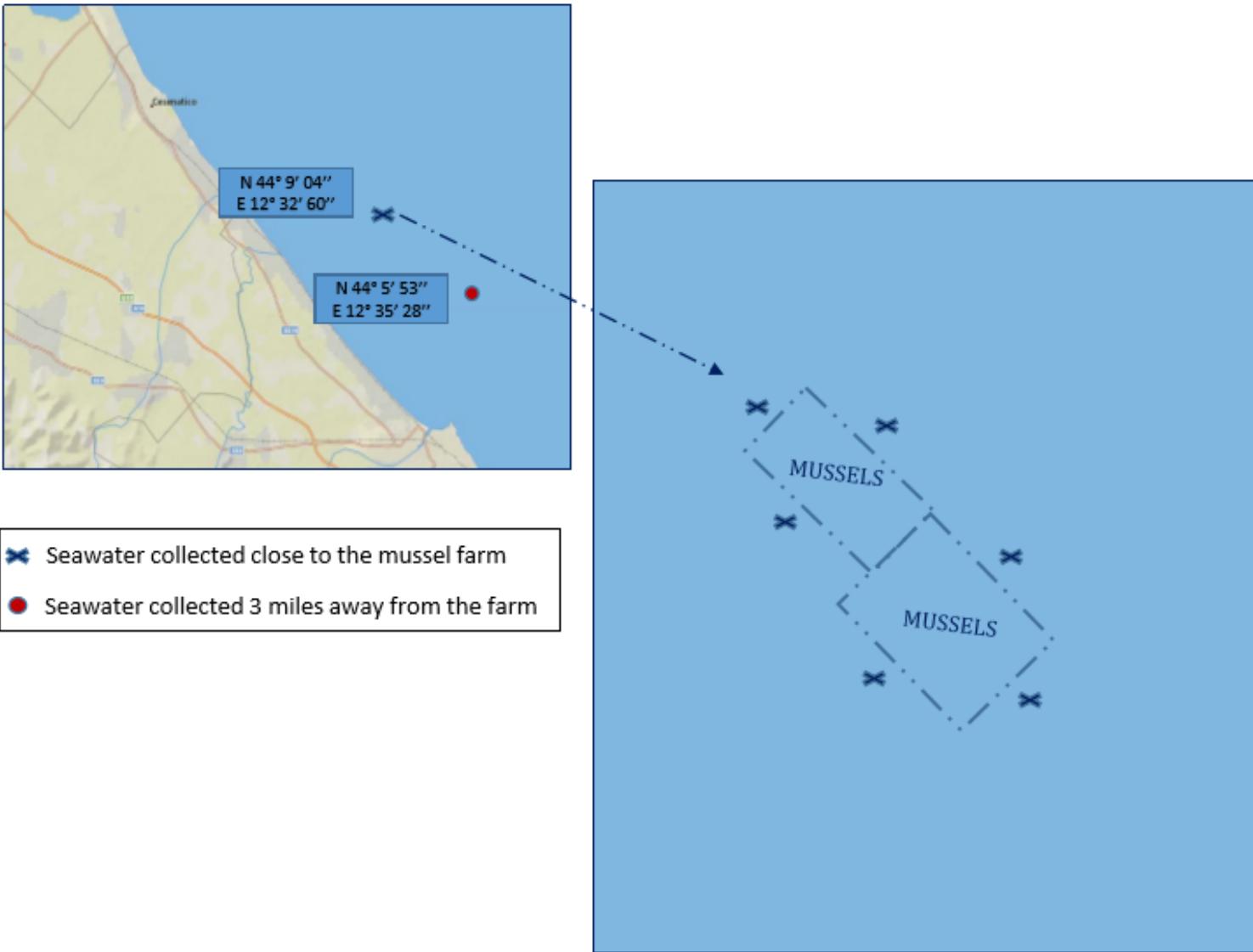


## ***Supplementary Material***

### **Tissue-scale microbiota of the Mediterranean mussel (*Mytilus galloprovincialis*) and its relationship with the environment**

