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

Authors:

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Highlights

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

Abstract

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

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

1. Introduction

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

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

66 additional maintenance activities (Blackwood et al., 2017; Loxton et al., 2017; Want et al.,
67 2017). In the aquaculture industry, biofouling hinders nutrient exchange and deforms cages
68 due to extra weight which can lower productivity (Fitridge et al., 2012). Therefore, the use of
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

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

2. Material and methods

2.1 Study species and animal maintenance

European sea bass, *Dicentrarchus labrax L.* (Moronidae, Perciformes), used in this experiment were sourced from a seabass aquaculture broodstock at the Ifremer aquaculture station (Palavas-les-Flots, France). A batch of 569 individuals was transferred to the experimental station at the CNAM Intechmer (Tourlaville, France) at 240 days-post-hatching (dph). After a 56-day acclimation period, all individuals were tagged with passive integrated transponder (PIT tag) at 296 dph to track individual fish weight. This operation was performed under benzocaine anaesthesia (200 ppm). A sub-sample of 360 individuals was randomly split into six 600 litre tanks (N = 60 individuals per tank) supplied with filtered seawater in a recirculating system (flow rate: 0.4 m^3h^{-1} and 100% water renewal per day). Mean water temperature, oxygen concentration and salinity were measured throughout the experiment with average values being 20.4 ± 1.7 °C, 7.21 ± 2.8 mg L^{-1}, and 35 ± 0 g L^{-1}, for each parameter respectively. The light was switched on for 14 h and off for 10 h of each 24-h cycle (light switched on at 07:00 U.T. + 1), with twilight transition periods of 30 min. The individuals were hand-fed twice a day (8:30 a.m. and 4:00 p.m.), with commercial food (Le Gouessant Aquaculture). The size of the pellets and the rations were adjusted after each weight measurement according to the manufacturer's recommendations.

2.2 Ultrasound equipment and experiment set-up

Ultrasonic frequencies were generated using ultrasound transducers (20 - 80 kHz, Model 2 Harsonic for Propellers, Harsonic) connected to a frequency generator (Virtual Bench, VB8012, National instruments). The LabVIEW (NXG 2017, National instrument) software was programmed to generate randomly alternated frequency sequence loops between 20 and 80 kHz. At 462 dph, an ultrasound transducer was placed at the centre-bottom of each tank to

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

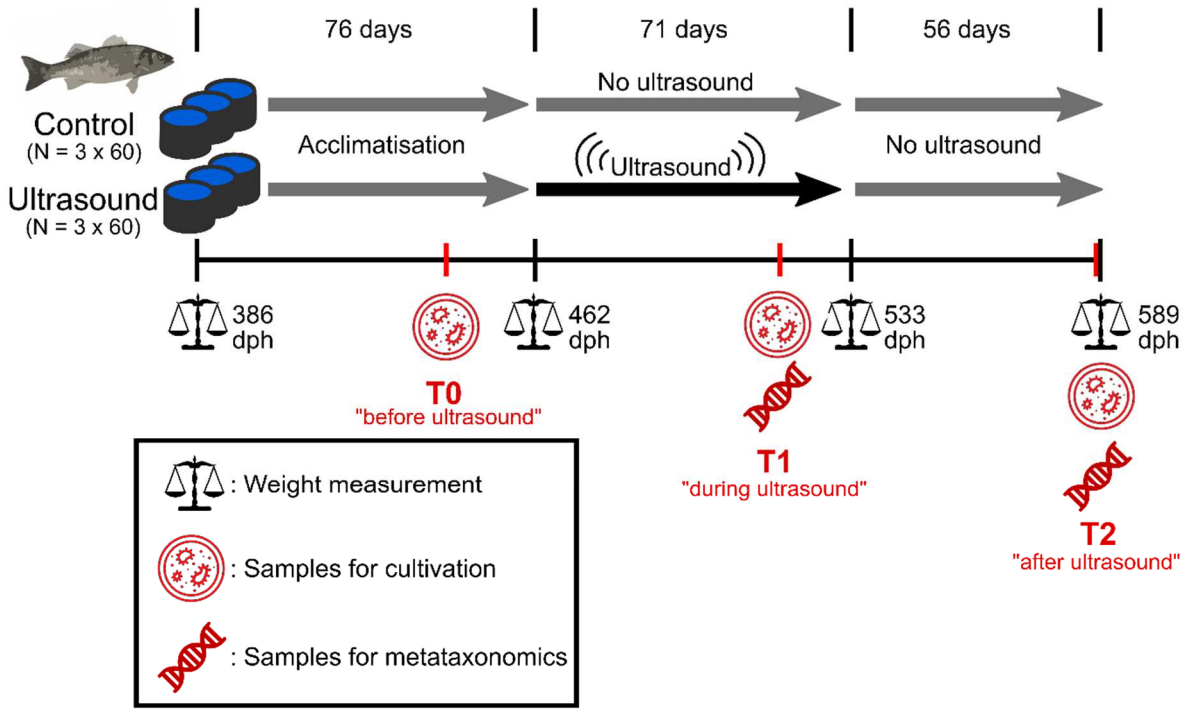


Figure 1: Overview of experimental timeline, weight measurements and sample collection.

