Wild gut microbiome suppresses the opportunistic pathogen *Aeromonas* in medaka under domesticated rearing conditions

Running head: Wild gut microbiome suppresses *Aeromonas*

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Keywords

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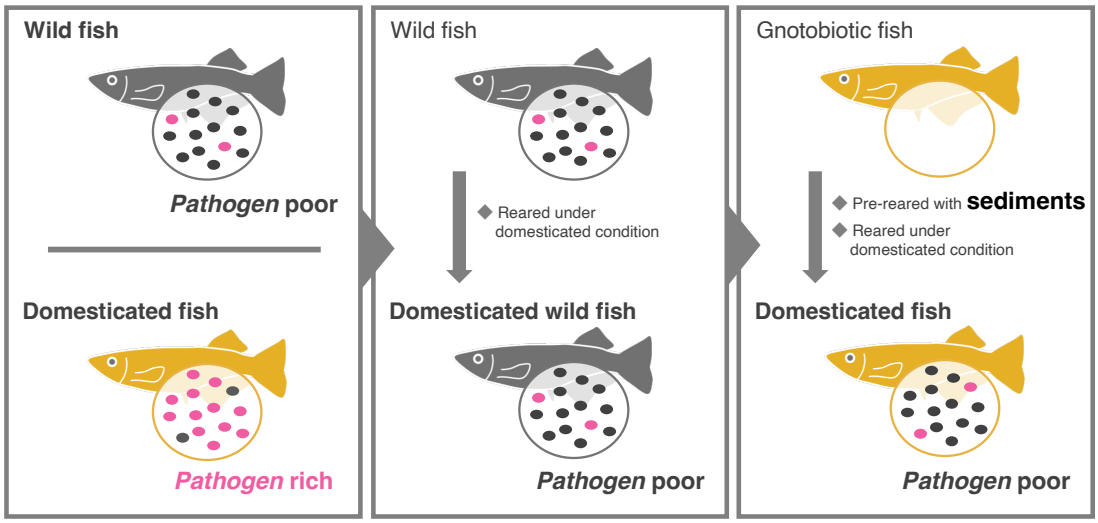
Graphical abstract



Abstract

The gut microbiome is crucial for the metabolic health and pathogen resistance of various host animals; however, the impact of the wild gut microbiome on host health remains poorly understood. This study examined the gut microbiome of medaka (*Oryzias latipes* species complex), comparing domesticated and wild populations, to assess the wild gut microbiome's potential to suppresses opportunistic pathogens in domesticated rearing environments. A comparative analysis of gut microbiomes from domesticated medaka, obtained from multiple laboratories and pet stores, and wild medaka from various habitats in Japan revealed lower microbial diversity in domesticated medaka, with the opportunistic pathogen *Aeromonas* frequently dominating. Conversely, when wild medaka were reared under the same conditions, *Aeromonas* did not proliferate. Additionally, exposing gnotobiotic medaka to sediments from their natural habitats, and thereby to environmental microbes, effectively prevented *Aeromonas* colonisation, even under domesticated conditions. These findings suggest that the habitat-derived wild gut microbiome can inhibit *Aeromonas* proliferation in domesticated fish, highlighting its potential to mitigate opportunistic diseases in aquaculture.

Introduction

Many animals host various microorganisms in their intestines [1]. These microbial communities, known as the gut microbiome, influence the metabolic physiology and development of the nervous and immune systems in their hosts [2, 3]. Focusing on these key functions, numerous probiotics (e.g. lactic acid bacteria and bifidobacteria), prebiotics (nutrients promoting beneficial bacteria growth) and symbiotics (combining both) have been developed and applied [4]. Moreover, the rapid advancement of DNA sequencing has broadened the scope of gut microbiome research across a wide range of species. Indeed, gut microbiota community structure and function are being analysed in both vertebrates and invertebrates, with the microbiome becoming a central research topic in human health, agriculture and fisheries [1, 5].

As gut microbiome research has expanded, it has become evident that the host's lifestyle and environment markedly influence its community structure. In humans, differences in the gut microbiome exist between Europeans, who consume large amounts of animal protein, and African indigenous populations, who follow grain-based diets with minimal animal protein [6]. Notably, pathogenic bacteria, such as *Escherichia*, *Salmonella* and *Shigella*, are more prevalent in Europeans than in indigenous Africans [6]. Similarly, the gut microbiome structure differs between laboratory-maintained and wild-caught mice [7]. Transplants of the gut microbiome into germ-free mice have shown that the recipients of the wild mouse gut microbiome display greater resistance to influenza and reduced susceptibility to induced colon cancer [7]. In invertebrates, soil-derived gut bacteria have been shown to activate the insect host immune system, enhancing their resistance to pathogens [8]. These findings suggest that the wild gut microbiome, shaped by diverse microbes in natural environments, can help prevent disease in hosts.

Gut microbiome research in fish is also advancing [9], with certain studies revealing key gut bacterial functions and mechanisms in fish [10, 11]. Habitat, including rearing conditions, has been shown to influence gut microbiome diversity [12]. Studies using model fish and aquaculture species have demonstrated that gut microbiome composition shifts depending on rearing conditions, such as diet, water temperature and salinity [13-16]. Comparative analyses between wild and domesticated

71 fish in the same species have also revealed differences in the gut microbiome [17-20]. However, it
72 remains unclear how the wild gut microbiome, acquired from natural habitats, affects pathogen
73 establishment or prevention in fish, as observed in previous mammal and insect studies.

74 In this study, we focused on medaka (*Oryzias latipes* species complex [21, 22]), a widely
75 used experimental model and ornamental fish that has wild populations across Japan [23]. Under
76 typical laboratory conditions, medaka are reared in plastic and glass tanks with stable water
77 temperatures (typically 25°C–28°C), using dechlorinated tap water and commercially formulated
78 artificial feed designed for rapid growth [24]. These conditions differ from the fluctuating temperatures
79 and diverse food sources (zooplankton, phytoplankton and small invertebrates) found in wild medaka
80 habitats, such as muddy puddles [25, 26]. We performed a comparative analysis of the gut microbiota
81 in medaka from artificial environments and natural habitats, aiming to clarify the distinct
82 characteristics of the gut microbiota in domesticated and wild medaka as well as explore the potential
83 role of wild gut microbiota in disease risk.

84

85 **Materials and methods**

86 **Sample collection**

87 Domesticated (D) medaka, including inbred/conserved lines (D1–4) and the orange–red commercial
88 strain (himedaka, D6–8), were purchased from the National BioResource Project Medaka
89 (www.shigen.nig.ac.jp/medaka/) and local pet stores, respectively (**Fig. 1; Table 1**). Three other small
90 fish species, the guppy (*Poecilia reticulata*), goldfish (*Carassius auratus*) and zebrafish (*Danio rerio*),
91 were acquired from the pet store where D8 medaka were purchased. All D medaka and other fish were
92 maintained using standardised methods [24]: feeding on fishmeal-based artificial foods in
93 dechlorinated tap water in glass or plastic tanks filled, where dechlorination was achieved via aeration
94 or chemical dechlorinators. Additionally, the F1 generation (D5) of D6 medaka was bred in the
95 laboratory under typical domesticated conditions: fish were kept at 25°C in dechlorinated tap water in
96 plastic tanks with aeration under long day conditions (i.e. 14 h of light per day) and fed TetraMin fish
97 flakes (Spectrum Brands Inc., Madison, WI, USA). Six individuals, including three females and three

98 males, from each D medaka were selected for gut microbiome analysis. Wild (W) medaka were caught
99 from summer to fall between 2014 and 2019 at 16 sites across Japan (**Fig. 1; Table 1**). Wild individuals
100 from each site were transported alive to the laboratory within two days for experiments. All medaka
101 analysed were adults over 2 cm in length. Sediments (0–5 cm depth) were collected from the sites
102 where W6, W12, W14 and W15 medaka were captured, transported to the laboratory and stored at 4°C
103 and −80°C for rearing experiment and DNA extraction, respectively. Ethical approval from the local
104 institutional animal care and use committee was not required, as the law does not mandate the
105 protection of fish. Sampling and experiments followed the 'Guidelines for the Use of Fishes in
106 Research' established by the Ichthyological Society of Japan in 2003 (http://www.fish-
107 isj.jp/english/guidelines.html).
108
109 **DNA extraction**
110 All fish were fasted for 1–3 days to remove transient gut contents and then anesthetised on ice. After
111 rinsing the bodies twice with ice-cold sterile distilled water, the entire gut was dissected using 70%
112 ethanol-treated forceps and tweezers under a binocular microscope, transferred to a microcentrifuge
113 tube and homogenised with 180 µL of AL Buffer (Qiagen, Hilden, Germany) using a pestle. After 1 h
114 at 37°C with 20 µL of 20 mg/mL lysozyme (FUJIFILM Wako Pure Chemical Corporation, Osaka,
115 Japan), the homogenates were incubated overnight at 56°C with 20 µL of 20 mg/mL protease K
116 (Qiagen). The crude extract was purified using the QIAamp DNA Mini Kit (Qiagen) following the
117 manufacturer's instructions. DNA extraction from sediment samples followed the same method as for
118 gut DNA extraction. All DNA samples were stored at −30°C until use.
119
120 **Diversity analysis of the microbiome**
121 The variable V4 region of the bacterial 16S rRNA gene was amplified using the universal primer set
122 515F with Illumina P5 sequences and 806R with 12-base indexes and Illumina P7 sequences [27], Q5
123 Hot Start High-Fidelity Master Mix (New England Biolabs, Beverly, MA, USA) and the prepared
124 DNA, as described previously [28]. Following 1.5% agarose gel electrophoresis using Novel Juice

