Seasonal dynamics of the microbiome-host response to pharmaceuticals and pesticides in *Mytilus galloprovincialis* farmed in the Northwestern Adriatic Sea

Supplementary material

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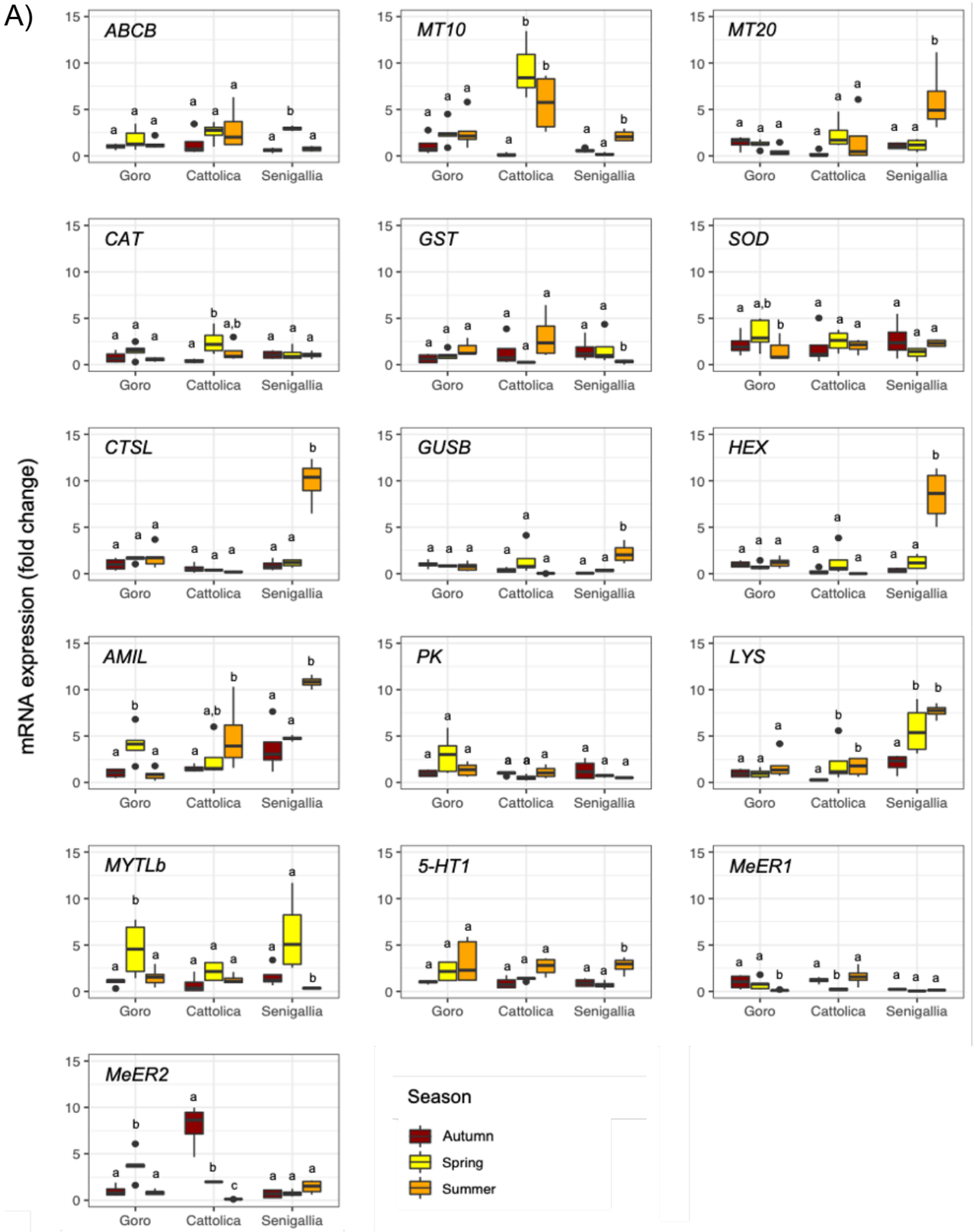
A)