2.4 Growth performance

Individual fish weight was assessed four times during the experiment (Figure 1). Before each measurement, the fish were fasted for 24 hours then anesthetized with Benzocaine (Sigma 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

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

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 µm pore size polycarbonate membrane (Whatman). All samples were immediately stored at -80°C in sterile vials until DNA extraction for metataxonomic analysis.

2.7.2 DNA extraction, PCR and sequencing

DNA from all samples was extracted using the MasterPure Complete DNA Purification Kit (Epicentre) according to the manufacturer's instructions for total DNA extraction. The gut samples were pre-processed as described in Benhaïm et al. (2020) to reduce PCR inhibition. The water sample residues were resuspended by adding 300µl of Tissue and Cell Lysis Solution to each filter and scraping the surface gently with a sterile scalpel before continuing with the manufacturer's protocol. DNA from each sample was subject to PCR using the universal prokaryotic primer pair S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21(5′- GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013) covering the V3-V4 region of the 16S rRNA gene and yielding an amplicon of approximately 465 base pairs. The PCR reactions were performed with Phusion High-Fidelity DNA polymerase (NEB) according to the manufacturer's recommendations using 10 ng of template DNA in a 25 µl reaction volume. The thermocycler conditions were set to an initial denaturation step at 98 °C for 30 s followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 52 °C for 30 s and extension at 72 °C for 30 s. Final extension was set to 72 °C for 5 min. The libraries were then multiplexed with Nextera XT v2 indices (Illumina), normalised using Sequel-Prep Normalisation Plates (ThermoFisher Scientific) and sequenced 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
236 the *learnError* command performed on a subset of 10^8 bases. Taxonomic assignment of ASVs
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

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

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

Plate counts (HPC, *Pseudomonas* spp. and *Vibrio* spp.) were subject to Student's Two Sample t-test at time T1 (control: n= 6; US: n = 6) and Welch's Two Sample t-test at time T2 (control: n= 3; US: n = 3). Results were considered significant when the p-value was below 0.05.

For metataxonomic analysis, differential abundance of taxa between groups was compared with DESeq2 (Love et al., 2014) implemented in the R package animalcules version 1.4.0 (Zhao et al., 2020) at genus level taxonomic assignment with default parameters and a minimum count cut-off of 10. Results were considered significant when the adjusted p-value was below 0.05. Significance of alpha-diversity indices (number of observed ASVs and Shannon diversity index) between groups was determined using Student's Two Sample t-test. Beta-diversity was plotted using Non-metric Multi-dimensional Scaling (NMDS) of Bray-Curtis dissimilarities and weighted UniFrac distances and significance of results between groups was determined using PERMANOVA implemented in the vegan package with 999 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
279 group and 13.36 ± 3.77 g in the ultrasound exposure group at the beginning of the experiment
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
282 ± 0.1 and 0.61 ± 0.11 % bodyweight d^{-1} before, during and after ultrasound exposure period,
283 respectively (Figure 2). In the ultrasound exposed group, the average SGR was 1.17 ± 0.16,
284 0.78 ± 0.1 and 0.61 ± 0.1 % bodyweight d^{-1} before, during and after ultrasound exposure,
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

