common dab (*Limanda limanda*) in the North Sea. J. Fish Dis. 41, 329–335.
- 727 https://doi.org/10.1111/jfd.12729
- 728 Wake, H., Takahashi, H., Takimoto, T., Takayanagi, H., Ozawa, K., Kadoi, H., Okochi, M.,
- 729 Matsunaga, T., 2006. Development of an electrochemical antifouling system for
- seawater cooling pipelines of power plants using titanium. Biotechnol. Bioeng. 95, 468–
- 731 473. https://doi.org/10.1002/bit.21022
- Want, A., Crawford, R., Kakkonen, J., Kiddie, G., Miller, S., Harris, R.E., Porter, J.S., 2017.
- 733 Biodiversity characterisation and hydrodynamic consequences of marine fouling
- communities on marine renewable energy infrastructure in the Orkney Islands
- Archipelago, Scotland, UK. Biofouling 33, 567–579.
- 736 https://doi.org/10.1080/08927014.2017.1336229
- 737 Weiss, C.V.C., Castellanos, O., Ondiviela, B., Juanes, J.A., Garcia, R.G., 2018. Development
- of a tool to identify potential zones for offshore aquaculture: A global case study for
- 739 greater amberjack, in: Proceedings of the International Conference on Offshore
- 740 Mechanics and Arctic Engineering OMAE. American Society of Mechanical Engineers
- 741 (ASME). https://doi.org/10.1115/OMAE2018-77870
- Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis Springer-Verlag. NewYork.
- Xie, Q., Pan, J., Ma, C., Zhang, G., 2019. Dynamic surface antifouling: Mechanism and
- 745 systems. Soft Matter 15, 1087–1107. https://doi.org/10.1039/c8sm01853g
- 746 Zhao, R., Symonds, J.E., Walker, S.P., Steiner, K., Carter, C.G., Bowman, J.P., Nowak, B.F.,
- 747 2020. Salinity and fish age affect the gut microbiota of farmed Chinook salmon

- 748 (Oncorhynchus tshawytscha). Aquaculture 528, 735539.
- 749 https://doi.org/10.1016/j.aquaculture.2020.735539
- 750 Zhao, Y., Federico, A., Faits, T., Manimaran, S., Monti, S., Johnson, W.E., 2020.
- 751 Animalcules: Interactive Microbiome Analytics and Visualization in R. bioRxiv
- 752 2020.05.29.123760. https://doi.org/10.1101/2020.05.29.123760
- 753 Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Chapter 1 Introduction
- 754 BT Mixed Effects Models and Extensions in Ecology with R. Springer Science &
- 755 Business Media. https://doi.org/https://doi.org/10.1007/978-0-387-87458-6
- 756





Supplemental Figure S1: Histogram of frequencies for the three tanks with ultrasound

760 exposure (high quality image submitted separately).



763 Supplemental Figure S2: Alpha diversity measures for gut, skin mucus and seawater

764 microbial communities (A and C). NMDS of weighted UniFrac distances (stress values <

765 0.08).

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