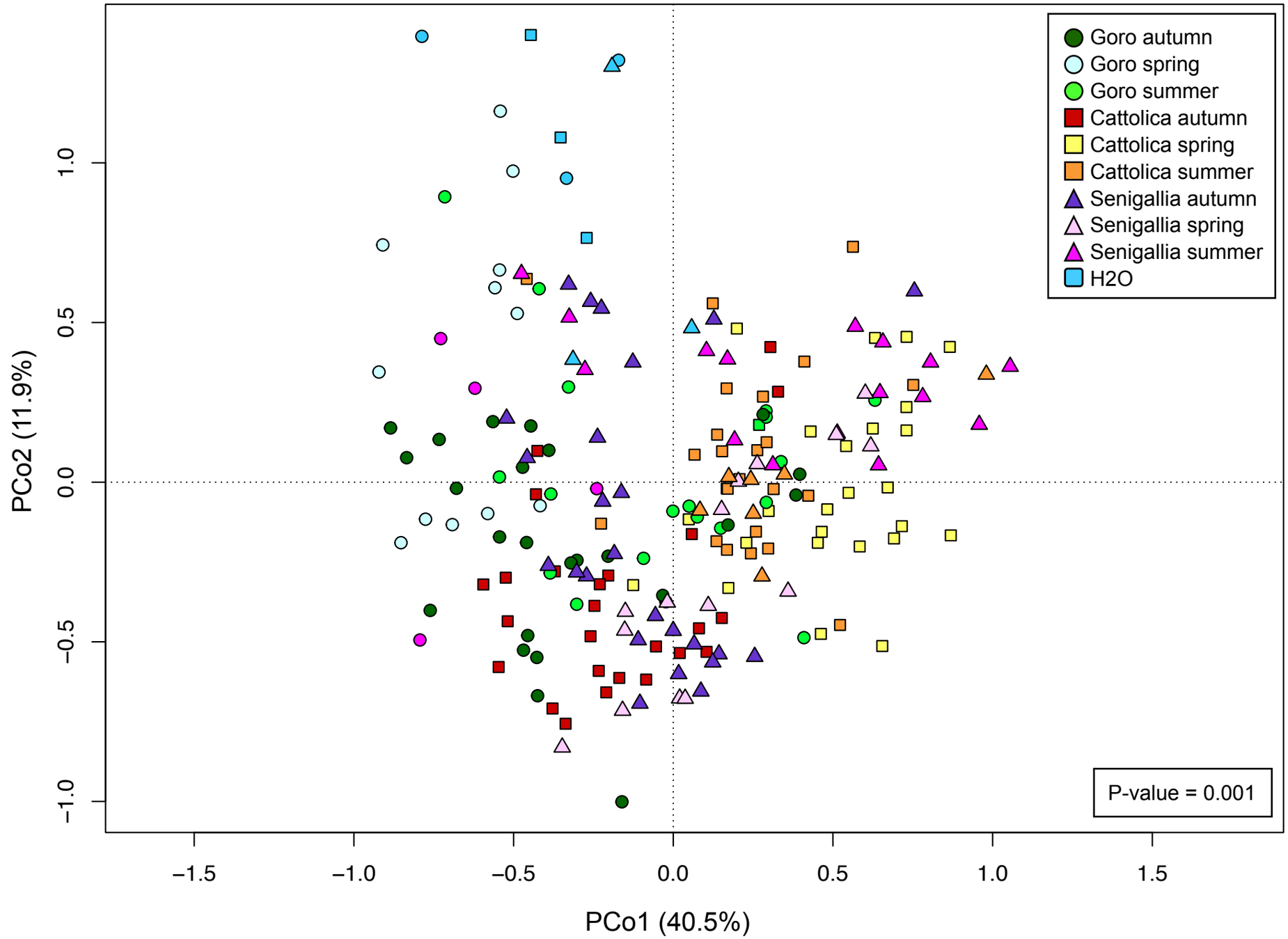


B)

Target transcripts		Autumn			Spring			Summer		
		Go-Ca	Go-Se	Ca-Se	Go-Ca	Go-Se	Ca-Se	Go-Ca	Go-Se	Ca-Se
ABCB	t	0.17	1.97	0.99	0.99	2.01	0.83	1.55	2.08	**2.31**
	P(perm)	0.971	0.127	0.410	0.353	0.121	0.614	0.172	0.082	0.030
MT10	t	2.25	0.91	**3.21**	**4.89**	**5.06**	**11.51**	1.85	0.26	2.46
	P(perm)	0.059	0.526	0.038	0.007	0.025	0.026	0.122	0.829	0.056
MT20	t	3.01	0.53	**3.61**	1.49	0.38	1.45	0.69	**5.72**	2.36
	P(perm)	0.059	0.742	0.032	0.199	0.74	0.23	0.648	0.012	0.117
CAT	t	1.12	0.85	2.88	1.27	0.55	1.79	0.64	0.34	0.40
	P(perm)	0.298	0.436	0.053	0.261	0.573	0.114	0.537	0.734	0.801
GST	t	0.77	1.41	0.35	**4.16**	0.61	**2.29**	0.50	**4.32**	**3.18**
	P(perm)	0.471	0.192	0.651	0.013	0.67	0.03	0.626	0.01	0.033
SOD	t	0.55	0.27	0.69	0.61	2.40	1.90	0.79	1.47	0.80
	P(perm)	0.61	0.77	0.49	0.57	0.06	0.11	0.44	0.15	0.41
CTSL	t	1.06	0.19	0.95	**8.84**	1.71	**3.94**	**3.90**	**6.25**	**16.73**
	P(perm)	0.27	0.91	0.327	0.01	0.149	0.037	0.017	0.006	0.03
GUSB	t	2.52	**5.53**	**2.63**	0.63	**5.17**	1.54	**3.87**	**2.88**	**6.29**
	P(perm)	0.058	0.029	0.032	0.783	0.005	0.09	0.019	0.03	0.024
HEX	t	3.28	**2.95**	0.72	0.37	1.07	0.26	**6.08**	**7.52**	**13.34**
	P(perm)	0.058	0.036	0.491	0.832	0.278	0.827	0.013	0.012	0.026
AMIL	t	1.67	2.45	1.76	1.41	0.86	2.23	**3.33**	**11.55**	**2.67**
	P(perm)	0.200	0.080	0.148	0.227	0.496	0.147	0.02	0.007	0.06
PK	t	0.02	0.34	0.35	**3.11**	2.75	1.23	0.59	**2.74**	1.86
	P(perm)	0.948	0.698	0.776	0.034	0.052	0.305	0.534	0.035	0.151
LYS	t	**3.63**	1.75	**4.20**	1.04	**5.27**	2.30	0.06	5.46	5.83
	P(perm)	0.025	0.09	0.031	0.4	0.011	0.09	0.98	**0.016**	**0.029**
MYTLb	t	0.79	0.91	1.38	1.53	0.59	2.07	0.28	**2.88**	**4.43**
	P(perm)	0.444	0.463	0.195	0.158	0.524	0.173	0.753	0.026	0.029
5-HT1	t	0.55	0.45	0.19	1.45	**3.01**	2.64	0.13	0.01	0.21
	P(perm)	0.67	0.731	0.823	0.18	0.047	0.064	0.933	1	0.795
MeER1	t	0.65	2.04	**6.69**	1.93	**2.83**	**2.83**	**4.32**	0.55	**3.69**
	P(perm)	0.608	0.124	0.033	0.094	0.019	0.026	0.008	0.589	0.026
MeER2	t	**7.10**	0.79	**7.95**	**7.10**	0.79	**7.95**	**2.36**	**4.87**	**5.61**
	P(perm)	0.025	0.486	0.027	0.025	0.486	0.027	0.049	0.03	0.028