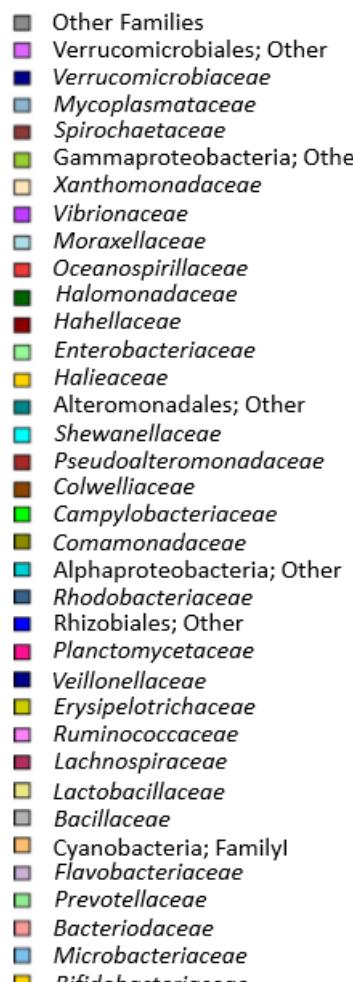
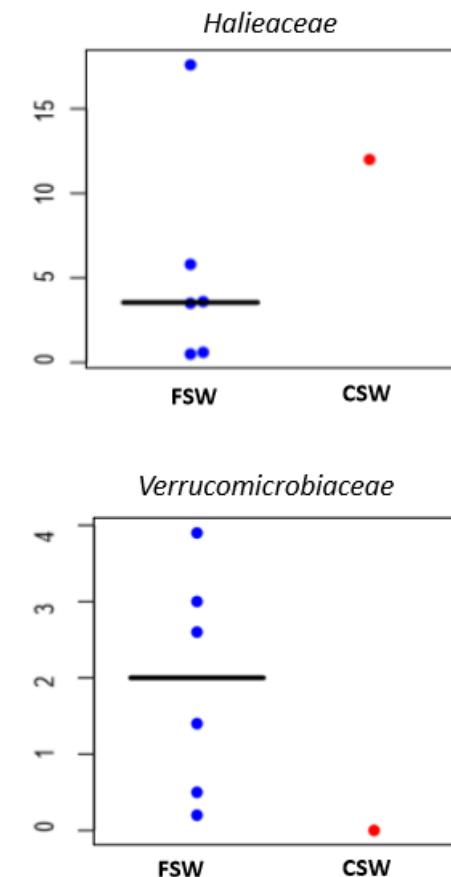
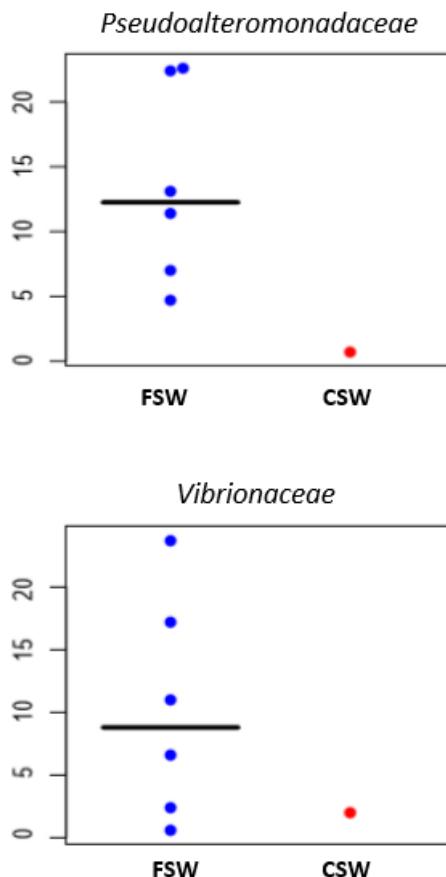
Margherita Musella, Rasika Wathsala, Teresa Tavella, Simone Rampelli, Monica Barone, Giorgia Palladino, Elena Biagi, Patrizia Brigidi, Silvia Turroni, Silvia Franzellitti \*, Marco Candela\*