125 dye (GeneDireX, Las Vegas City, NV, USA), appropriately sized PCR amplicons were cut from the
126 gels and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA).
127 Each purified DNA sample was quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA,
128 USA) with Qubit DNA HS Assay Kits (Invitrogen), diluted and mixed equally. Paired-end sequencing
129 of the PCR amplicon libraries was performed on a MiSeq or iSeq sequencing platform (Illumina) using
130 the MiSeq Reagent Kit v2 or iSeq 100 Reagent Kit (both Illumina), respectively, following the
131 manufacturer's instructions. Raw sequence preprocessing and quality filtering, including paired-end
132 sequence joining and removal of low-quality and chimeric sequences, were performed as previously
133 described [29]. Taxonomic assignment was performed using the RDP classifier ver. 2.13 [30] with a
134 50% confidence threshold.
135
136 **Quantitative analysis of the microbiome**
137 Quantitative PCR (qPCR) was performed to amplify the V4 region of the bacterial 16S rRNA gene
138 using the universal primers 515F and 806R, along with a LightCycler 96 System (Roche Diagnostics,
139 Basel, Switzerland) and THUNDERBIRD SYBR qPCR Mix (Toyobo, Otsu, Shiga, Japan), following
140 the manufacturers' instructions. The thermal cycling conditions were as follows: initial denaturation
141 at 95°C for 30 s, followed by 45 cycles at 95°C for 10 s, 60°C for 30 s and 72°C for 30 s. The gene
142 copy number of the bacterial 16S rRNA gene was calculated based on a standard curve created from
143 a dilution series of PCR products for the 16S rRNA gene of *Aeromonas hydrophila* NBRC13286.
144
145 **Rearing wild medaka under domestic conditions**
146 Wild medaka samples were collected from four sites in Japan (W10–13; **Table 1**), transported alive to
147 the laboratory within two days and used in rearing experiments. W samples (<8 individuals per tank)
148 were kept in a 10-L plastic tank (15.3 × 27.8 × 16.5 cm) for 30 days under domesticated conditions.
149 W medaka were maintained in 8 L of autoclaved dechlorinated tap (ADT) water at 25°C under long
150 day conditions and fed 0.5 g of TetraMin fish food flakes (Spectrum Brands) daily. Additionally,
151 ammonium and nitrite concentrations in the rearing water were monitored daily using pack tests

152 (Kyoritsu Chemical Lab., Tokyo, Japan); if they exceeded the safe standards for fish (ammonium, 0.2
153 mg/L; nitrite, 0.01 mg/L), one-third of the rearing water was replaced with fresh ADT water. After 30
154 days of rearing, all domestically reared wild (DW) medaka were dissected for whole gut analysis and
155 subjected to DNA extraction and diversity analysis of the microbiome, as described above.
156
157 **Antibiotic treatment of medaka**
158 Two medaka purchased from a pet store (D8 medaka) were placed in a 250-mL sterile plastic flask
159 with a vented cap (ASONE, Osaka, Japan) filled with 200 mL of autoclaved gnotobiotic zebrafish
160 medium (GZM) [31] containing a 1% penicillin/streptomycin/amphotericin B mixture (FUJIFILM
161 Wako Pure Chemical Corporation) and 100 μL/mg erythromycin (Tokyo Chemical Industry Co.,
162 Tokyo, Japan) for 24 h. After antibiotic treatment, each medaka was immersed in 200 mL of fresh
163 sterilised GZM for 10 min, with this step repeated three times to remove residual antibiotics. The
164 treated medaka were used in the subsequent experiment, i.e. for 'pretreatment with stream sediments'.
165 The sterility of the treated medaka was verified using a culture-based method, similar to that for
166 producing germ-free zebrafish [31]. The whole gut was dissected from each treated medaka (as
167 described above) and homogenised in 100 μL of sterile GZM using a pestle. The homogenates were
168 diluted with sterile GZM and spread on 1.5% agar plates containing Luria–Bertani broth (Becton,
169 Dickinson and Company, Sparks, MD, USA), Nutrient Broth (Becton, Dickinson and Company) and
170 R2A Broth (Nihon Pharmaceutical Co., Tokyo, Japan). After incubation at 25°C for 3 days, colony-
171 forming units (CFUs) were measured.
172
173 **Rearing experiment with stream sediment pretreatment**
174 Initially, 200 g of each sediment (S6, S12, S14 and S15) collected from four streams where wild
175 medaka (W6, W12, W14 and W15) were caught (**Table 1**) was added to 6 L of ADT water in a 10-L
176 plastic tank (15.3 × 27.8 × 16.5 cm) and maintained with aeration at 25°C for one week. The antibiotic-
177 treated medaka, prepared as described above, were then reared in these tanks for one week (six
178 individuals per tank). As a control, antibiotic-treated medaka were reared in 6 L of ADT water without

179 sediment for one week. Following this period, medaka pre-reared with sediments (DS6, DS12, DS14
180 and DS15) or without (C1 and C2) were transferred to a 17-L plastic tank (20.8 × 31.4 × 27.0 cm)
181 filled with 10 L of rearing water routinely used for D5 medaka. At this time, medaka pre-reared with
182 sediments were immersed in 200 mL of ADT water for 10 min, repeated three times to remove residual
183 sediment from their surface. The medaka were then reared at 25°C with aeration under long day
184 conditions for three weeks. During this period, 0.5 g of TetraMin fish food flakes (Spectrum Brands)
185 was provided daily per tank. Additionally, ammonium and nitrite concentrations in the rearing water
186 were checked daily using pack tests (Kyoritsu Chemical Lab.); if concentrations exceeded the safe
187 ammonium and nitrite standards for fish (mentioned earlier), one-third of the rearing water was
188 replaced with domesticated rearing water. DS and C medaka samples were collected before and after
189 rearing in domesticated rearing water, and their whole guts were dissected for diversity and
190 quantitative analyses of the gut microbiome, as described above.
191
192 **Isolation and haemolysis assay of *Aeromonas* strains from the medaka gut**
193 Whole guts were dissected from D8 samples and homogenised in 100 μL of GZM using a pestle. The
194 homogenates were diluted with GZM and spread on Trypticase soy agar containing 5% sheep blood
195 (Becton, Dickinson and Company). After incubation at 25°C for 3 days, halo formation around the
196 colonies was monitored for haemolysis activity. Colonies of haemolytic bacteria were picked and
197 subjected to 16S rRNA gene sequencing, as described previously [32]. Haemolytic *Aeromonas* strains
198 were cultured overnight in Luria–Bertani medium at 27°C, collected via centrifugation and washed
199 three times with GZM. After adjusting the concentration to OD_{600} = 0.01 using GZM, 10 μL of the
200 bacterial suspension was dropped onto Trypticase soy agar with 5% sheep blood (Becton, Dickinson
201 and Company) and cultured at 27°C for 3 days. Following incubation, haemolysis activity was
202 evaluated based on the diameter of the halo formed around the drop. Halo size was measured using
203 Fiji [33]. *Aeromonas hydrophilia* NBRC 13286 was used as the control in the haemolysis assay.
204
205 **Statistical analysis**