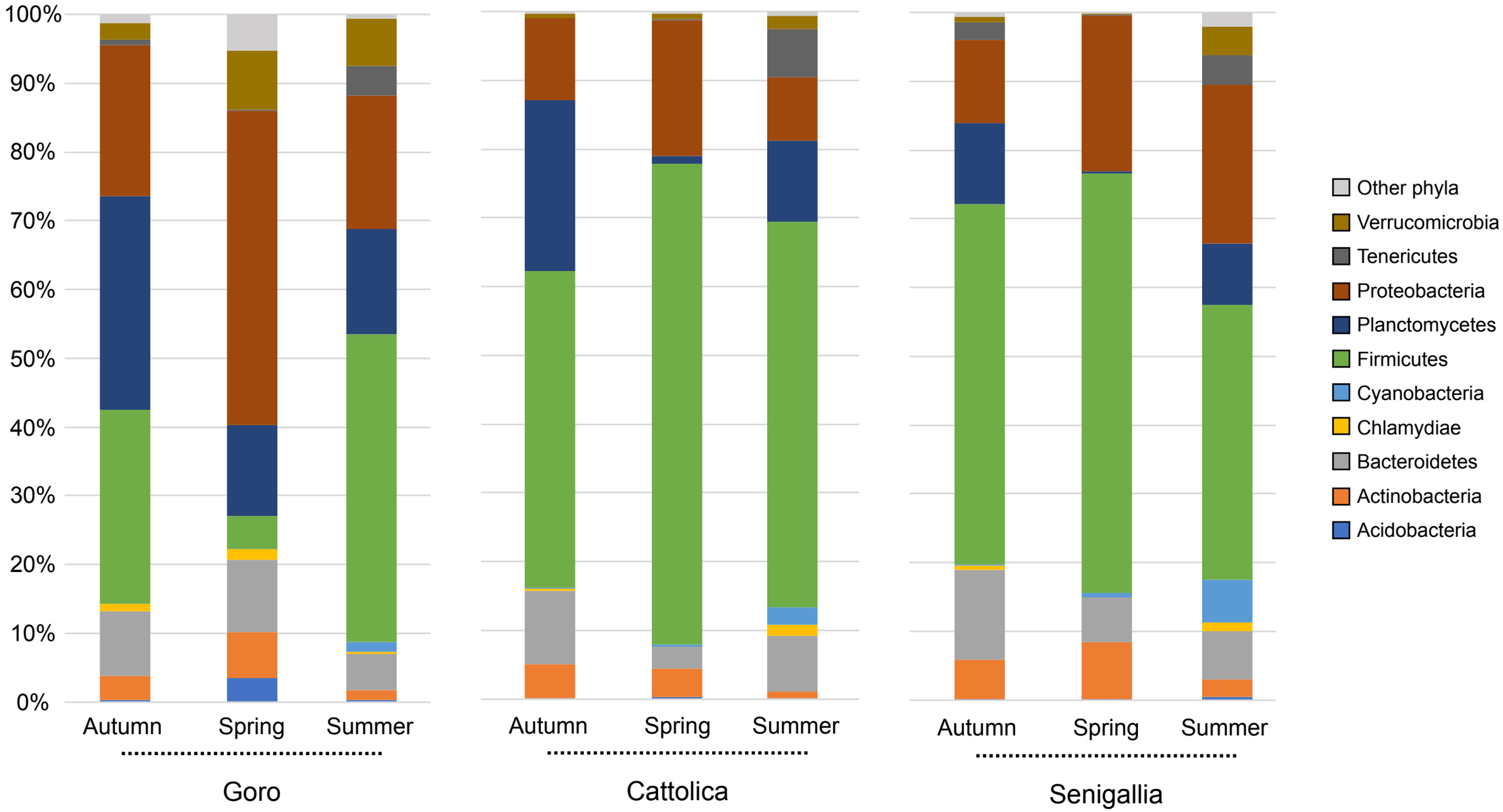
Abbreviations: Go = Goro; Ca = Cattolica; Se = Senigallia.

Supplementary Figure S1 - Transcriptional profiles assessed in mussels sampled at the different farm sites and seasons in the Adriatic Sea. A) Transcriptional profiles of cytoprotective/ detoxification (ABCB, MT10, MT20), antioxidant (CAT, GST, SOD), lysosomal (*HEX, CTSL, GUSB*), metabolic (*AMIL, PK, LYS, MYTLb*), and neuroendocrine-relates (5-HT1, MeER1, MeER2) mRNAs. For each target transcript box plots (median, upper and lower quartiles; N = 21) show transcriptional profiles across the sampling sites and seasons. Different letters indicate statistical differences between sampling seasons within each site ($P < 0.05$, pairwise PERMANOVA comparisons). B) Season-based comparisons (PERMANOVA) among sites. Abbreviations: ABCB, P-glycoprotein; MT10, 10kDa metallothionein; MT20, 20kDa metallothionein; CAT, catalase; GST, glutathione s-transferase; SOD, superoxide dismutase; CTSL, cathepsin L; GUSB, ß-glucuronidase; HEX, hexosaminidase; AMIL, amilase; PK, pyruvate kinase; LYS, lysozyme; MYTLb, mytilin b; 5-HT1, type 1 serotonin receptor; MeER1, type 1 estrogen receptor; MeER2, type 2 estrogen receptor.