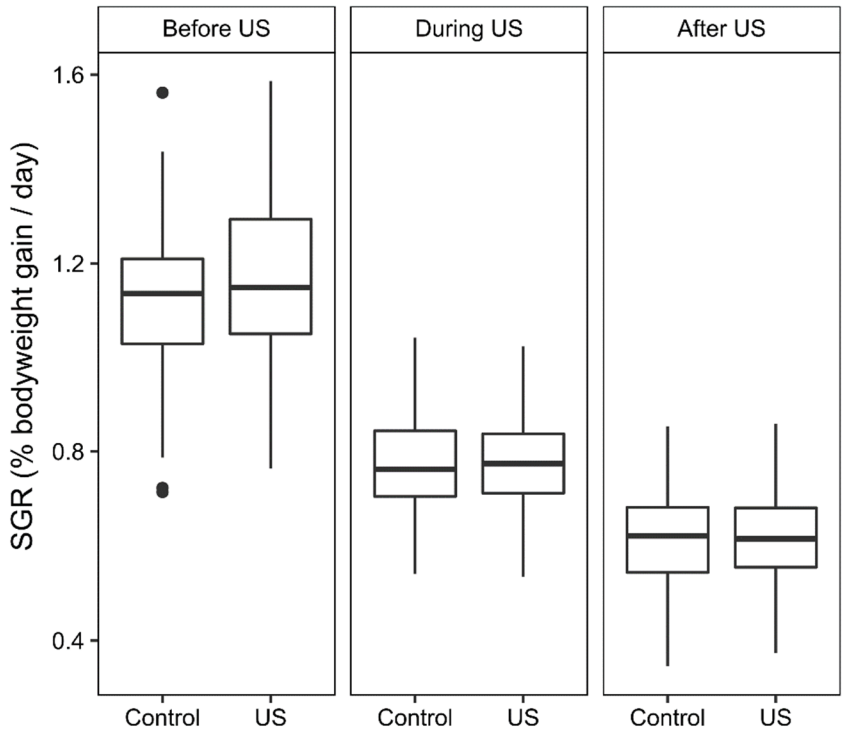


Figure 2: Specific growth rate (SGR) of sea bass in the control group (Control, N = 180) and ultrasound exposure group (US, N = 180) for the periods before (386 – 462 dph), during (462 – 533 dph) and after (533 – 589 dph) ultrasound (US) exposure.

3.3 Cultivation-based microbiological analyses

Bacterial counts of gills, skin mucus and tank seawater at times T0, T1 and T2 are presented in Figure 3 and Table 1. Ultrasound exposure had an impact on the cultivable bacterial flora on the gills at time T1, with the counts of HPC, *Vibrio* spp. and *Pseudomonas* spp. being 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. and *Vibrio* spp., respectively. At time T2, the mean counts remained higher for fish in the ultrasound exposure group than for the control fish, although the differences between the two groups were not significant anymore. For fish in the control group, the mean counts of *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.

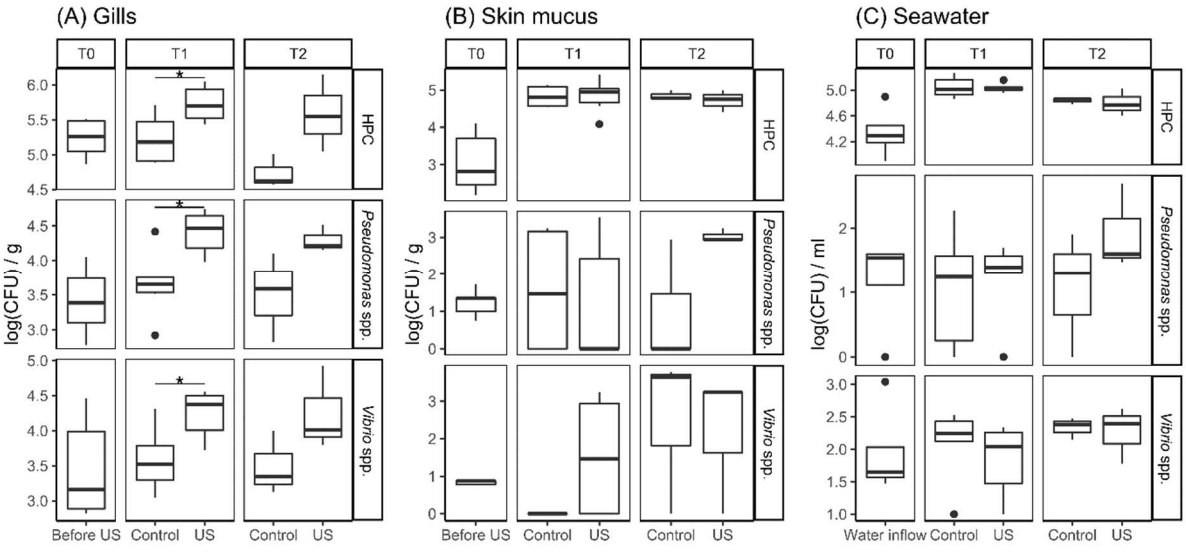


Figure 3: Heterotrophic plate counts (HPC) and counts of Pseudomonas *spp. and* Vibrio *spp. at times T0, T1 and T2 in sea bass gills (A), skin mucus (B) and seawater (C) of the control group (Control) and ultrasound exposed group (US), presented as log CFU per g for gills and skin mucus samples and per ml for seawater samples.*

Counts of cultivable bacteria in the tank seawater were not significantly impacted by ultrasound exposure (Figure 3C, Table 1). The concentration of HPC and *Vibrio* spp. remained constant and similar between the two groups (US and Control), throughout the study (about log 4.8 CFU mL^{-1} and log 2.3 CFU mL^{-1} for HPC and *Vibrio* spp., respectively). The mean counts of *Pseudomonas* spp. remained the same at all three times for the control group, while it increased by log 0.8 CFU mL^{-1} between T1 and T2 for the ultrasound exposed group.