\*Corresponding Authors: silvia.franzellitti@unibo.it; marco.candela@unibo.it

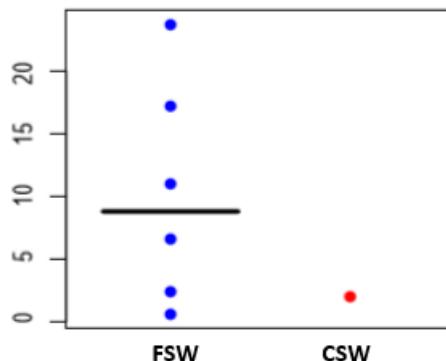


**Supplementary Figure S1.**

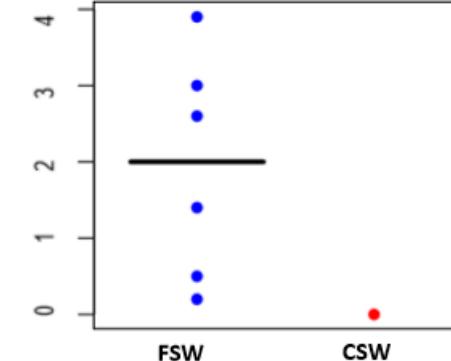
The sampling site of seawater located in North Adriatic Sea coast. Blue cross, seawater collected close to the mussel farm. Red circle, seawater collected 3 miles away from the mussel farm. Samples were collected at a depth of 3 m.

**A****B**

### Vibrionaceae



### Verrucomicrobiaceae



## Supplementary Figure S2.

Family-level relative abundance profiles of bacterial communities in the seawater surrounding the mussel farm (FSW) and in seawater taken 3 miles from the farm (CSW). (A). Family-level relative abundance profiles of FSW and CSW. Only families with a relative abundance of  $\geq 1.5\%$  in at least 10% of samples (B). Scatter plots showing the relative abundance values of the main families differently represented between FSW and CSW. The black bar in the graphs indicates the median.

**Table S1.** Description of the samples. For each tissue type, number of samples, quantity analyzed, and method of extraction are reported. All samples were collected on April 3, 2019.

tissue	samples number	amount analyzed	extraction method
DIGESTIVE GLAND	25	20-30 mg	DNeasy PowerSoil kit
GILL	25	20-30 mg	DNeasy PowerSoil kit
FOOT	25	20-30 mg	DNeasy PowerSoil kit
STOMACH	21	20-30 mg	DNeasy PowerSoil kit
HEMOLYMPH	18	200 µl	DNeasy PowerSoil kit
WATER	7	2 l	DNeasy PowerWater kit

**Table S2.** Results of adonis statistics applied to the ordination analysis of figure 2 (A).

organs	R <sup>2</sup>	P-value
Digestive gland vs Foot	0.01	0.03
Digestive gland vs Gill	0.01	0.003
Digestive gland vs Hemolymph	0.02	0.002
Digestive gland vs Stomach	0.02	0.002
Foot vs Gill	0.02	0.002
Foot vs Hemolymph	0.02	0.002
Foot vs Stomach	0.02	0.002
Gill vs Hemolymph	0.01	0.006
Gill vs Stomach	0.01	0.03
Stomach vs Hemolymph	0.02	0.002