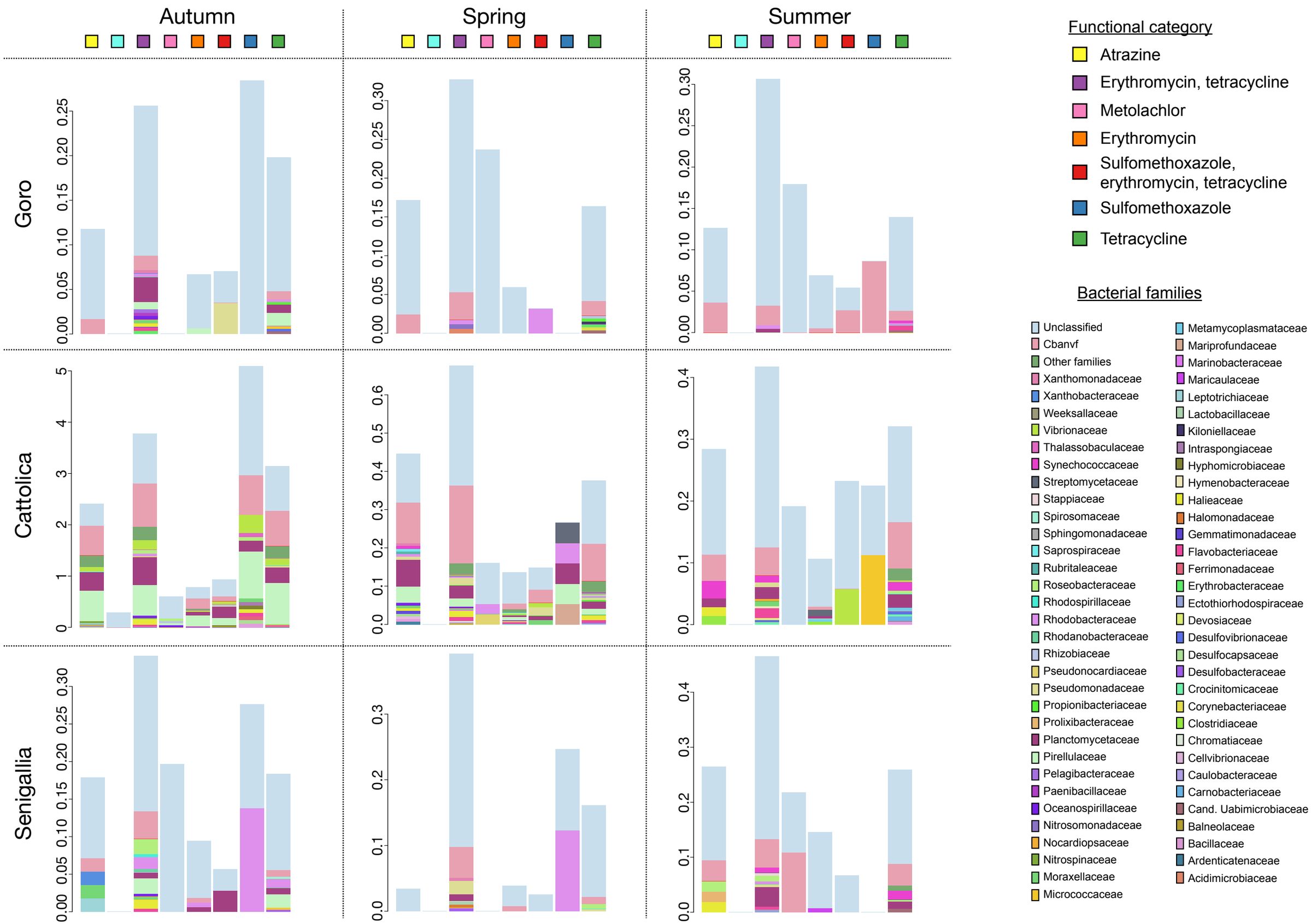


Supplementary Figure S2 - Overall microbiome compositional structure of *M. galloprovincialis* digestive gland and the surrounding seawater. Principal Coordinates Analysis (PCoA) based on weighted UniFrac distances between microbial profiles of mussel digestive gland and seawater samples in all sampling sites (Goro, Cattolica, Senigallia) and seasons (autumn, spring, summer) shows a significant separation between the groups (permutation test with pseudo-F ratio, p-value = 0.001).

Supplementary Figure S3 - Microbiome alpha diversity of *M. galloprovincialis* digestive gland. Box-and-whiskers distribution of alpha diversity metrics, estimated by Faith's Phylogenetic Diversity (Faith PD), Chao1 and number of observed ASVs, of the mussel digestive gland-associated microbiome from different sites (Goro, Cattolica, and Senigallia) and seasons (autumn, spring, and summer). Significant p-values (Wilcoxon rank-sum test controlled for multiple testing using FDR) are reported with the following symbols: p-value ≤ 0.05 *; p-value ≤ 0.01 **, p-value ≤ 0.001 ***. Go = Goro, Ca = Cattolica, Se = Senigallia.



Supplementary Figure S4 - *M. galloprovincialis* digestive gland microbiome composition at phylum level. Bar plots of the average microbiome composition at the phylum level, associated with mussel digestiva gland by sampling site (Goro, Cattolica, and Senigallia) and season (autumn, spring, and summer). Phyla with relative abundance >0.5% in at least 10% of samples are shown.



Supplementary Figure S5 - Taxonomic classification of metagenomics reads assigned to xenobiotic degradation genes. Reads assigned to xenobiotic degradation genes were taxonomically characterized using Kaiju “marDB” database, in order to retrieve the mussels digestiva gland associated microbiome components involved in xenobiotic degradation. For each of the 8 different functional categories of xenobiotic degradation genes, bar plots representing the associated microbiome families are shown. On the x-axis the functional categories of xenobiotic degradation are represented. On the y-axis, the CPM assigned to xenobiotic degradation genes are represented as the sum of the gene belonging to each category on the total functional genes CPM assigned to the respective family. Abbreviations: Cbanvf = cannot be assigned to a non-viral family; Cand = Candidatus.

Supplementary Table S1 - Seawater parameters at the selected sampling sites as monitored by the Regional Agency for Prevention, Environment and Energy of Emilia-Romagna and Marche regions.

Site	Season	SST (°C)	Chl-a (µg/L)	Turbidity (m)	Salinity (psu)
Goro	Autumn	12.9	6.6	0.435	27.5
	Spring	19.0	5.9	0.559	26.2
	Summer	27.0	6.0	0.691	28.1
Cattolica	Autumn	13.8	4.2	0.435	29.4
	Spring	17.5	3.6	0.559	35.0
	Summer	25.7	2.1	0.552	33.7
Senigallia	Autumn	14.1	5.2	0.285	29.7
	Spring	17.3	5.1	0.184	35.2
	Summer	25.6	1.9	0.139	35.9

Supplementary Table S2 - Biometric parameters of mussels sampled at different sites and seasons. SD = standard deviation.

Site	Season		Shell weight (g)	Soft tissue weight (g)	Shell length (cm)
Goro	Autumn	Mean	2.99	5.81	4.96
		SD	0.34	1.17	0.21
	Spring	Mean	6.66	5.87	6.21
		SD	1.69	2.06	0.49
	Summer	Mean	4.07	4.99	5.15
		SD	0.64	0.87	0.32
Cattolica	Autumn	Mean	2.66	5.82	5.50
		SD	2.93	4.17	5.42
	Spring	Mean	8.10	11.89	7.35
		SD	1.53	3.41	0.48
	Summer	Mean	8.02	7.70	6.91
		SD	1.83	2.83	0.58
Senigallia	Autumn	Mean	5.77	3.58	5.50
		SD	5.04	3.75	5.41
	Spring	Mean	7.24	7.51	6.88
		SD	1.03	2.25	0.38
	Summer	Mean	7.33	6.76	6.26
		SD	1.60	1.96	0.46

Supplementary Table S3 - LC-MS experimental conditions.

COMPOUND	RETENTION TIME (min)	TRANSITIONS	COLLISION ENERGY (eV)	CONE (Volts)
Amoxicillin trihydrate	4.28	[365.69] > [349.03]; [365.69] > [208.14]	12	15
Carbamazepine	19.10	[237.12] > [194.05]; [237.12] > [237.12]	20	30
Carbamazepine- $^{13}C_6$	19.06	[243.02] > [200.13]; [243.02] > [243.02]	20	30
Sulfamethoxazole	17.60	[253.63] > [155.99]; [253.63] > [108.46]	12	30
Sulfamethoxazole-(phenyl-$^{13}C_6$)	17.60	[260.05]> [162.06]; [260.05]> [260.05]	12	30
Erythromycin A dehydrate	16.54	[733.84] > [576.35]; [733.84] > [158.20]	15	35
Tetracycline	16.42	[444.59] > [427.38]; [444.59] > [409.99]	17	20
Doxycycline hyclate	17.40	[444.62] > [428.20]; [444.62] > [282.85]	14	20
N,N-Diethyl-meta-toluamide (DEET)	20.13	[192.13] > [119.10]; [192.13] > [91.07]	15	25
Metolachlor	22.48	[284.04] > [252.03]; [253.63] > [155.89]	12	25
Metolachlor-(2-ethyl-6-methylphenyl-d_{11})	22.37	[295.1] > [263.13]; [295.1] > [295.1]	15	25
Alachlor	22.41	[238.32] > [162.13]; [270.12] > [161.35]	15	30
Alachlor-d_{13}	22.48	[251.13]> [175.23]; [251.13] > [251.13]	15	30
Atrazine	20.02	[216.34] > [174.02]; [216.34] > [104.13]	12	30
Atrazine-d_5	19.98	[221.09] > [179.11]; [221.09] > [221.09]	15	30
Atrazine-desethyl-desisopropyl	5.77	[146.03] > [104.13]; [146.03] > [110.08]	18	30

Supplementary Table S4 - Manually curated dataset of bacterial enzymes of xenobiotic degradation and resistance. In the first column, the name of the gene enconding the correspondent enzyme is reported. The second column represents the functional category into which the enzymes were grouped. In the third column, targeted xenobiotics are reported. In the fourth column, the reference database is reported. In the fifth column, the accession number is reported.