206 Beta and alpha diversity were analysed by normalising read counts to match the sample with the
207 minimum read counts using MicrobiomeAnalyst 2.0 [34]. Beta diversity among the gut microbiota
208 was assessed using nonmetric multidimensional scaling (NMDS) with the Bray–Curtis dissimilarity
209 index, applying the PERMANOVA statistical method. Alpha diversity was calculated using the Chao
210 and Shannon indices. To identify specific bacterial groups in medaka under each rearing condition,
211 linear discriminant effect size (LEfSe) analysis was conducted using MicrobiomeAnalyst 2.0 [34]. For
212 the alpha indices, the proportion and abundance of *Aeromonas* in the gut of D and W medaka, as well
213 as the haemolytic activity of *Aeromonas* strains and CFUs before and after antibiotic treatment, were
214 first assessed for normality using the Shapiro–Wilk test and for variance homogeneity using the F-test
215 or Bartlett test. Subsequently, the most appropriate statistical analysis among three methods (Welch's
216 t-test, the Mann–Whitney U-test and the Brunner–Munzel test) was conducted, as indicated in the
217 figure legends. The proportion and abundance of *Aeromonas* in W and WS medaka, as well as in C
218 and DS medaka, were compared using the Tukey HSD test for multiple comparisons, with analysis
219 conducted in R studio 4.2.2. [35].
220
221 **Nucleotide sequence data availability**
222 Illumina sequencing data from all medaka gut samples were deposited in the DDBJ/GenBank/EBI
223 databases under accession PRJNA1102999. Additionally, Sanger sequencing data from isolated
224 *Aeromonas* strains were deposited in the same databases under accessions PP474260, PP474261 and
225 PP549924–PP549964.
226
227 **Results**
228 **Domesticated and wild medaka gut microbiome profiles**
229 To investigate gut microbiome diversity in medaka, we analysed 48 D medaka from 3 laboratories and
230 3 pet stores, as well as 122 W medaka from 16 locations across Japan (**Fig. 1a–g; Table 1**). The NMDS
231 plot based on the Bray–Curtis distance revealed a clear distinction in gut microbiome community
232 structures between domesticated and wild populations (PERMANOVA: $F = 42.043$, $r^2 = 0.200$, p

233 < 0.05; **Fig. 2a**). Alpha diversity indices showed that D medaka had lower microbiome richness and
234 evenness compared with W medaka (**Fig. 2b,c**), indicating greater diversity in wild populations.
235 Taxonomic analysis at the phylum level revealed Proteobacteria as the dominant group in both D and
236 W medaka guts (**Fig. 2d**), whereas Fusobacteria were also abundant in half of the D medaka (D5–D8)
237 (**Fig. 2d**). Depending on the collection site, W medaka exhibited a more diverse microbiome, including
238 phyla such as Planctomycetes, Verrucomicrobia, Firmicutes, Chloroflexi, Acidobacteria and
239 Actinobacteria, which were less prominent in D medaka (**Fig. 2d**).

240 LEfSe analysis identified genera specific to each group. *Aeromonas*, *Cetobacterium* and
241 *Flavobacterium* were specific to D medaka, whereas *Diplorickettsia*, *Luteolibacter* and *Aquishaera*
242 were unique to W medaka (**Fig. 2e; Supplementary fig. S1**). Notably, the proportion of
243 *Cetobacterium* was high (>30%) in most D5–D8 medaka (91.7%, 22/24 individuals), but lower (<5%)
244 in most D1–D4 medaka (95.8%, 23/24 individuals) (**Fig. 2e,f**). In W medaka, *Diplorickettsia* was
245 predominantly found in W11 medaka (33.7%–83.1%, 8 individuals) but rarely in other W populations
246 (0%–8.4%, 116 individuals) (**Fig. 2e**). *Aeromonas*, with the highest linear discriminant analysis (LDA)
247 score and average proportion (**Supplementary fig. S1a,b**), was consistently detected in D medaka
248 (**Fig. 2e**; **Supplementary fig. S1b**), confirming it as the most common genus in domesticated medaka,
249 as further supported by the low coefficient of variation within the specific dominant genera of D
250 medaka (**Fig. 2f**).

251

252 **Predominance of potentially pathogenic *Aeromonas* in the gut of domesticated fish**

253 *Aeromonas* was the dominant bacterial group in the gut of D medaka (**Fig. 2e,f; Supplementary fig.**
254 **S1b**), accounting for 4.2%–80.4% of the gut microbiome (mean ± SD = 23.8% ± 14.8%, n = 48; **Fig.**
255 **3a**). However, *Aeromonas* levels in W medaka were consistently low (0%–8.6%; mean ± SD = 0.5%
256 ± 1.2%, n = 116), except for the W16 population where they were slightly higher (1.9%–15.8%; mean
257 ± SD = 9.1% ± 4.5%, n = 6; **Fig. 3a**). Overall, *Aeromonas* levels were significantly higher in D medaka
258 compared with W medaka (**Fig. 3b**). Quantitively, *Aeromonas* levels in D medaka ranged from 1.3 ×
259 10^6 to 1.3 × 10^9 copies/individual (mean ± SD = 2.0 ± 2.8 × 10^8 copies/individual, n = 48), whereas in

260 W medaka they were substantially lower (3.0×10^7 to 1.2×10^7; mean ± SD = $0.4 \pm 1.5 \times 10^6$
261 copies/individual, n = 116) (**Fig. 3c**). Both the proportion and quantity of *Aeromonas* were
262 significantly higher in D medaka guts (**Fig. 3d**).

263 To assess whether *Aeromonas* dominance occurs in other fish species reared under
264 domesticated conditions, we analysed guppy (*Poecilia* sp.), goldfish (*Carassius* sp.) and zebrafish
265 (*Danio rerio*) from the pet store as the D8 medaka. *Aeromonas* proportions in the gut microbiome
266 were 5.0%–10.9% in guppy (mean ± SD = 7.5% ± 2.2%, n = 6), 10.4%–92.3% in goldfish (mean ±
267 SD = 49.6% ± 20.6 %, n = 6) and 15.0%–27.9% in zebrafish (mean ± SD = 17.0% ± 5.8%, n = 6)
268 (**Supplementary fig. S2**). These levels, higher than those in W medaka, suggest that domesticated
269 rearing conditions promote *Aeromonas* dominance not only in medaka but also in other small fish
270 species.

271 Specific *Aeromonas* species are opportunistic pathogens that cause diseases, such as
272 haemorrhagic inflammation, ulcers and fin rot in fish [36]. To examine the pathogenic potential of
273 *Aeromonas* in the D medaka gut, we isolated strains from D5 medaka and subjected them to a
274 haemolysis assay on blood agar (**Fig. 3e**). Of the 35 *Aeromonas* strains isolated from D5 medaka (**Fig.
275 3e; Supplementary table S1**), 88.6% (31/35 strains) showed haemolytic activity, forming halos on
276 the blood agar (**Fig. 3e**). Among 40 haemolytic isolates, 77.5% (31/40 strains) were *Aeromonas*
277 (**Supplementary table S1**). Two representative *Aeromonas* strains, designated KK93 and KK125 (**Fig.
278 3f; Supplementary table S1**), exhibited stronger haemolytic activity compared with *Aeromonas*
279 *hydrophila* NBRC13286 isolated from the intestine of juvenile silver salmon (**Fig. 3g**).

280

281 **Gut microbiome of wild medaka reared under domesticated conditions**

282 A comparative analysis of 170 individuals from 24 sites showed distinct differences in the gut
283 microbiota of D and W medaka, with each group harbouring unique dominant bacterial members (**Fig.
284 2**). *Aeromonas* was notably more prevalent in D medaka (**Fig. 3**), likely due to the domesticated rearing
285 conditions, such as stable water temperature and artificial feeding. To test whether similar conditions
286 would promote *Aeromonas* dominance in wild medaka, individuals from four field sites (W10–W13)

287 were reared for 30 days under the same conditions as D5 medaka (generating 'domesticated wild
288 medaka', DW10–DW13; **Fig. 4a**), and the resulting gut microbiomes were analysed.

289 An NMDS plot based on Bray–Curtis distances revealed that the gut microbiome of DW
290 medaka differed significantly from that of both W and D5 medaka (PERMANOVA: DW vs. W:
291 $F = 31.01$, $r^2 = 0.41342$, $p < 0.05$; DW vs. D: $F = 25.626$, $r^2 = 0.50618$, $p < 0.05$; **Fig. 4b**). The
292 predominant gut bacteria also shifted markedly after domesticated rearing (**Fig. 4c**). Although
293 *Pseudomonas*, *Acinetobacter* and *Diplorickettsia* were dominant in W medaka, these genera were
294 nearly absent in DW medaka, replaced by *Aquisphaera*, *Luteolibacter* and *Legionella*, among others.
295 The specificity of these genera to W or DW medaka was confirmed through LEfSe analysis (**Fig. 4d**).