Table 1: Statistical analysis of CFU counts between the control group (Control) and the ultrasound exposure group (US). Counts are presented as log CFU per g for gills and skin mucus samples and per ml for seawater samples. Values in bold indicate significant 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	-1.41	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

3.4 Metataxonomic analysis

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

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

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

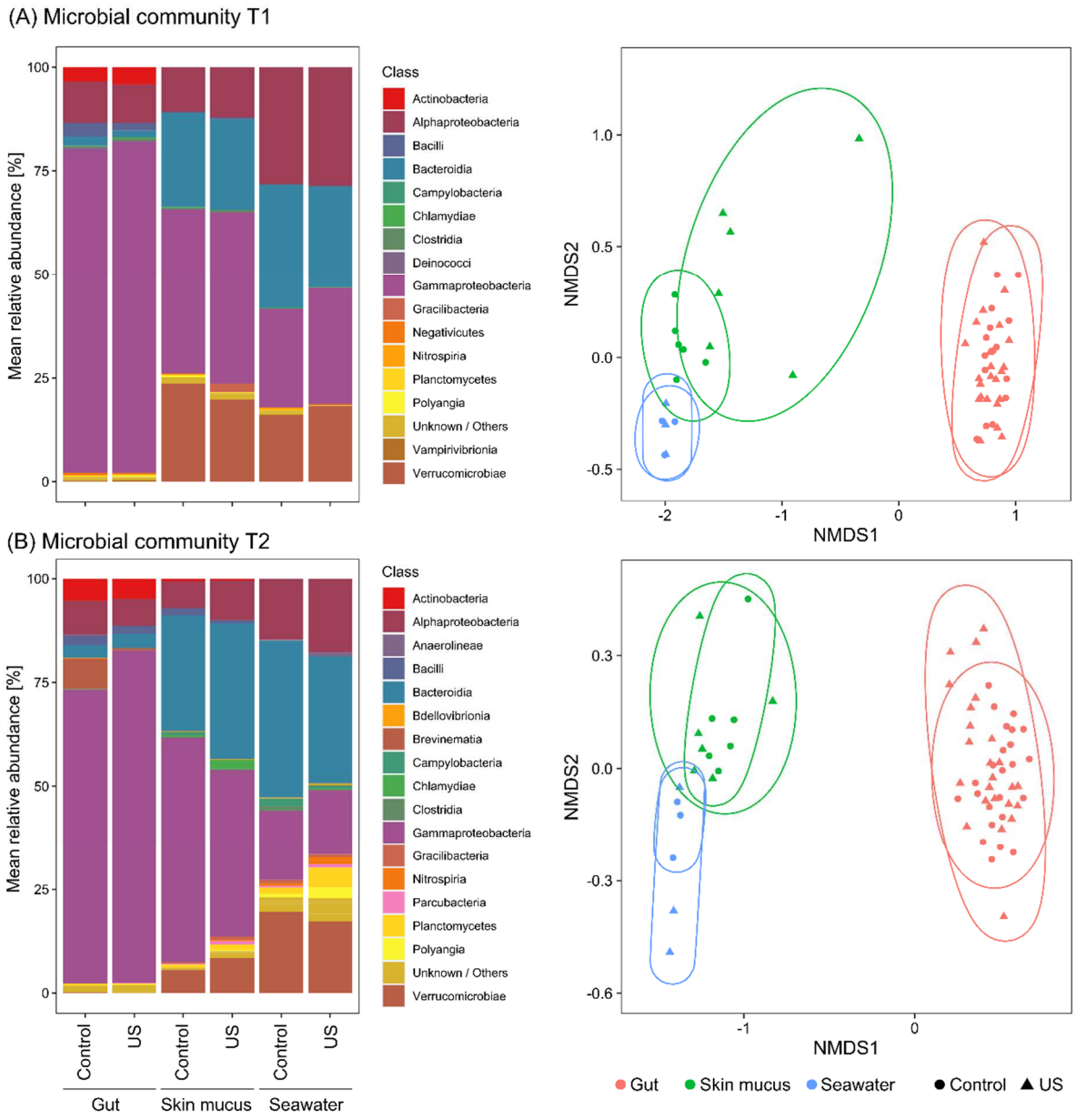


Figure 4: Microbial community profiles at class level assignment (left) and non-metric multidimensional scaling (NMDS) plot of Bray-Curtis distances (right) of gut, skin mucus and seawater microbiota at time T1 (A) and T2 (B) from fish in the control group (Control) or ultrasound exposed group (US).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