Genes	Functional cathegory	Target xenobiotics	Database	Accession n°
AtzE	ATRA	Atrazine	UniProt	Q936X3
atzF	ATRA	Atrazine	UniProt	Q936X2
M446_3816	ATRA	Atrazine	UniProt	B0UET5
BRAO375_2640015	ATRA	Atrazine	UniProt	H0SH23
Rleg_6452	ATRA	Atrazine	UniProt	C6BAU4
trzD	ATRA	Atrazine	UniProt	P0A3V4
Moth_2120	ATRA	Atrazine	UniProt	Q2RGM7
atzD	ATRA	Atrazine	UniProt	P58329
Psed_3188	ATRA	Atrazine	UniProt	F4CUJ4
CAH_RHOSO	ATRA	Atrazine	UniProt	H8ZKW0
blr7281	ATRA	Atrazine	UniProt	Q89E06
pRL100353	ATRA	Atrazine	UniProt	Q1M7F3
BN5_3198	ATRA	Atrazine	UniProt	W6RJ11
AZC_3892	ATRA	Atrazine	UniProt	A8IKD2
atzC	ATRA	Atrazine	UniProt	O52063
atzB	ATRA	Atrazine	UniProt	P95442
atzA	ATRA	Atrazine	UniProt	P72156
dthA	DEET	N,N-diethyl-meta-toluamide	NCBI	ABO15581.1
adeI	MAC	Erythromycin, tetracycline	CARD	ARO:3000780
adeJ	MAC	Erythromycin, tetracycline	CARD	ARO:3000781
adeK	MAC	Erythromycin, tetracycline	CARD	ARO:3000782
adeN	MAC	Erythromycin, tetracycline	CARD	ARO:3000559
carA	MAC	Erythromycin, tetracycline	CARD	ARO:3002817
eatAv	MAC	Erythromycin, tetracycline	CARD	ARO:3003761
evgA	MAC	Erythromycin, tetracycline	CARD	ARO:3000832
evgS	MAC	Erythromycin, tetracycline	CARD	ARO:3000833
H-NS	MAC	Erythromycin, tetracycline	CARD	ARO:3000676
lmrC	MAC	Erythromycin, tetracycline	CARD	ARO:3002881
lmrP	MAC	Erythromycin, tetracycline	CARD	ARO:3003969
lsaA	MAC	Erythromycin, tetracycline	CARD	ARO:3000300
lsaB	MAC	Erythromycin, tetracycline	CARD	ARO:3003111
lsaC	MAC	Erythromycin, tetracycline	CARD	ARO:3003112
lsaE	MAC	Erythromycin, tetracycline	CARD	ARO:3003206
mel	MAC	Erythromycin, tetracycline	CARD	ARO:3000616
MexC	MAC	Erythromycin, tetracycline	CARD	ARO:3000800
MexD	MAC	Erythromycin, tetracycline	CARD	ARO:3000801
MexJ	MAC	Erythromycin, tetracycline	CARD	ARO:3003692
MexK	MAC	Erythromycin, tetracycline	CARD	ARO:3003693
MexL	MAC	Erythromycin, tetracycline	CARD	ARO:3003710
mexP	MAC	Erythromycin, tetracycline	CARD	ARO:3003698
mexQ	MAC	Erythromycin, tetracycline	CARD	ARO:3003699
MexV	MAC	Erythromycin, tetracycline	CARD	ARO:3003030
MexW	MAC	Erythromycin, tetracycline	CARD	ARO:3003031
mexX	MAC	Erythromycin, tetracycline	CARD	ARO:3003034
mexY	MAC	Erythromycin, tetracycline	CARD	ARO:3003033
MexZ	MAC	Erythromycin, tetracycline	CARD	ARO:3003709
msrA	MAC	Erythromycin, tetracycline	CARD	ARO:3000251
msrC	MAC	Erythromycin, tetracycline	CARD	ARO:3002819
msrE	MAC	Erythromycin, tetracycline	CARD	ARO:3003109
MuxA	MAC	Erythromycin, tetracycline	CARD	ARO:3004073
MuxB	MAC	Erythromycin, tetracycline	CARD	ARO:3004074
MuxC	MAC	Erythromycin, tetracycline	CARD	ARO:3004075
oleB	MAC	Erythromycin, tetracycline	CARD	ARO:3003036
OpmB	MAC	Erythromycin, tetracycline	CARD	ARO:3004072
opmE	MAC	Erythromycin, tetracycline	CARD	ARO:3003700
OprA	MAC	Erythromycin, tetracycline	CARD	ARO:3003039
optrA	MAC	Erythromycin, tetracycline	CARD	ARO:3003746
poxtA	MAC	Erythromycin, tetracycline	CARD	ARO:3004470
smeD	MAC	Erythromycin, tetracycline	CARD	ARO:3003055
smeE	MAC	Erythromycin, tetracycline	CARD	ARO:3003056
smeF	MAC	Erythromycin, tetracycline	CARD	ARO:3003057
srmB	MAC	Erythromycin, tetracycline	CARD	ARO:3002828
tlrC	MAC	Erythromycin, tetracycline	CARD	ARO:3002827
TolC	MAC	Erythromycin, tetracycline	CARD	ARO:3000237
tva(A)	MAC	Erythromycin, tetracycline	CARD	ARO:3004730
Type A NfxB	MAC	Erythromycin, tetracycline	CARD	ARO:3004059
Type B NfxB	MAC	Erythromycin, tetracycline	CARD	ARO:3004060
vgaA	MAC	Erythromycin, tetracycline	CARD	ARO:3002829
vgaALC	MAC	Erythromycin, tetracycline	CARD	ARO:3002830
vgaB	MAC	Erythromycin, tetracycline	CARD	ARO:3000118
vgaC	MAC	Erythromycin, tetracycline	CARD	ARO:3002831
vgaD	MAC	Erythromycin, tetracycline	CARD	ARO:3002832
vgaE	MAC	Erythromycin, tetracycline	CARD	ARO:3002833
vmlR	MAC	Erythromycin, tetracycline	CARD	ARO:3004476
ethR	METOL	Metolachlor	NCBI	AIP89953.1
ethA	METOL	Metolachlor	NCBI	QTK22471.1
ethB	METOL	Metolachlor	NCBI	AIP89955.1
ethC	METOL	Metolachlor	NCBI	AIP89956.1
ethD	METOL	Metolachlor	NCBI	AIP89957.1
chrB	MLS	Erythromycin	CARD	ARO:3001302
emtA	MLS	Erythromycin	CARD	ARO:3004669
EreA	MLS	Erythromycin	CARD	ARO:3000361
EreA2	MLS	Erythromycin	CARD	ARO:3002826
EreD	MLS	Erythromycin	CARD	ARO:3004608
Erm(30)	MLS	Erythromycin	CARD	ARO:3001265
Erm(31)	MLS	Erythromycin	CARD	ARO:3000598
erm(32)	MLS	Erythromycin	CARD	ARO:3004592
Erm(36)	MLS	Erythromycin	CARD	ARO:3000605
Erm(37)	MLS	Erythromycin	CARD	ARO:3000392
erm(40)	MLS	Erythromycin	CARD	ARO:3004606
Erm(42)	MLS	Erythromycin	CARD	ARO:3003106
Erm(43)	MLS	Erythromycin	CARD	ARO:3003205
Erm(44)v	MLS	Erythromycin	CARD	ARO:3003971
erm(45)	MLS	Erythromycin	CARD	ARO:3004595
erm(46)	MLS	Erythromycin	CARD	ARO:3004596
Erm(47)	MLS	Erythromycin	CARD	ARO:3003908
Erm(48)	MLS	Erythromycin	CARD	ARO:3004094
Erm(49)	MLS	Erythromycin	CARD	ARO:3004626
Erm(K)	MLS	Erythromycin	CARD	ARO:3004643
Erm(O)-Irm	MLS	Erythromycin	CARD	ARO:3004652
ErmD	MLS	Erythromycin	CARD	ARO:3000495
ErmN	MLS	Erythromycin	CARD	ARO:3000592
ErmO-srmA	MLS	Erythromycin	CARD	ARO:3001303
ErmQ	MLS	Erythromycin	CARD	ARO:3000593
ErmR	MLS	Erythromycin	CARD	ARO:3000594
ErmS	MLS	Erythromycin	CARD	ARO:3001304
ErmT	MLS	Erythromycin	CARD	ARO:3000595
ErmU	MLS	Erythromycin	CARD	ARO:3001305
ErmV	MLS	Erythromycin	CARD	ARO:3002824
ErmW	MLS	Erythromycin	CARD	ARO:3001306
ErmX	MLS	Erythromycin	CARD	ARO:3000596
ErmY	MLS	Erythromycin	CARD	ARO:3002825
ermZ	MLS	Erythromycin	CARD	ARO:3004605
gimA	MLS	Erythromycin	CARD	ARO:3000463
LpeA	MLS	Erythromycin	CARD	ARO:3004099
LpeB	MLS	Erythromycin	CARD	ARO:3004100
macA	MLS	Erythromycin	CARD	ARO:3000533