**Table S3.** Relative abundance values of the most represented families in *M. galloprovincialis* organs and seawater.

family	r. a. in digestive gland (%)	r. a. in foot (%)	r. a. in gill (%)	r. a. in stomach (%)	r. a. in hemolymph (%)	r. a. in seawater (%)
<i>Bifidobacteriaceae</i>	1.04	2.92	0.09	1.17	0.46	0.00
<i>Microbacteriaceae</i>	0.32	0.13	0.07	1.02	1.02	4.88
<i>Bacteroidaceae</i>	2.06	2.38	0.17	1.34	1.34	0.00
<i>Prevotellaceae</i>	1.69	1.86	0.03	0.83	0.83	0.00
<i>Flavobacteriaceae</i>	7.69	6.93	4.27	19.64	19.64	11.01
<i>Cyanobacteria; Familyl</i>	0.88	0.04	0.09	1.33	1.33	3.02
<i>Bacillaceae</i>	4.14	0.79	0.68	8.38	8.38	0.00
<i>Lactobacillaceae</i>	1.24	2.11	0.12	0.01	0.01	0.00
<i>Lachnospiraceae</i>	10.42	4.74	1.26	1.70	1.70	0.00
<i>Ruminococcaceae</i>	13.97	5.34	1.55	1.00	1.00	0.03
<i>Erysipelotrichaceae</i>	1.34	0.74	0.43	0.04	0.04	0.00
<i>Veillonellaceae</i>	1.37	0.60	0.11	0.29	0.29	0.00
<i>Planctomycetaceae</i>	3.81	2.67	0.40	0.34	0.34	2.12
<i>Rhizobiales;Other</i>	0.50	0.23	0.21	0.38	0.38	2.70
<i>Rhodobacteraceae</i>	2.89	1.86	1.38	9.43	9.43	8.80
<i>Alphaproteobacteria;Other</i>	1.72	0.14	0.81	0.36	0.36	0.21
<i>Comamonadaceae</i>	2.34	1.56	0.20	0.29	0.29	0.00
<i>Campylobacteraceae</i>	0.07	0.47	1.66	0.80	0.80	2.01
<i>Colwelliaceae</i>	0.34	0.95	0.99	0.88	0.88	0.17
<i>Pseudoalteromonadaceae</i>	0.59	3.27	3.49	0.13	0.13	11.66
<i>Shewanellaceae</i>	0.89	0.77	0.64	0.04	0.04	0.00
<i>Alteromonadales;Other</i>	0.16	0.55	43.43	0.81	0.81	0.01
<i>Halieaceae</i>	1.42	0.16	0.41	4.34	4.34	6.14
<i>Enterobacteriaceae</i>	1.58	0.90	0.41	0.08	0.08	0.00
<i>Hahellaceae</i>	0.08	0.86	11.53	0.84	0.84	0.00
<i>Halomonadaceae</i>	2.18	2.24	0.09	1.49	1.49	0.00
<i>Oceanospirillaceae</i>	0.07	0.51	1.08	0.60	0.60	2.25
<i>Moraxellaceae</i>	1.95	0.97	0.27	0.32	0.32	0.00
<i>Vibrionaceae</i>	2.93	2.18	4.95	1.52	1.52	9.34
<i>Xanthomonadaceae</i>	1.76	1.33	0.22	0.44	0.44	0.00
<i>Gammaproteobacteria;Other</i>	0.36	0.52	1.76	1.17	1.17	3.37
<i>Spirochaetaceae</i>	0.09	22.53	0.26	0.73	0.73	0.00
<i>Mycoplasmataceae</i>	0.39	0.73	0.18	0.07	0.07	0.00
<i>Verrucomicrobiaceae</i>	2.79	2.01	0.96	9.21	9.21	1.63
<i>Verrucomicrobiales;Other</i>	0.70	0.25	0.27	0.79	0.79	0.01
Other Families	24.24	23.73	15.56	28.94	28.94	30.60