296 Notably, the proportion of *Aeromonas* did not increase in DW medaka after rearing under
297 domesticated conditions. Instead, it tended to decrease across all individuals from the four field sites:
298 DW10 (1.2% ± 1.8%), DW11 (1.1% ± 2.1%), DW12 (0.04% ± 0.03%) and DW13 (0.4% ± 0.4%; **Fig.**
299 **4e**). Although the quantity of *Aeromonas* increased significantly in DW12 (**Fig. 4f**), it remained lower
300 than that observed in domesticated medaka (D5), which were reared under the same conditions (**Fig.**
301 **4f**). These findings suggest that, unlike D medaka, *Aeromonas* does not accumulate and/or dominate
302 in the gut of wild medaka, even those reared under domesticated conditions (**Fig. 3**).

303

304 **Effect of sediment pretreatment on the gut microbiome of medaka reared under domesticated**
305 **conditions**

306 The comparative analysis of domesticated and wild medaka suggested that a 'wild-type' gut
307 microbiome, established in the natural environment, may inhibit the dominance of *Aeromonas*, which
308 tends to thrive in domesticated medaka. However, given that the morphologies of domesticated and
309 wild medaka differ (**Fig. 1**), it remained unclear whether host genotype also influenced *Aeromonas*
310 dominance. To address this, we investigated whether *Aeromonas* would still accumulate in
311 domesticated medaka (D8, in which *Aeromonas* dominated; **Fig. 2**) following a pre-rearing period in
312 an environment more similar to the wild (**Fig. 5a**).

313 Prior to rearing with field-derived environmental microbes, D8 medaka underwent antibiotic

314 treatment to remove their existing gut microbiota (**Treatment 1 in Fig. 5a**). This reduced CFUs on
315 three types of agar media to less than 1/10,000 of the initial CFUs (**Supplementary fig S3**), with no
316 adverse effects on medaka health. The antibiotic-treated medaka were then pre-reared for one week
317 with sediments (S6, S12, S14 and S15) from the four rivers where wild medaka (W6, W12, W14 and
318 W15) were captured (**Treatment 2 in Fig. 5a; Table 1**), allowing them to acquire gut microbiomes
319 from environmental microbes. After this period, *Aquisphaera*, *Zavarzinella*, *Pirellula* and *Reyranella*,
320 among other genera derived from the sediments, colonised the gut (**Supplementary tables S2–S5**).
321 When these sediment-treated medaka (DS6, DS12, DS14 and DS15) were reared for an
322 additional three weeks under domesticated conditions (**Treatment 3 in Fig. 5a**), their gut microbiome
323 structure differed significantly from that of control medaka (C1 and C2) reared without sediment
324 exposure (PERMANOVA: DS vs. C: $F = 100.71$, $r^2 = 0.6727$, $p < 0.05$; **Fig. 5b**). The control groups
325 exhibited gut microbiomes dominated by *Aeromonas* (C1: 64.5% ± 12.2%, n = 8; C2: 57.7% ± 11.8%,
326 n = 7), similar to other domesticated medaka (D1–D8; **Fig. 2**), with *Aeromonas* being the most
327 dominant genus in almost all control samples (14/15 individuals; **Fig. 5c**). In contrast, the gut
328 microbiomes of DS medaka were dominated by *Pirellula*, *Aquisphaera* and/or *Luteolibacter* (**Fig. 5c**).
329 The specificity of the predominant genera in C or DS medaka was confirmed statistically via LEfSe
330 analysis (**Fig. 5d**). The proportion of *Aeromonas* in the gut of DS medaka was significantly lower than
331 that in the controls: DS6, 0.9% ± 1.6%, n = 9; DS12, 0.3% ± 0.2%, n = 9; DS14, 0.7% ± 0.3%, n = 9;
332 and DS15, 3.2% ± 3.4%, n = 9 (**Fig. 5e**). These levels were comparable to those found in wild medaka
333 (**Fig. 3a**). Additionally, the quantity (copies/individual) of *Aeromonas* in the DS medaka gut (DS6, 5.3
334 ± 5.8×10^6, n = 9; DS12, 5.8 ± 4.1×10^5, n = 9; DS14, 7.8 ± 9.4×10^6, n = 9; DS15, 1.0 ± 1.5×10^7,
335 n = 9; **Fig. 5f**) was one to three orders of magnitude lower ($p < 0.05$) than that in the controls (C1, 3.2
336 ± 2.2×10^8, n = 8; C2, 1.9 ± 2.9×10^8, n = 7; **Fig. 5f**). Despite *Aeromonas* dominance in D8 medaka
337 under domesticated conditions (**Figs. 2 and 3**), this genus did not dominate when pre-exposed to
338 sediment-derived microbes (**Fig. 5e,f**). These findings suggest that, regardless of the medaka genotype,
339 the establishment of a gut microbiome from wild environmental microbes can inhibit the colonisation
340 and accumulation of *Aeromonas*, even under domesticated rearing conditions.
341

342 **Discussion**

343 A key finding of this study is the difference in gut microbiome diversity between domesticated and
344 wild medaka (**Fig. 1**). Wild medaka had a significantly higher microbial diversity than domesticated
345 medaka (**Fig. 2b,c**), with a broader range of bacterial phyla, including Planctomycetes,
346 Verrucomicrobia, Firmicutes and Acidobacteria, which were largely absent in domesticated medaka
347 (**Fig. 2d**). In contrast, domesticated medaka exhibited reduced diversity and a higher abundance of
348 Proteobacteria and Fusobacteria, along with consistent *Aeromonas* dominance (**Fig. 2e,f;**
349 **Supplementary fig. S1a,b**). This trend aligns with previous studies showing higher gut microbiome
350 diversity in wild versus domesticated animals [37, 38], with the pattern likely due to more varied diets
351 and exposure to diverse environmental microbes.

352 The dominance of *Aeromonas* in the gut of domesticated medaka, reported in the present
353 study (**Supplementary fig. S2**) and others [39, 40], is also common in other domesticated fish species,
354 such as zebrafish, guppy, molly, rainbow trout, grass carp and silver carp [17, 41-45]. However, in
355 wild fish, including guppy, grass carp and silver carp, *Aeromonas* is less dominant [17, 42, 43]. Thus,
356 the reduced dominance of *Aeromonas* in wild populations relative to their domesticated counterparts
357 appears to be a widespread phenomenon across species.

358 Several factors may explain *Aeromonas* proliferation in the guts of domesticated fish. First,
359 *Aeromonas* is a mesophilic bacterium, with the rearing temperature for medaka (24°C–28°C)
360 providing optimal conditions for its growth [46]. Additionally, the provision of nutrient-rich artificial
361 feed supports its growth, with *Aeromonas* known to thrive under nutrient-abundant conditions [46].
362 Furthermore, overcrowded conditions in domesticated rearing environments may weaken immune
363 defences, making it easier for opportunistic pathogens, including *Aeromonas*, to proliferate. Although
364 these factors likely encourage *Aeromonas* dominance in domesticated fish, with this phenomenon
365 observed in various fish species, the present study revealed that wild medaka reared under
366 domesticated conditions did not exhibit *Aeromonas* dominance (**Fig. 4**). This suggests that the wild
367 gut microbiome may exert stronger selective pressure on *Aeromonas* relative to domesticated rearing
368 conditions. Given that the rearing period was limited to 30 days in the present study, longer-term

369 studies are needed to determine how long *Aeromonas* dominance can be suppressed under
370 domesticated conditions.

371 The most notable finding of this study is that introducing environmental microbes into
372 medaka successfully suppressed *Aeromonas* dominance in the gut, even under domesticated rearing
373 conditions (**Fig. 5**). In D8 medaka, which typically exhibit *Aeromonas* dominance in the gut under
374 such conditions (**Figs. 2e and 3a**), pretreatment with sediment from wild medaka habitats prevented
375 *Aeromonas* from becoming dominant (**Fig. 5**). This suggests that the established gut microbiota has a
376 stronger influence on *Aeromonas* suppression than the medaka's genetic type or rearing environment.
377 This phenomenon, known as the priority effect, has been observed in the guts of various animals and
378 in plants' rhizospheres [47], where established microbial communities prevent the invasion of new
379 microbes, potentially maintaining stability in the gut environment. Therefore, this study emphasises
380 the critical role gut microbiota composition plays in preventing *Aeromonas* overgrowth, highlighting
381 the importance of the priority effect.

382 *Pirellula*, *Aquisphaera* and *Luteolibacter* were consistently associated with wild medaka,
383 domesticated wild-reared medaka and sediment-exposed medaka (**Figs. 2, 4 and 5**), suggesting that
384 they may inhibit *Aeromonas* through priority effects and/or direct antagonistic interactions. Both
385 *Pirellula* and *Aquisphaera* belong to the phylum Planctomycetes, commonly found in aquatic and
386 terrestrial environments [48, 49], and are frequently detected in fish gut microbiomes in natural or
387 semi-natural settings [50, 51]. *Luteolibacter*, a Verrucomicrobia member, is found in diverse
388 environments, including seawater, soil and activated sludge [52-54], as well as the guts of annelids,
389 including the medicinal leech [55]. Given their association with lower *Aeromonas* levels, these
390 bacteria may use antibiotics or injectors (such as those from the Type VI secretion system) to attack
391 *Aeromonas*, or they may form robust communities, such as biofilms, that prevent its colonisation.
392 However, the ecological roles of these bacteria are not well understood; therefore, future research
393 should isolate strains from medaka guts and test their antagonism against *Aeromonas* through
394 coculture experiments *in vitro* and i*n vivo*.