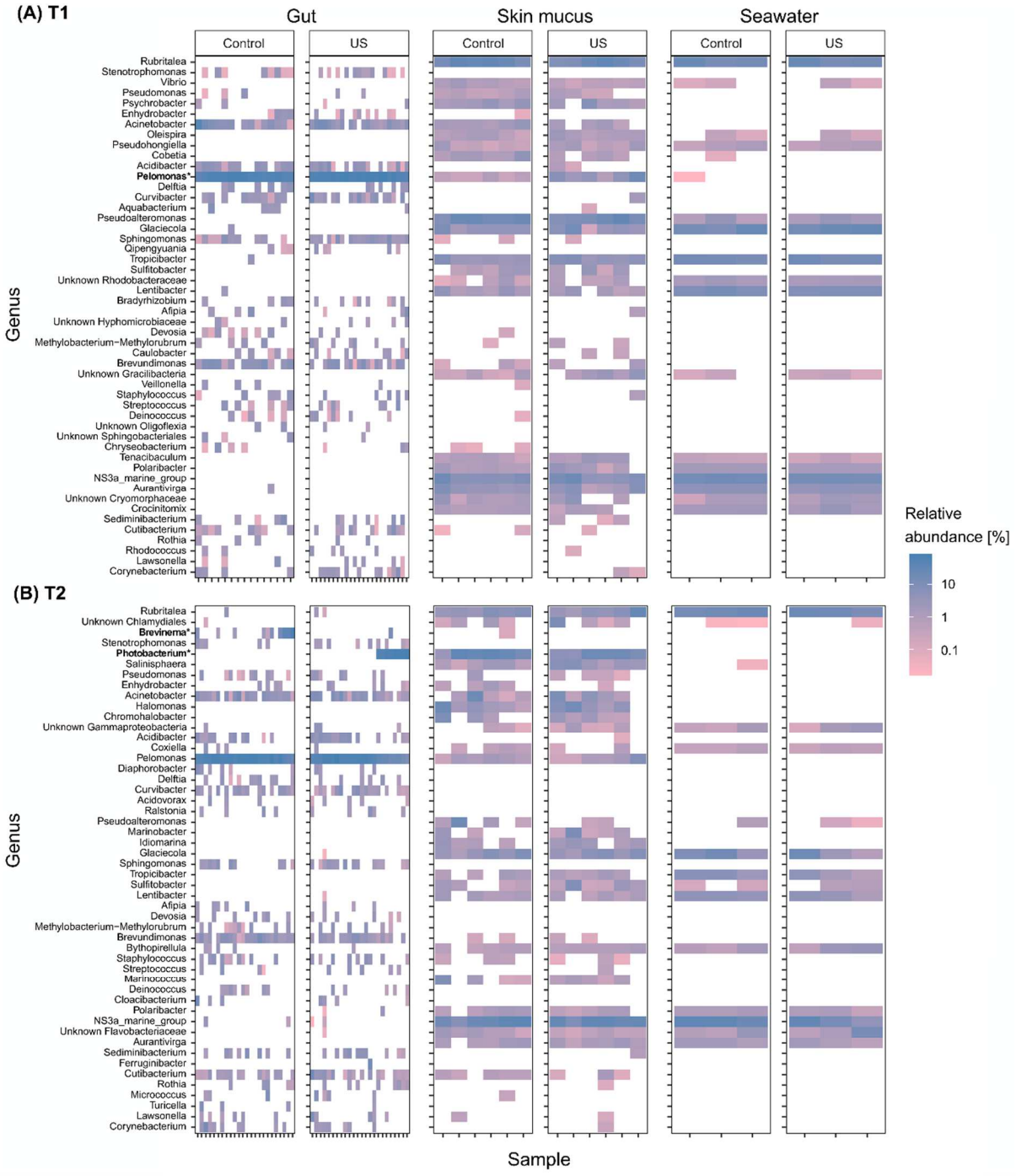


Figure 5: Heatmap showing the relative abundance of the 50 most abundance genera across gut, skin mucus and seawater samples with logarithmic colour scale. Genera which showed significantly differential abundance between the control group (Control) and ultrasound exposed group (US) in one of the sample types are highlighted in bold with an asterisk.