macB	MLS	Erythromycin	CARD	ARO:3000535
mef(B)	MLS	Erythromycin	CARD	ARO:3003107
Mef(En2)	MLS	Erythromycin	CARD	ARO:3004659
mefC	MLS	Erythromycin	CARD	ARO:3003745
mefE	MLS	Erythromycin	CARD	ARO:3000614
mgtA	MLS	Erythromycin	CARD	ARO:3000462
mphA	MLS	Erythromycin	CARD	ARO:3000316
mphB	MLS	Erythromycin	CARD	ARO:3000318
mphC	MLS	Erythromycin	CARD	ARO:3000319
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mphM	MLS	Erythromycin	CARD	ARO:3003767
mphN	MLS	Erythromycin	CARD	ARO:3004542
mphO	MLS	Erythromycin	CARD	ARO:3004543
myrA	MLS	Erythromycin	CARD	ARO:3001300
oleC	MLS	Erythromycin	CARD	ARO:3003748
oleD	MLS	Erythromycin	CARD	ARO:3000865
oleI	MLS	Erythromycin	CARD	ARO:3000866
RlmA(II)	MLS	Erythromycin	CARD	ARO:3001301
ArmR	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3004056
MexA	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3000377
MexB	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3000378
MexR	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3000506
nalC	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3004051
nalD	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3004051
OprM	SULF	Sulfomethoxazole, erythromycin, tetracycline	CARD	ARO:3000379
sul1	SULFO	Sulfomethoxazole	CARD	ARO:3000410
sul2	SULFO	Sulfomethoxazole	CARD	ARO:3000412
sul3	SULFO	Sulfomethoxazole	CARD	ARO:3000413
sul4	SULFO	Sulfomethoxazole	CARD	ARO:3004361
salA	TET	Tetracycline	CARD	ARO:3003749
acrA_1	TET	Tetracycline	CARD	ARO:3004041
acrA_2	TET	Tetracycline	CARD	ARO:3004042
acrA_3	TET	Tetracycline	CARD	ARO:3004043
acrB	TET	Tetracycline	CARD	ARO:3000216
AcrS	TET	Tetracycline	CARD	ARO:3000656
adeA	TET	Tetracycline	CARD	ARO:3000774
adeB	TET	Tetracycline	CARD	ARO:3000775
adeC	TET	Tetracycline	CARD	ARO:3003811
adeF	TET	Tetracycline	CARD	ARO:3000777
adeG	TET	Tetracycline	CARD	ARO:3000778
adeH	TET	Tetracycline	CARD	ARO:3000779
adeL	TET	Tetracycline	CARD	ARO:3000620
adeR	TET	Tetracycline	CARD	ARO:3000553
adeS	TET	Tetracycline	CARD	ARO:3000549
emrK	TET	Tetracycline	CARD	ARO:3000206
emrY	TET	Tetracycline	CARD	ARO:3000254
hp1181	TET	Tetracycline	CARD	ARO:3003964
LAP-1	TET	Tetracycline	CARD	ARO:3004825
LAP-2	TET	Tetracycline	CARD	ARO:3004826
marA	TET	Tetracycline	CARD	ARO:3000263
mdfA	TET	Tetracycline	CARD	ARO:3001328
mepA	TET	Tetracycline	CARD	ARO:3000026
mepR	TET	Tetracycline	CARD	ARO:3000746
MexG	TET	Tetracycline	CARD	ARO:3000806
MexH	TET	Tetracycline	CARD	ARO:3000807
mgrA	TET	Tetracycline	CARD	ARO:3000815
OpmD	TET	Tetracycline	CARD	ARO:3000809
oqxA	TET	Tetracycline	CARD	ARO:3003922
oqxB	TET	Tetracycline	CARD	ARO:3003923
otr(A)	TET	Tetracycline	CARD	ARO:3002891
otr(B)	TET	Tetracycline	CARD	ARO:3002892
otrC	TET	Tetracycline	CARD	ARO:3002894
ramA	TET	Tetracycline	CARD	ARO:3000823
rpsJ	TET	Tetracycline	CARD	ARO:3003930
sdiA	TET	Tetracycline	CARD	ARO:3000826
tap	TET	Tetracycline	CARD	ARO:3000343
tcr3	TET	Tetracycline	CARD	ARO:3002893
tet(30)	TET	Tetracycline	CARD	ARO:3000561
tet(31)	TET	Tetracycline	CARD	ARO:3000476
tet(33)	TET	Tetracycline	CARD	ARO:3000478
tet(35)	TET	Tetracycline	CARD	ARO:3000481
tet(38)	TET	Tetracycline	CARD	ARO:3000565
tet(39)	TET	Tetracycline	CARD	ARO:3000566
tet(40)	TET	Tetracycline	CARD	ARO:3000567
tet(41)	TET	Tetracycline	CARD	ARO:3000569
tet(42)	TET	Tetracycline	CARD	ARO:3000572
tet(43)	TET	Tetracycline	CARD	ARO:3000573
tet(44)	TET	Tetracycline	CARD	ARO:3000556
tet(45)	TET	Tetracycline	CARD	ARO:3003196
Tet(47)	TET	Tetracycline	CARD	ARO:3004613
tet(48)	TET	Tetracycline	CARD	ARO:3004581
tet(49)	TET	Tetracycline	CARD	ARO:3004582
tet(50)	TET	Tetracycline	CARD	ARO:3004584
tet(51)	TET	Tetracycline	CARD	ARO:3004586
tet(52)	TET	Tetracycline	CARD	ARO:3004587
tet(53)	TET	Tetracycline	CARD	ARO:3004589
tet(54)	TET	Tetracycline	CARD	ARO:3004590
tet(55)	TET	Tetracycline	CARD	ARO:3004591
tet(56)	TET	Tetracycline	CARD	ARO:3004603
tet(57)	TET	Tetracycline	CARD	ARO:3005006
tet(59)	TET	Tetracycline	CARD	ARO:3004441
tet(A)	TET	Tetracycline	CARD	ARO:3000165
tet(B)	TET	Tetracycline	CARD	ARO:3000166
tet(C)	TET	Tetracycline	CARD	ARO:3000167
tet(D)	TET	Tetracycline	CARD	ARO:3000168
tet(E)	TET	Tetracycline	CARD	ARO:3000173
tet(G)	TET	Tetracycline	CARD	ARO:3000174
tet(H)	TET	Tetracycline	CARD	ARO:3000175
tet(J)	TET	Tetracycline	CARD	ARO:3000177
tet(K)	TET	Tetracycline	CARD	ARO:3000178
tet(L)	TET	Tetracycline	CARD	ARO:3000179
tet(V)	TET	Tetracycline	CARD	ARO:3000181
tet(W/N/W)	TET	Tetracycline	CARD	ARO:3004442
Tet(X3)	TET	Tetracycline	CARD	ARO:3004719
Tet(X4)	TET	Tetracycline	CARD	ARO:3004720
tet(Y)	TET	Tetracycline	CARD	ARO:3000182
tet(Z)	TET	Tetracycline	CARD	ARO:3000183
tet32	TET	Tetracycline	CARD	ARO:3000196
tet36	TET	Tetracycline	CARD	ARO:3000197
tet37	TET	Tetracycline	CARD	ARO:3002871
tetA	TET	Tetracycline	CARD	ARO:3000165
tetA(46)	TET	Tetracycline	CARD	ARO:3004032
tetA(58)	TET	Tetracycline	CARD	ARO:3003980
tetA(60)	TET	Tetracycline	CARD	ARO:3004035
tetA(P)	TET	Tetracycline	CARD	ARO:3000180
tetB(46)	TET	Tetracycline	CARD	ARO:3004033
tetB(58)	TET	Tetracycline	CARD	ARO:3003981
tetB(60)	TET	Tetracycline	CARD	ARO:3004036
tetB(P)	TET	Tetracycline	CARD	ARO:3000195
tetM	TET	Tetracycline	CARD	ARO:3000186
tetO	TET	Tetracycline	CARD	ARO:3000190
tetQ	TET	Tetracycline	CARD	ARO:3000191
tetR	TET	Tetracycline	CARD	ARO:3003479
tetS	TET	Tetracycline	CARD	ARO:3000192
tetT	TET	Tetracycline	CARD	ARO:3000193
tetU	TET	Tetracycline	CARD	ARO:3004650
tetW	TET	Tetracycline	CARD	ARO:3000194
tetX	TET	Tetracycline	CARD	ARO:3000205
ykkC	TET	Tetracycline	CARD	ARO:3003063
ykkD	TET	Tetracycline	CARD	ARO:3003064