395 The consistent dominance of *Aeromonas* in domesticated medaka is a key finding. Although

396 *Aeromonas* can aid digestion and immune activation [56], it is more commonly recognised as an
397 opportunistic pathogen in fish [57, 58]. Environmental stressors, such as water quality or temperature
398 changes, can trigger *Aeromonas*-related infections [59], with a high abundance of *Aeromonas* in the
399 gut increasing infection risk. In this study, 88.6% of *Aeromonas* isolates from domesticated medaka
400 exhibited haemolytic activity (**Fig. 3e**), indicating a potential health risk for these fish. In aquaculture,
401 efforts are being made to reduce the prevalence of *Aeromonas* in fish and their environments given the
402 pathogen's role in disease outbreaks [57, 58, 60]. Current *Aeromonas* control methods rely on
403 antibiotics, but this bacterium is highly prone to developing antibiotic resistance, and fish farms are
404 major sources of resistant *Aeromonas* strains [61-65]. Therefore, there is an urgent need for alternative
405 control strategies.

406 This study introduces a novel concept as a promising approach for aquaculture: temporarily
407 rearing fish in sediment to introduce 'wild-type' gut microbiota. The 'dirt is good' hypothesis [66]
408 challenges traditional aquaculture practices that emphasise sterile environments. Although
409 maintaining hygiene remains important, this study shows that introducing beneficial environmental
410 microbes can prevent the overgrowth of opportunistic pathogens, such as *Aeromonas*, in the guts of
411 domesticated fish. Previous studies have shown that exposure to diverse microbial communities
412 enhances host immunity in medaka and other species [40, 67, 68], supporting the notion that controlled
413 microbial exposure may be a key strategy for disease prevention. This approach could reduce the
414 dependence on antibiotics and chemical treatments in aquaculture, offering more sustainable disease
415 management practices.

416 In conclusion, this study provides compelling evidence that 'wild-type' gut microbiota can
417 suppress the opportunistic pathogen *Aeromonas* in medaka under domesticated conditions. These
418 findings highlight the crucial role of microbial diversity in maintaining gut health and suggest that
419 introducing environmental microbes could offer a sustainable strategy for disease prevention in
420 aquaculture. Furthermore, by shedding light on the relationship between animals and the
421 environmental microbes in their habitats, this research underscores the significance of understanding
422 these interactions for improving animal health and welfare. While we observed marked differences in

microbiome composition, the underlying mechanisms, especially those related to *Aeromonas* suppression in 'wild-type' gut microbiota, remain unclear. Further research is needed to identify specific bacterial genera in wild gut microbiomes that inhibit pathogen growth, as well as to assess host responses, including immunity and stress, through survival tests, histological analyses, and immune response evaluations.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

Illumina sequencing data from all medaka gut and sediment samples were available in the DDBJ/GenBank/EBI databases under accession PRJNA1102999. Additionally, Sanger sequencing data from isolated *Aeromonas* strains were available in the same databases under accessions PP474260, PP474261 and PP549924–PP549964.

450 **Figure legends**

451 **Fig. 1** Habitat and morphological comparison of domesticated (D) medaka and wild (W) medaka,
452 *Oryzias* sp., in Japan. (A) Geographical locations of collection sites for D and W medaka in Japan.
453 Numbers in parentheses indicate the number of individuals used in the gut microbiome analysis, as
454 shown in **Figs. 2 and 3**. (B) Laboratory rearing conditions of D medaka. Inset photo shows D medaka
455 (closed triangles). (C) Stream habitat of W medaka. Inset photo showing W medaka in a mud puddle
456 (closed triangles). Photographs of D8 medaka (D) and W7 medaka (E). Icons representing D medaka
457 (F) and W medaka (G) used in subsequent figures. Map data sourced from the Geospatial Information
458 Authority of Japan (GSI).

459

460 **Fig. 2** Diversity and specificity of the gut microbiome between domesticated (D) medaka and wild
461 (W) medaka. (A) Nonmetric multidimensional scaling (NMDS) plot illustrating gut microbiome
462 dissimilarity based on the Bray–Curtis distance. Microbial diversity comparison using Chao1 (B) and
463 Shannon (C) indices. Asterisks indicate significant differences ($p < 0.05$, Brunner–Munzel test). (D)
464 Community structure of the gut microbiome according to bacterial phylum proportions. (E) Specific
465 bacterial groups associated with D or W medaka, identified by LEfSe analysis, listing the top 10 genera
466 with the highest LDA scores. (F) Histograms showing the top 5 groups in D medaka. SD, standard
467 deviation; CV, coefficient of variation.

468

469 **Fig. 3** Distribution and potential toxicity of *Aeromonas* in the medaka gut. (A) Proportion of
470 *Aeromonas* in the gut microbiome of each medaka sample. (B) Average proportion of *Aeromonas*
471 compared between domesticated (D) and wild (W) medaka. Asterisks indicate a statistically significant
472 difference ($p < 0.05$, Brunner–Munzel test). (C) Abundance of *Aeromonas* in the gut microbiome,
473 estimated via amplicon sequencing and qPCR analyses. (D) Average abundance of *Aeromonas*
474 compared between D and W medaka. Asterisks indicate a statistically significant difference ($p < 0.05$,
475 Brunner–Munzel test). (E) Schematic overview of haemolytic bacterial strain isolation from the D
476 medaka gut, highlighting the composition of 35 *Aeromonas* strains from D5 medaka. Isolates other

477 than *Aeromonas* are summarised in **Supplementary table S1**. (F) Phylogenetic tree of *Aeromonas*
478 strains isolated from the guts of domesticated medaka, based on 16S rRNA gene sequences.
479 *Pseudaeromonas sharmana* GPTSA-6^{T} was used as the outgroup. Scale bar represents 0.01
480 substitutions per nucleotide position. (G) Haemolytic activity of *A. hydrophila* and medaka-derived
481 *Aeromonas* strains, assessed via halo formation on blood agar (right photo: *A. hydrophila* NBRC
482 13286; left photo: *Aeromonas* sp. strain KK93). Data are from triplicate experiments, with different
483 letters indicating no significant difference (Mann–Whitney U-test with Bonferroni correction).
484
485 **Fig. 4** Gut microbiome of wild medaka reared under domesticated conditions. (A) Schematic overview
486 of the experimental design for preparing domestically reared wild (DW) medaka. (B) NMDS plot
487 based on the Bray–Curtis distance, illustrating dissimilarity in the community structure of the gut
488 microbiome. (C) Community structure of the gut microbiome at the bacterial genus level before (W10–
489 13) and after (DW10–13) rearing under domesticated conditions. (D) Specific bacterial groups in W
490 or DW medaka identified via LEfSe analysis. Genera with an LDA score >4.0 are shown. (E)
491 Proportion of *Aeromonas* in the gut microbiome of each medaka sample. (F) *Aeromonas* abundance
492 in the gut microbiome of each medaka sample, estimated through amplicon sequencing and qPCR
493 analyses. Different letters indicate no significant difference ($p > 0.05$, Tukey's HSD test). Data for D5
494 and W10–13 medaka are the same as in **Fig. 2**.
495
496 **Fig. 5** Effect of sediment pretreatment on the gut microbiome of medaka reared under domesticated
497 conditions. (A) Schematic overview of the experimental design for DS medaka (DS6, DS12, D14 and
498 D15), i.e. D medaka pre-reared with sediment from W6, W12, W14 and W15 medaka habitats. (B)
499 NMDS plot illustrating gut microbiome dissimilarity based on the Bray–Curtis distance. (C)
500 Community structure of the gut microbiome at the bacterial genus level with (DS medaka) or without
501 (C medaka) sediment pretreatment. (D) Specific bacterial groups in DS or C medaka identified via
502 LEfSe analysis. Genera with an LDA score >4.0 are shown. (E) Proportion of *Aeromonas* in the gut
503 microbiome of each medaka sample. (F) *Aeromonas* abundance in the gut microbiome of each medaka

504 sample, estimated through amplicon sequencing and qPCR analyses. Different letters indicate no

505 significant difference ($p > 0.05$, Tukey's HSD test).

Table 1. Medaka examined in this study.