4. Discussion

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

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

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

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

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

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Competing interest statement

The authors declare that there are no competing interests.

References

Abhinav, K.A., Collu, M., Benjamins, S., Cai, H., Hughes, A., Jiang, B., Jude, S., Leithead, W., Lin, C., Liu, H., Recalde-Camacho, L., Serpetti, N., Sun, K., Wilson, B., Yue, H., Zhou, B.Z., 2020. Offshore multi-purpose platforms for a Blue Growth: A technological, environmental and socio-economic review. Sci. Total Environ. 734, 138256. https://doi.org/10.1016/j.scitotenv.2020.138256

Bakopoulos, V., Volpatti, D., Gusmani, L., Galeotti, M., Adams, A., Dimitriadis, G.J., 2003. Vaccination trials of sea bass, Dicentrarchus labrax (L.), against Photobacterium damsela subsp. piscicida, using novel vaccine mixtures. J. Fish Dis. 26, 77–90. https://doi.org/10.1046/j.1365-2761.2003.00438.x

Benassai, G., Mariani, P., Stenberg, C., Christoffersen, M., 2014. A Sustainability Index of potential co-location of offshore wind farms and open water aquaculture. Ocean Coast. Manag. 95, 213–218. https://doi.org/10.1016/j.ocecoaman.2014.04.007

Benhaïm, D., Leblanc, C.A.L., Horri, K., Mannion, K., Galloway, M., Leeper, A., Knobloch, S., Sigurgeirsson, Ó., Thorarensen, H., 2020. The effect of triploidy on the performance, gut microbiome and behaviour of juvenile Atlantic salmon (Salmo salar) raised at low temperature. Appl. Anim. Behav. Sci. 229, 105031. https://doi.org/10.1016/j.applanim.2020.105031

Benhaïm, D., Péan, S., Brisset, B., Leguay, D., Bégout, M.L., Chatain, B., 2011. Effect of size grading on sea bass (Dicentrarchus labrax) juvenile self-feeding behaviour, social structure and culture performance. Aquat. Living Resour. 24, 391–402. https://doi.org/10.1051/alr/2011140

Blackwood, D.J., Lim, C.S., Teo, S.L.M., Hu, X., Pang, J., 2017. Macrofouling induced localized corrosion of stainless steel in Singapore seawater. Corros. Sci. 129, 152–160. 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
524 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
534 Chen, L., Lam, J.C.W., 2017. SeaNine 211 as antifouling biocide: A coastal pollutant of
535 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
564 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
567 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.,

2008. Sonication of bacteria, phytoplankton and zooplankton: Application to treatment of ballast water. Mar. Pollut. Bull. 56, 1201–1208. https://doi.org/10.1016/j.marpolbul.2008.02.007

Holm, P., Buck, B.H., Langan, R., 2017. Introduction: New Approaches to Sustainable Offshore Food Production and the Development of Offshore Platforms, in: Buck, B.H., Langan, R. (Eds.), Aquaculture Perspective of Multi-Use Sites in the Open Ocean: The Untapped Potential for Marine Resources in the Anthropocene. Springer International Publishing, Cham, pp. 1–20. https://doi.org/10.1007/978-3-319-51159-7_1

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric 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. NCBI BLAST: a better web interface. Nucleic Acids Res. 36. https://doi.org/10.1093/nar/gkn201

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1–e1. https://doi.org/10.1093/nar/gks808

Kokou, F., Sasson, G., Mizrahi, I., Cnaani, A., 2020. Antibiotic effect and microbiome persistence vary along the European seabass gut. Sci. Rep. 10, 10003. https://doi.org/10.1038/s41598-020-66622-5

Kormas, K.A., Meziti, A., Mente, E., Frentzos, A., 2014. Dietary differences are reflected on the gut prokaryotic community structure of wild and commercially reared sea bream (Sparus aurata). Microbiologyopen 3, 718–728. https://doi.org/10.1002/mbo3.202

Lasa, A., Avendaño-Herrera, R., Estrada, J.M., Romalde, J.L., 2015. Isolation and 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/s13059-
610 014-0550-8
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

623 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
634 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