Supplementary Table S5 - Levels of pharmaceuticals and pesticides measured by LC-MS-based multiresidue analysis. All values are in ng/g of dry weight. DEET = N,N-Diethyl-m-toluamide

Target compound	Goro						Cattolica						Senigallia					
	Autumn		Sping		Summer		Autumn		Spring		Summer		Autumn		Spring		Summer	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sulfamethoxazole	41.4	37.1	39.0	23.1	25.5	20.3	37.5	20.8	33.9	12.0	46.2	36.8	42.6	19.0	44.1	25.6	56.2	45.2
Erythromycin	9.5	3.4	6.5	4.4	15.9	14.6	11.1	4.5	7.4	7.2	10.1	6.6	9.1	6.8	9.6	3.7	4.8	4.9
Tetracycline	33.4	12.0	106.9	140.8	44.6	41.9	29.9	16.4	24.3	27.2	56.8	63.4	34.8	15.7	26.3	16.6	71.8	92.9
DEET	0.005	0.0007	0.004	0.002	0.007	0.001	0.006	0.004	0.006	0.002	0.009	0.0008	0.006	0.003	0.005	0.002	0.007	0.0001
Atrazine	34.7	14.3	12.7	5.9	25.2	17.9	30.7	10.3	18.1	10.5	22.2	12.7	20.6	18.4	19.5	8.6	11.8	4.2
Metolachlor	0.06	0.05	0.04	0.03	0.04	0.03	0.06	0.05	0.04	0.001	0.05	0.03	0.07	0.01	0.04	0.03	0.06	0.02
Carbamazepine	0	0	0	0	0	0	0	0	0	0	1	1	2	4	0	0	0	0
Atrazina-desethyl deisopropyl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alachlor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Doxycycline hyclate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table S6 - Abundance profiles of xenobiotic degradation and resistance enzymes in mussel digestive gland metagenome.
Numerical values corresponding to Fig. 6. In the first column, the name of the gene enconding the correspondent enzyme is reported.