Sample ID	No. of individuals[a]	Condition[b]	Purchase and collection site[c]	Collection date	Note[d]
Domesticated populations					
D1	6	Conventional lab aquaculture (indoor)	NIBB, Okazaki, Aichi pref.	4 Jul, 2013	Inbred strains HNI-II, originated from northern Japan population
D2	6	Conventional lab aquaculture (indoor)	NIBB, Okazaki, Aichi pref.	4 Jul, 2013	Inbred strains HdrR-II1, originated from southern Japan population
D3	6	Conventional lab aquaculture (outdoor)	Niigata University, Niigata, Niigata pref.	4 Jul, 2013	Inbred strains HSOK, originated from the east Korean population
D4	6	Conventional lab aquaculture (outdoor)	Niigata University, Niigata, Niigata pref.	4 Jul, 2013	Conserved strains WS267, originated from the China-west Korean population
D5	6	Conventional lab aquaculture (indoor)	AIST Hokkaido, Sapporo, Hokkaido pref.	28 Nov, 2014	F1 generations originated from commercial strains D6
D6	6	Aquaculture (indoor)	A pet store 1, Sapporo, Hokkaido pref.	11 Jan, 2014	Commercial strains, himedaka
D7	6	Aquaculture (indoor)	A pet store 2, Sapporo, Hokkaido pref.	8 Dec, 2019	Commercial strains, himedaka
D8	6	Aquaculture (indoor)	A pet store 3, Sapporo, Hokkaido pref.	19 May, 2020	Commercial strains, himedaka
Wild populations[†]					
W1	4	-	Setouchi, Okayama pref.	12 Jul, 2014	Southern Japan population
W2	9	-	Niigata, Niigata pref.	17 Jul, 2014	Northern Japan population
W3	9	-	Mito, Ibaraki pref.	19 Jul, 2014	Southern Japan population
W4	8	-	Myoko, Niigata pref.	10 Oct, 2015	Northern Japan population
W5	4	-	Kanzaki, Saga pref.	29 Oct, 2015	Southern Japan population
W6	10	-	Onga-gun, Fukuoka pref.	30 Oct, 2015	Southern Japan population
W7	9	-	Tamana, Kumamoto pref.	30 Oct, 2015	Southern Japan population

W8	9	-	Ozu, Ehime pref.	1 Nov, 2015	Southern Japan population
W9	9	-	Kusu-gun, Oita pref.	2 Nov, 2015	Southern Japan population
W10	8	-	Shimanto, Kochi pref.	20 Nov, 2017	Southern Japan population
W11	8	-	Sukumo, Kochi pref.	20 Nov, 2017	Southern Japan population
W12	3	-	Matsuyama, Ehime pref.	20 Nov, 2017	Southern Japan population
W13	6	-	Uwajima, Ehime pref.	20 Nov, 2017	Southern Japan population
W14	10	-	Site 1, Hamamatsu, Shizuoka pref.	6 Sep, 2019	Southern Japan population
W15	10	-	Site 2, Hamamatsu, Shizuoka pref.	6 Sep, 2019	Southern Japan population
W16	6	-	Iwata, Shizuoka, pref.	10 Oct, 2019	Southern Japan population

[a] Numbers indicate the number of individuals used for the gut microbiome analysis shown in Figs. 2 and 3.

[b] All of domesticated fish were reared in dechlorinated tap water and fed with artificial food. D1, D2, and D5-D8 were kept at around 25°C. D3 and D4 were maintained at variable water temperature because they reared in outdoor tanks.

[c] NIBB, National Institute for Basic Biology; AIST, National Institute of Advanced Industrial Science and Technology.

[d] A category of wild medaka population was referred to Takehana et al., 2003 [23].

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Figure 1

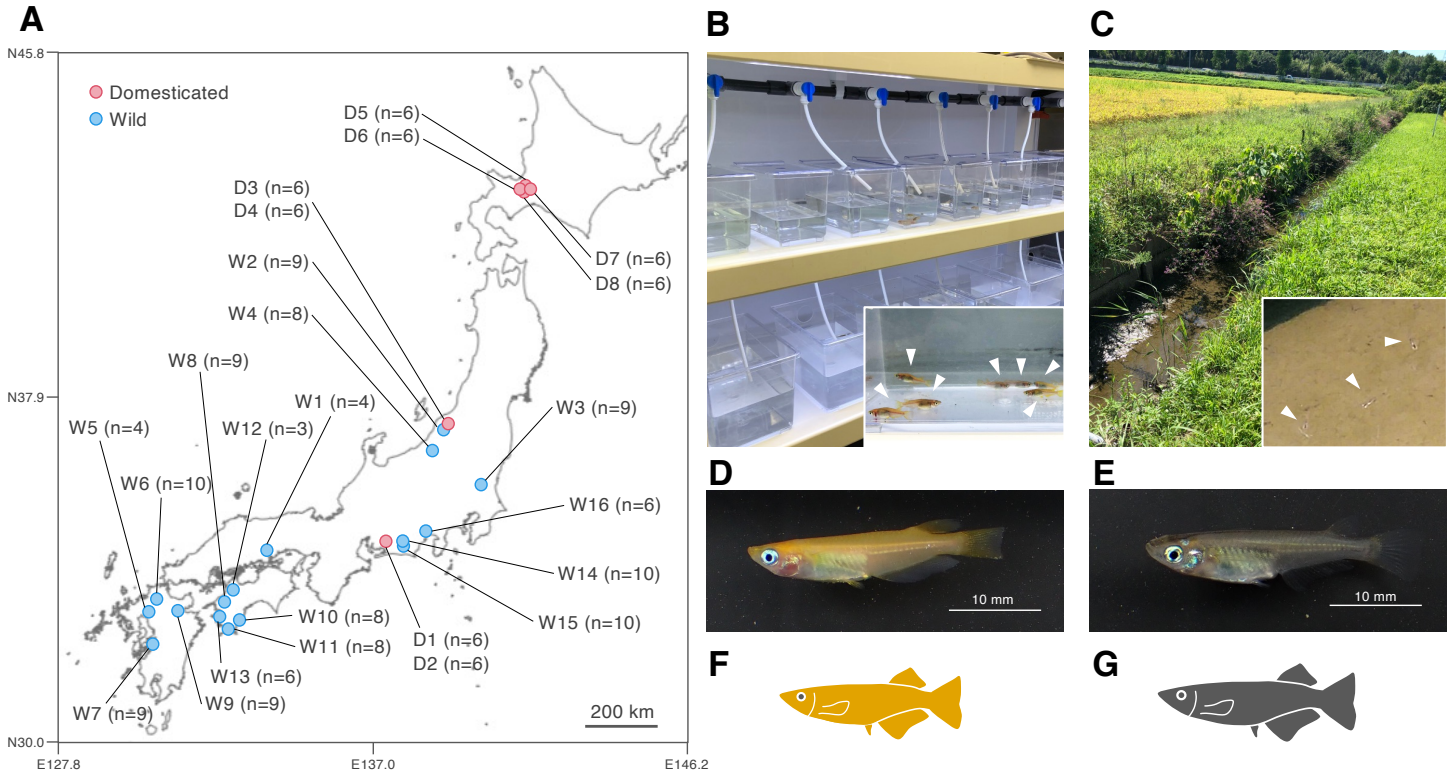

A
B
C
D
E
F
G
Domesticated
Wild
D5 (n=6)
D6 (n=6)
D3 (n=6)
D4 (n=6)
W2 (n=9)
W4 (n=8)
W8 (n=9)
W1 (n=4)
W5 (n=4)
W12 (n=3)
W6 (n=10)
W3 (n=9)
W16 (n=6)
W14 (n=10)
W15 (n=10)
D7 (n=6)
D8 (n=6)
W10 (n=8)
W11 (n=8)
D1 (n=6)
D2 (n=6)
W13 (n=6)
W9 (n=9)
W7 (n=9)
200 km
N45.8
N37.9
N30.0
E127.8
E137.0
E146.2
10 mm
10 mm

Figure 2

A
Stress = 0.1271
NMDS2
NMDS1
Domesticated
Wild
B
Chao1
Domesticated
Wild
C
Shannon
Domesticated
Wild
D
Proportion (%)
Proteobacteria
Planctomycetes
Verrucomicrobia
Fusobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Chloroflexi
Acidobacteria
Others
Domesticated medaka
Wild medaka
E
Aeromonas
Cetobacterium
Flavobacterium
Pelomonas
Pseudomonas
Vibrio
Ralstonia
Bosea
Shewanella
Comamonas
Litorilinea
Pirellula
Caldilinea
Methylocystis
Corynebacterineae
Alsobacter
Gemmobacter
Aquisphaera
Luteolibacter
Diplorickettsia
Specific to D medaka
Specific to W medaka
Genera with top 20 LDA score (4.7-6.1)
Proportion %
Domesticated medaka
Wild medaka
F
Aeromonas
mean: 23.8%
SD: 14.8%
CV: 0.62
Cetobacterium
mean: 22.3%
SD: 22.9%
CV: 1.03
Flavobacterium
mean: 8.6%
SD: 9.0%
CV: 1.04
Pseudomonas
mean: 7.4%
SD: 9.4%
CV: 1.28
Pelomonas
mean: 4.5%
SD: 8.3%
CV: 1.85
No. of individuals in D medaka
Proportion (%)