Papandroulakis, N., Thomsen, C., Mintenbeck, K., Mayorga, P., Hernández-Brito, J.J., 2017. The EU-Project ``TROPOS'', in: Buck, B.H., Langan, R. (Eds.), Aquaculture Perspective of Multi-Use Sites in the Open Ocean: The Untapped Potential for Marine Resources in the Anthropocene. Springer International Publishing, Cham, pp. 355–374. https://doi.org/10.1007/978-3-319-51159-7_12

Park, J.S., Lee, J.H., 2018. Sea-trial verification of ultrasonic antifouling control. Biofouling 34, 98–110. https://doi.org/10.1080/08927014.2017.1409347

Pérez-Sánchez, T., Balcázar, J.L., García, Y., Halaihel, N., Vendrell, D., de Blas, I., Merrifield, D.L., Ruiz-Zarzuela, I., 2011. Identification and characterization of lactic acid bacteria isolated from rainbow trout, Oncorhynchus mykiss (Walbaum), with inhibitory activity against Lactococcus garvieae. J. Fish Dis. 34, 499–507. https://doi.org/10.1111/j.1365-2761.2011.01260.x

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/S0168-1605(03)00075-8

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, 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/.

Rasheeda, M.K., Rangamaran, V.R., Srinivasan, S., Ramaiah, S.K., Gunasekaran, R., Jaypal, S., Gopal, D., Ramalingam, K., 2017. Comparative profiling of microbial community of three economically important fishes reared in sea cages under tropical offshore environment. Mar. Genomics 34, 57–65. https://doi.org/10.1016/j.margen.2017.04.003

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
682 of disease, antibiotic treatment and recovery trajectory on the microbiome of farmed
683 seabass (Dicentrarchus labrax). Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-
684 55314-4
685 RStudio Team, 2016. Rstudio: Integrated development environment for r [Computer software
686 manual]. Boston, MA.
687 Schütz, S.E., Slater, A.M., 2019. From strategic marine planning to project licences – Striking
688 a balance between predictability and adaptability in the management of aquaculture and
689 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

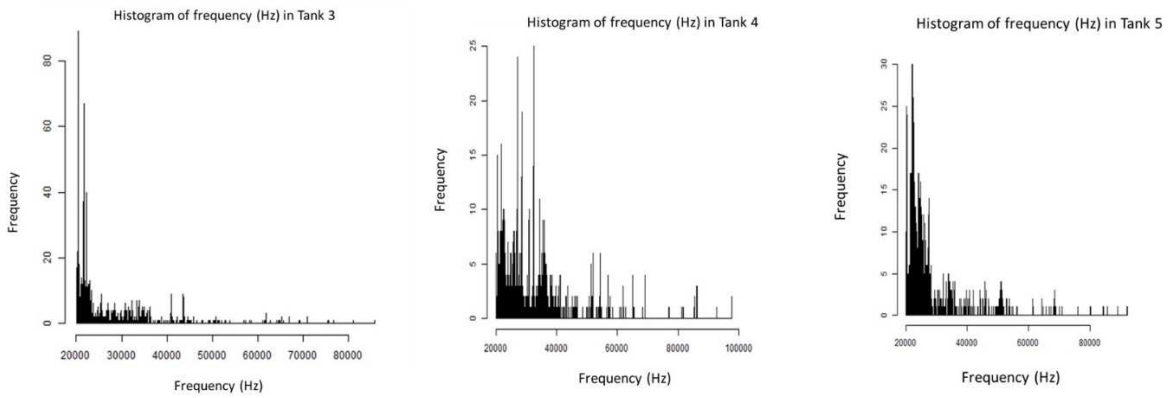
698 Soroldoni, S., Vieira da Silva, S., Castro, Í.B., de Martinez Gaspar Martins, C., Leães Pinho,
699 G.L., 2020. Antifouling paint particles cause toxicity to benthic organisms: Effects on
700 two species with different feeding modes. Chemosphere 238, 124610.
701 https://doi.org/10.1016/j.chemosphere.2019.124610
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
709 Terceti, M.S., Ogut, H., Osorio, C.R., 2016. Photobacterium damselae subsp. Damselae, an
710 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
712 Tian, L., Yin, Y., Jin, H., Bing, W., Jin, E., Zhao, J., Ren, L., 2020. Novel marine antifouling
713 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, M., De Swaef, E., Declercq, A., Bosseler, L., Gulla, S., Balboa, S., Romalde,
724 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
726 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
730 seawater cooling pipelines of power plants using titanium. Biotechnol. Bioeng. 95, 468–
731 473. https://doi.org/10.1002/bit.21022
732 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
734 communities on marine renewable energy infrastructure in the Orkney Islands
735 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
738 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
742 Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis Springer-Verlag. New
743 York.
744 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