**Table S4.** Over-abundance metagenomic inferred pathways.

pathways	pathways	pathways
1,4-dihydroxy-2-naphthoate biosynthesis I	superpathway of UDP-N-acetylglucosamine-derived O-antigen building blocks biosynthesis	superpathway of glycol metabolism and degradation
1,4-dihydroxy-6-naphthoate biosynthesis I	2-methylcitrate cycle I	mevalonate pathway I
superpathway of menaquinol-10 biosynthesis	glutaryl-CoA degradation	mono-trans, poly-cis decaprenyl phosphate biosynthesis
superpathway of menaquinol-6 biosynthesis I	coenzyme M biosynthesis I	mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
superpathway of demethylmenaquinol-6 biosynthesis I	enterobacterial common antigen biosynthesis	mycothiol biosynthesis
superpathway of demethylmenaquinol-9 biosynthesis	enterobactin biosynthesis	peptidoglycan biosynthesis IV ( <i>Enterococcus faecium</i> )
superpathway of menaquinol-9 biosynthesis	ethylmalonyl-CoA pathway	peptidoglycan biosynthesis V ( $\beta$ -lactam resistance)
acetylene degradation	formaldehyde assimilation II (RuMP Cycle)	teichoic acid (poly-glycerol) biosynthesis
Bifidobacterium shunt	formaldehyde oxidation I	NAD salvage pathway II
heterolactic fermentation	fucose degradation	nitrate reduction VI (assimilatory)
hexitol fermentation to lactate, formate, ethanol and acetate	gallate degradation I	octane oxidation
superpathway of Clostridium acetobutylicum acidogenic fermentation	gallate degradation II	palmitate biosynthesis II (bacteria and plants)
pyruvate fermentation to acetate and lactate II	methylgallate degradation	polymyxin resistance
pyruvate fermentation to acetone	protocatechuate degradation II (ortho-cleavage pathway)	reductive acetyl coenzyme A pathway
pyruvate fermentation to butanoate	nicotinate degradation I	S-methyl-5-thio-& $\alpha$ -D-ribose 1-phosphate degradation
allantoin degradation to glyoxylate III	superpathway of salicylate degradation	sitosterol degradation to androstenedione
creatinine degradation I	toluene degradation IV (aerobic) (via catechol)	starch biosynthesis
androstenedione degradation	glycine betaine degradation I	superpathway of (Kdo)2-lipid A biosynthesis
aromatic biogenic amine degradation (bacteria)	methyl ketone biosynthesis	superpathway of (R,R)-butanediol biosynthesis
aromatic compounds degradation via $\beta$ -ketoadipate	glycolysis V ( <i>Pyrococcus</i> )	superpathway of 2,3-butanediol biosynthesis
4-hydroxyphenylacetate degradation	isoprene biosynthesis II (engineered)	superpathway of geranylgeranyl diphosphate biosynthesis I (via mevalonate)
catechol degradation III (ortho-cleavage pathway)	L-arginine degradation II (AST pathway)	superpathway of methylglyoxal degradation
catechol degradation to $\beta$ -ketoadipate	L-glutamate and L-glutamine biosynthesis	superpathway of ornithine degradation
biotin biosynthesis II	L-glutamate degradation V (via hydroxyglutarate)	superpathway of sulfolactate degradation
cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)	L-histidine degradation II	superpathway of sulfur oxidation ( <i>Acidianus ambivalens</i> )
cob(II)yrinate a,c-diamide biosynthesis II (late cobalt incorporation)	superpathway of L-arginine and L-ornithine degradation	superpathway of taurine degradation
adenosylcobalamin biosynthesis II (late cobalt incorporation)	superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation	thiazole biosynthesis II ( <i>Bacillus</i> )
chondroitin sulfate degradation I (bacterial)	superpathway of L-tryptophan biosynthesis	
lactose and galactose degradation I	L-lysine biosynthesis II	
mannan degradation	L-rhamnose degradation I	
superpathway of fucose and rhamnose degradation	4-deoxy-L-threo-hex-4-enopyranuronate degradation	
1,4-dihydroxy-2-naphthoate biosynthesis I	ketogluconate metabolism	
1,4-dihydroxy-6-naphthoate biosynthesis I	superpathway of hexitol degradation (bacteria)	
superpathway of menaquinol-10 biosynthesis	methanogenesis from acetate	
superpathway of menaquinol-6 biosynthesis I	methylphosphonate degradation I	