Figure 3

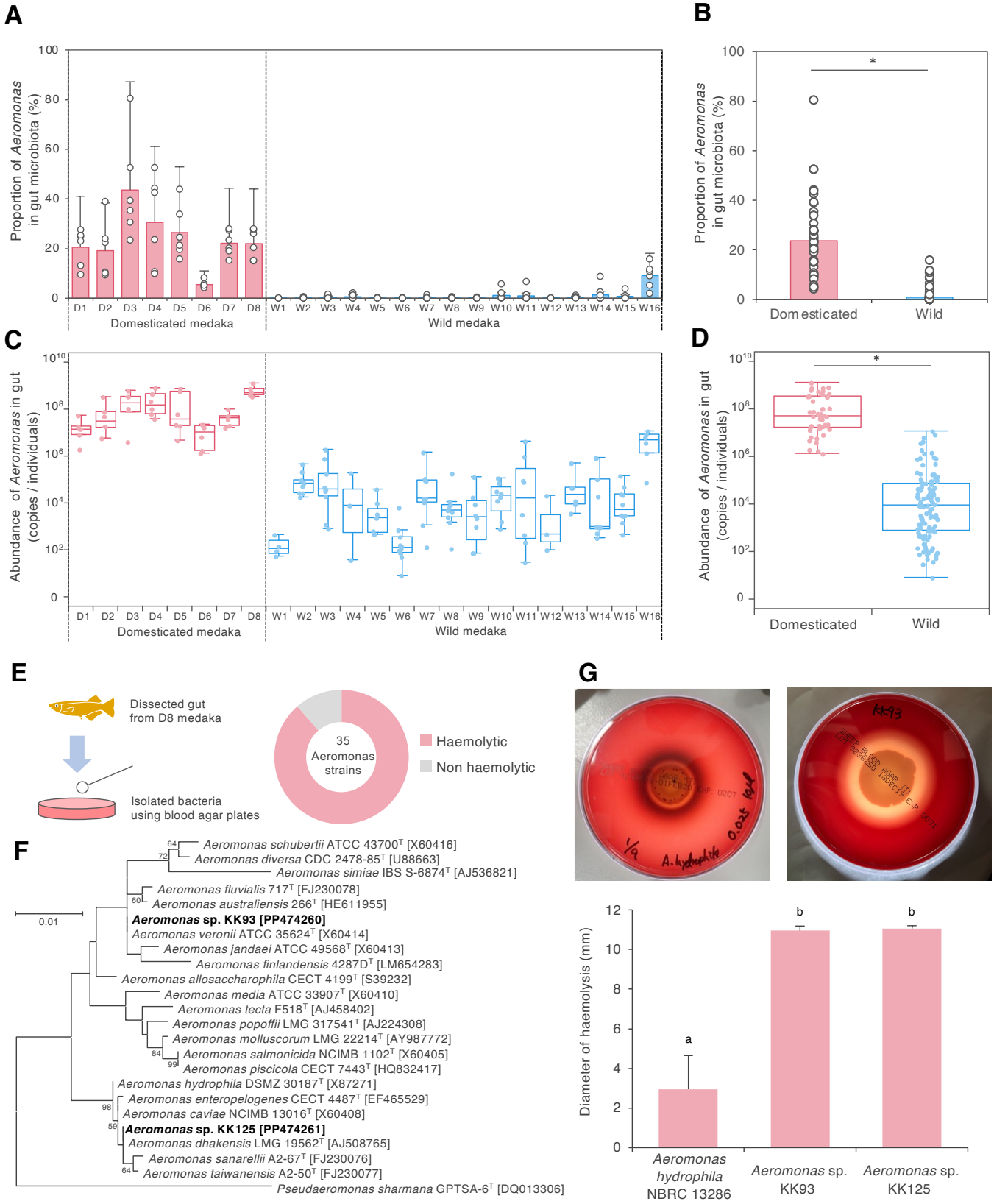

A
Proportion of Aeromonas in gut microbiota (%)
D1 D2 D3 D4 D5 D6 D7 D8
Domesticated medaka
W1 W2 W3 W4 W5 W6 W7 W8 W9 W10 W11 W12 W13 W14 W15 W16
Wild medaka
B
Domesticated
Wild
C
Abundance of Aeromonas in gut (copies / individuals)
D
E
Dissected gut from D8 medaka
Isolated bacteria using blood agar plates
35 Aeromonas strains
Haemolytic
Non haemolytic
F
0.01
Aeromonas schubertii ATCC 43700T [X60416]
Aeromonas diversa CDC 2478-85T [U88663]
Aeromonas simiae IBS S-6874T [AJ536821]
Aeromonas fluvialis 717T [FJ230078]
Aeromonas australiensis 266T [HE611955]
Aeromonas sp. KK93 [PP474260]
Aeromonas veronii ATCC 35624T [X60414]
Aeromonas jandaei ATCC 49568T [X60413]
Aeromonas finlandensis 4287DT [LM654283]
Aeromonas allosaccharophila CECT 4199T [S39232]
Aeromonas media ATCC 33907T [X60410]
Aeromonas tecta F518T [AJ458402]
Aeromonas popoffii LMG 317541T [AJ224308]
Aeromonas molluscorum LMG 22214T [AY987772]
Aeromonas salmonicida NCIMB 1102T [X60405]
Aeromonas piscicola CECT 7443T [HQ832417]
Aeromonas hydrophila DSMZ 30187T [X87271]
Aeromonas enteropelogenes CECT 4487T [EF465529]
Aeromonas caviae NCIMB 13016T [X60408]
Aeromonas sp. KK125 [PP474261]
Aeromonas dhakensis LMG 19562T [AJ508765]
Aeromonas sanarellii A2-67T [FJ230076]
Aeromonas taiwanensis A2-50T [FJ230077]
Pseudaeromonas sharmana GPTSA-6T [DQ013306]
G
Diameter of haemolysis (mm)
Aeromonas hydrophila NBRC 13286
Aeromonas sp. KK93
Aeromonas sp. KK125