(Oncorhynchus tshawytscha). Aquaculture 528, 735539. https://doi.org/10.1016/j.aquaculture.2020.735539

Zhao, Y., Federico, A., Faits, T., Manimaran, S., Monti, S., Johnson, W.E., 2020. Animalcules: Interactive Microbiome Analytics and Visualization in R. bioRxiv 2020.05.29.123760. https://doi.org/10.1101/2020.05.29.123760

Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Chapter 1 - Introduction BT - Mixed Effects Models and Extenstions in Ecology with R. Springer Science & Business Media. https://doi.org/https://doi.org/10.1007/978-0-387-87458-6

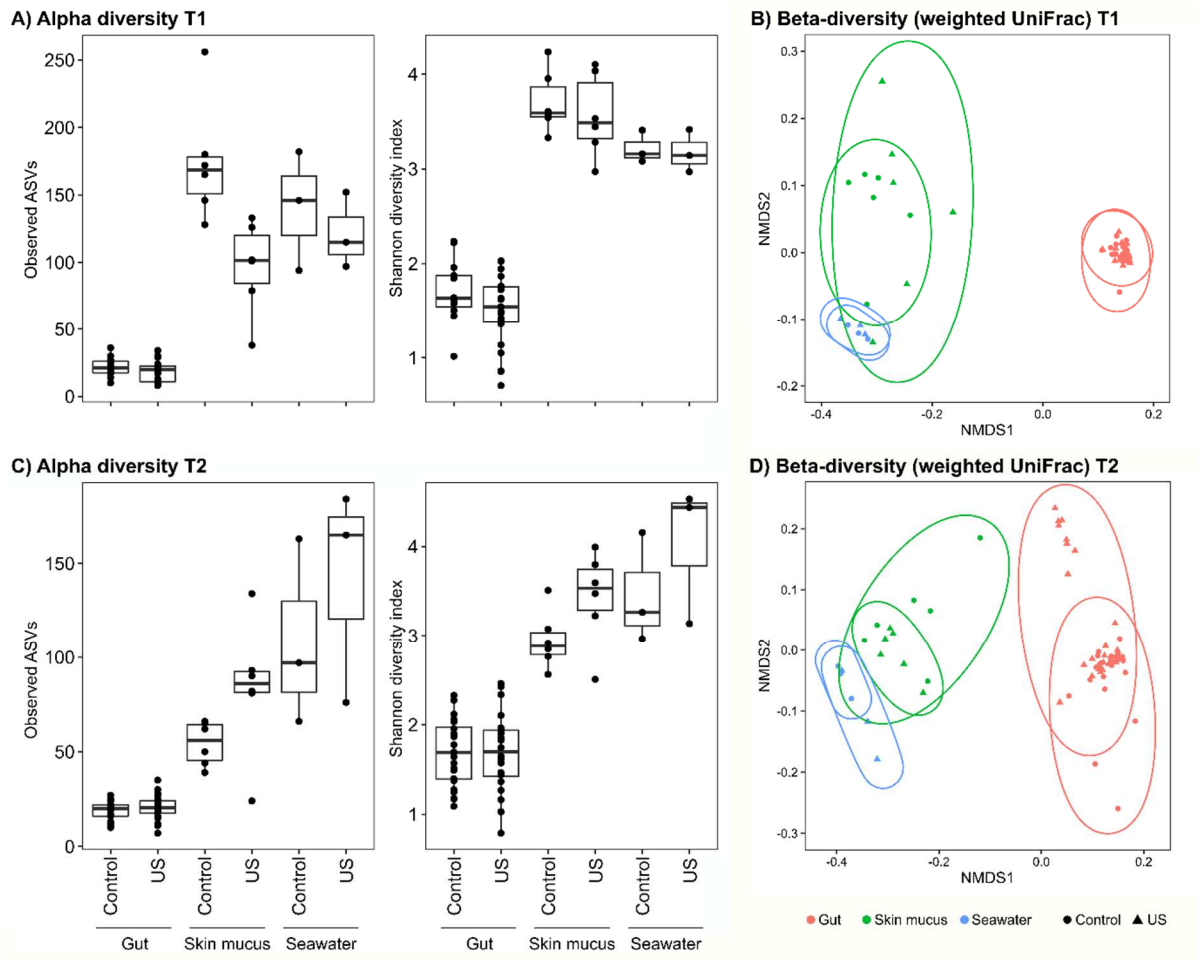


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759 **Supplemental Figure S1**: Histogram of frequencies for the three tanks with ultrasound

760 exposure (high quality image submitted separately).

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762

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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