Figure 4

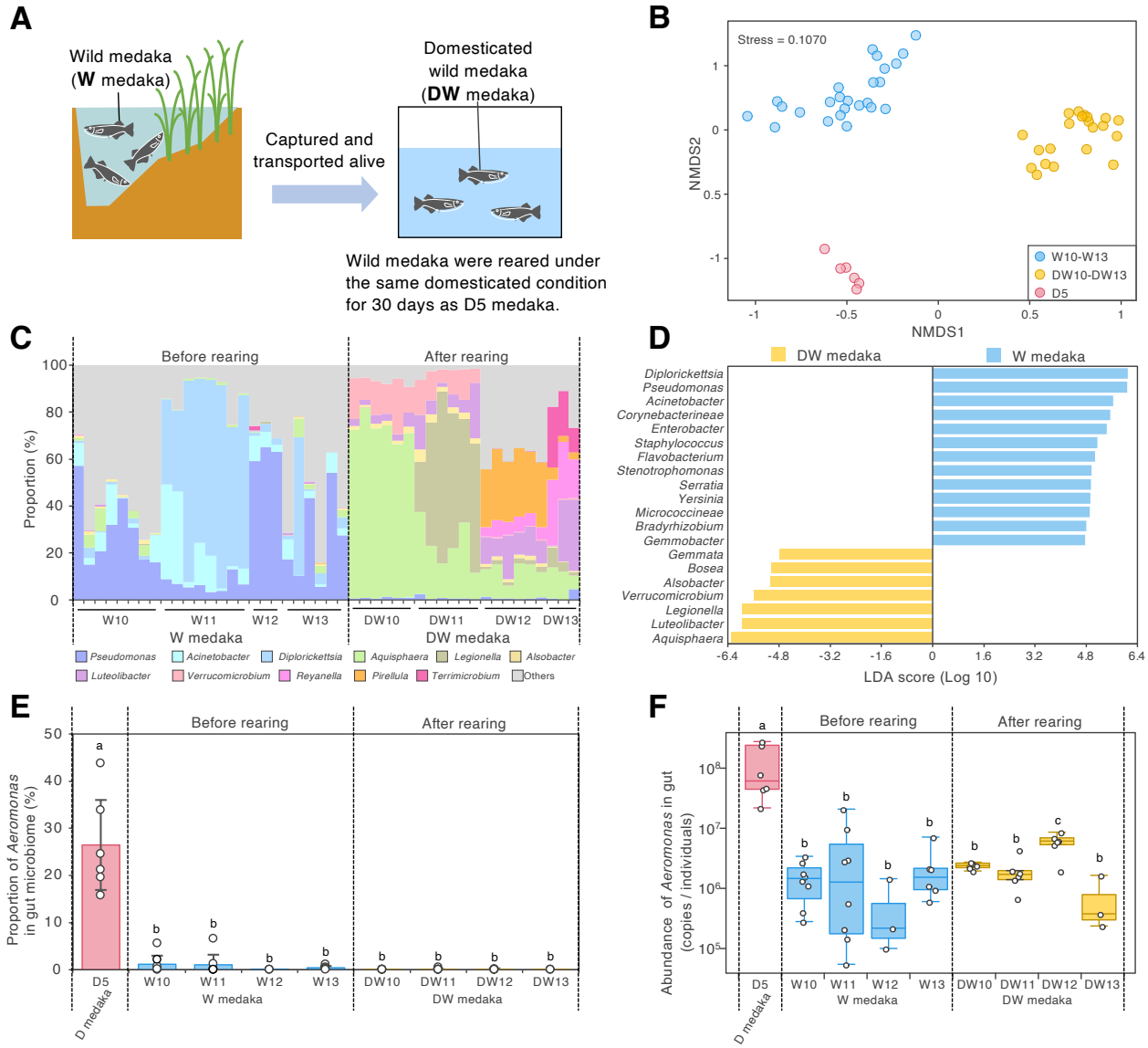
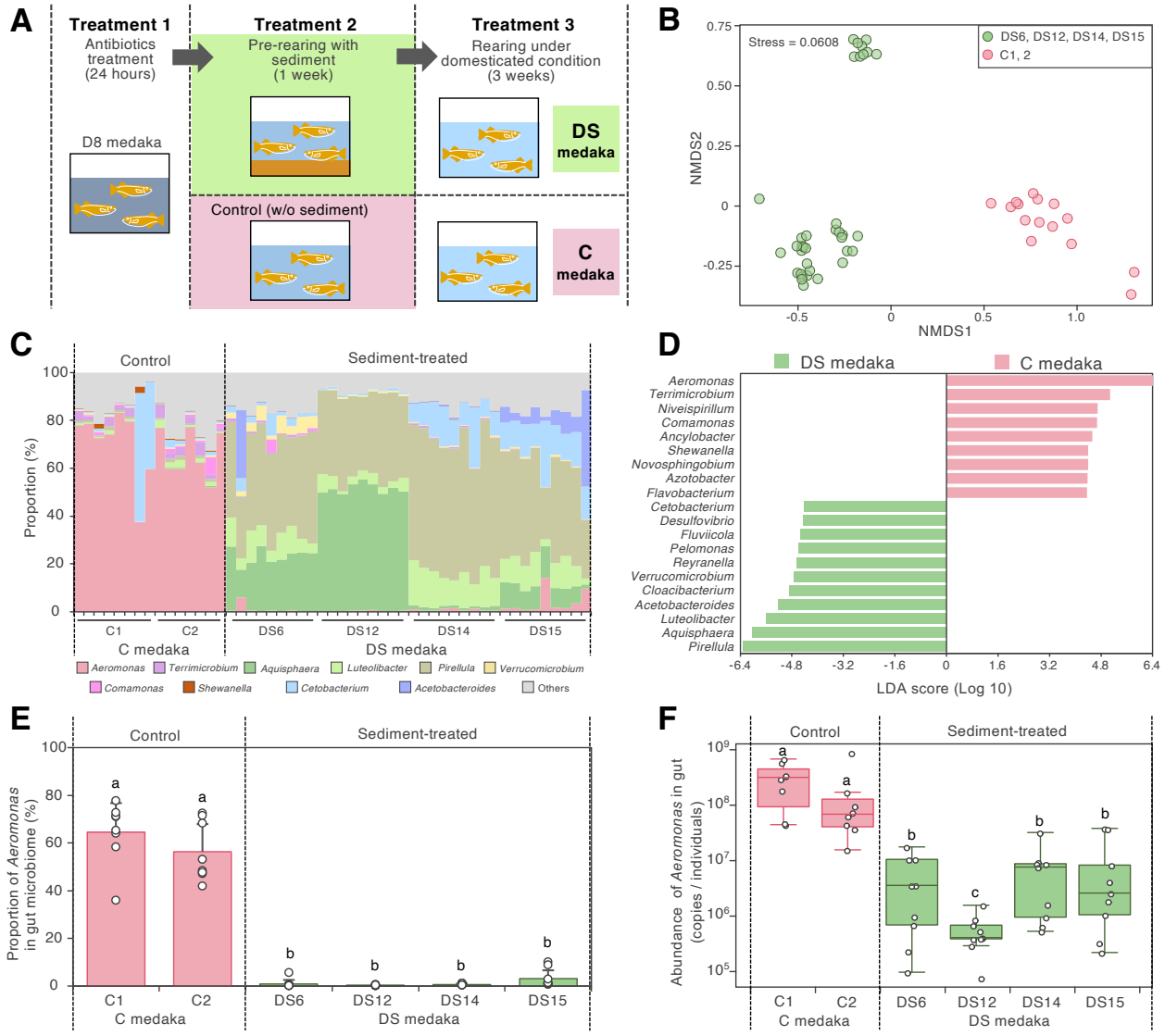

A
Wild medaka
(W medaka)
Captured and
transported alive
Domesticated
wild medaka
(DW medaka)
Wild medaka were reared under
the same domesticated condition
for 30 days as D5 medaka.
B
Stress = 0.1070
NMDS2
NMDS1
W10-W13
DW10-DW13
D5
C
Before rearing
After rearing
Proportion (%)
W10
W11
W12
W13
W medaka
DW10
DW11
DW12
DW13
DW medaka
Pseudomonas
Acinetobacter
Diplorickettsia
Aquisphaera
Legionella
Alsobacter
Luteolibacter
Verrucomicrobium
Reyanella
Pirellula
Terrimicrobium
Others
D
DW medaka
W medaka
Diplorickettsia
Pseudomonas
Acinetobacter
Corynebacterineae
Enterobacter
Staphylococcus
Flavobacterium
Stenotrophomonas
Serratia
Yersinia
Micrococcineae
Bradyrhizobium
Gemmobacter
Gemmata
Bosea
Alsobacter
Verrucomicrobium
Legionella
Luteolibacter
Aquisphaera
LDA score (Log 10)
E
Before rearing
After rearing
Proportion of Aeromonas
in gut microbiome (%)
D5
D medaka
W10
W11
W12
W13
W medaka
DW10
DW11
DW12
DW13
DW medaka
F
Before rearing
After rearing
Abundance of Aeromonas in gut
(copies / individuals)
D5
D medaka
W10
W11
W12
W13
W medaka
DW10
DW11
DW12
DW13
DW medaka

Figure 5


A
Treatment 1
Antibiotics treatment (24 hours)
Treatment 2
Pre-rearing with sediment (1 week)
Treatment 3
Rearing under domesticated condition (3 weeks)
D8 medaka
DS medaka
Control (w/o sediment)
C medaka
B
Stress = 0.0608
DS6, DS12, DS14, DS15
C1, 2
NMDS2
NMDS1
C
Control
Sediment-treated
Proportion (%)
C1
C2
C medaka
DS6
DS12
DS14
DS15
DS medaka
Aeromonas
Terrimicrobium
Aquisphaera
Luteolibacter
Pirellula
Verrucomicrobium
Comamonas
Shewanella
Cetobacterium
Acetobacteroides
Others
D
DS medaka
C medaka
Aeromonas
Terrimicrobium
Niveispirillum
Comamonas
Ancylobacter
Shewanella
Novosphingobium
Azotobacter
Flavobacterium
Cetobacterium
Desulfovibrio
Fluviicola
Pelomonas
Reyranella
Verrucomicrobium
Cloacibacterium
Acetobacteroides
Luteolibacter
Aquisphaera
Pirellula
LDA score (Log 10)
E
Control
Sediment-treated
Proportion of Aeromonas in gut microbiome (%)
C1
C2
C medaka
DS6
DS12
DS14
DS15
DS medaka
F
Control
Sediment-treated
Abundance of Aeromonas in gut (copies / individuals)
C1
C2
C medaka
DS6
DS12
DS14
DS15
DS medaka