Gene	Cattolica autumn	Cattolica spring	Goro spring	Senigallia autumn	Senigallia spring	Goro autumn	Cattolica summer	Goro summer	Senigallia summer
AtzE	0.17	0.55	0.15	-0.63	-0.54	-0.58	1.26	-0.18	-0.34
atzF	-0.56	-0.35	-0.41	-0.21	-0.54	-0.58	-0.45	-0.55	-0.38
atzC	-0.45	-0.46	-0.41	-0.63	-0.54	-0.25	-0.79	0.69	-0.54
atzA	-0.55	-0.24	0.4	-0.63	0.11	-0.58	0.3	0.15	-0.54
adeJ	-0.47	-0.38	-0.29	0.08	-0.54	-0.33	0.29	-0.4	-0.01
carA	-0.03	-0.43	0.06	-0.17	-0.54	-0.08	-0.41	-0.55	-0.21
eatAv	-0.65	0.74	0.11	-0.13	0.08	-0.31	-0.58	-0.21	0.2
evgA	2.7	1	0.22	1.84	1.69	0.09	-0.28	-0.55	2.2
evgS	0.4	0.4	-0.19	0.44	-0.02	0.56	-0.45	-0.42	-0.38
H-NS	-0.54	-0.32	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
lmrC	-0.26	-0.15	0.06	-0.4	0.01	-0.33	-0.22	-0.25	-0.38
lsaA	0.74	0.03	-0.41	1.15	1.61	0.52	-0.16	-0.21	-0.36
lsaB	-0.64	-0.51	0.12	-0.37	1.63	1.09	-0.16	1.48	-0.16
lsaC	-0.48	-0.57	-0.41	-0.37	-0.54	-0.3	0.26	0.46	-0.16
MexD	-0.05	-0.01	-0.17	-0.63	0.33	-0.31	-0.79	-0.4	-0.54
MexK	-0.38	-0.28	-0.29	-0.38	-0.25	-0.58	-0.68	-0.38	-0.54
mexP	-0.49	-0.59	-0.08	-0.63	-0.54	-0.58	-0.25	-0.55	-0.54
mexQ	0.23	0.39	-0.17	-0.38	-0.39	-0.06	-0.09	-0.4	-0.09
MexW	0.07	0.4	-0.41	-0.5	0.06	-0.31	-0.17	-0.55	-0.54
mexX	-0.57	-0.52	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
mexY	-0.44	-0.69	-0.29	-0.27	-0.54	-0.58	-0.59	-0.55	-0.45
msrA	-0.15	-0.32	0.38	-0.11	0.09	-0.58	-0.15	-0.55	-0.54
msrC	-0.11	1.67	8.47	5.78	2.26	2.75	3	5.85	6.49
msrE	-0.23	0.83	0.38	0.41	2.87	-0.03	0.9	-0.21	0.03
MuxA	-0.51	-0.61	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
MuxB	-0.54	-0.41	-0.41	-0.38	-0.54	-0.58	-0.79	-0.23	-0.45
MuxC	-0.52	-0.58	-0.41	-0.38	-0.54	-0.18	-0.79	-0.55	-0.54
oleB	-0.49	-0.38	-0.41	-0.17	-0.01	-0.58	-0.6	-0.55	-0.22
optrA	0.4	1.44	0.38	1.5	1.32	1.93	3.33	1.23	1.75
poxtA	0.16	0.57	-0.41	0.78	0.03	-0.08	1.13	0.97	1.01
salA	0.85	5.26	1.02	1.94	3.13	0.67	-0.41	0.97	0.32
smeE	-0.51	-0.6	-0.16	-0.63	0.19	-0.45	-0.68	-0.55	-0.37
srmB	-0.41	-0.69	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.38
TolC	-0.52	-0.75	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.36
tva(A)	0.71	1.79	0.1	0.13	1.89	-0.31	0.85	1.09	0.01
vgaA	0.9	-0.18	-0.41	-0.14	-0.54	0.21	-0.59	0.09	0.18
vgaB	0.25	0.82	-0.41	0.29	0.29	-0.08	2.78	1.85	-0.21
vgaC	0.06	-0.18	-0.41	-0.38	-0.54	-0.31	-0.79	-0.55	-0.54
vgaD	-0.27	-0.35	-0.17	-0.38	0.04	-0.06	-0.79	0.41	-0.54
vgaE	0.49	1.01	2.05	2.26	5.57	4.37	2.58	4.51	1.78
vmlR	-0.25	-0.64	-0.41	-0.63	-0.26	-0.08	-0.41	0.35	0.14
ethA	-0.49	-0.46	-0.41	-0.32	-0.54	-0.58	0.47	-0.55	-0.09
ethD	-0.69	-0.75	0.9	0.65	-0.54	-0.58	-0.79	-0.55	-0.54
Erm(30)	-0.57	-0.66	-0.41	-0.63	-0.09	-0.58	-0.79	-0.55	-0.54
erm(32)	-0.58	0.1	0.05	-0.63	-0.54	0.39	-0.79	0.04	1.12
Erm(42)	-0.69	-0.55	-0.41	-0.63	-0.54	-0.13	0.25	0.56	0.7
erm(45)	-0.59	-0.51	-0.41	0.92	-0.54	1.65	0.05	1.48	0.22
Erm(48)	-0.16	-0.75	-0.41	1.45	-0.54	0.54	0.06	-0.55	-0.16
ErmU	-0.36	-0.54	0.05	-0.63	-0.54	-0.58	-0.04	-0.55	0.13
LpeB	-0.46	-0.58	-0.16	-0.38	-0.39	-0.58	-0.68	-0.55	-0.36
macB	1.61	1.01	-0.41	0.36	-0.54	-0.58	0.82	0.74	-0.1

Gene	Cattolica autumn	Cattolica spring	Goro spring	Senigallia autumn	Senigallia spring	Goro autumn	Cattolica summer	Goro summer	Senigallia summer
oleC	0.4	0.54	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
MexA	-0.41	-0.68	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
MexB	-0.35	-0.26	-0.17	-0.51	-0.25	-0.31	-0.49	0.09	-0.54
MexR	-0.69	-0.75	-0.41	-0.63	-0.54	-0.58	0.61	-0.55	-0.54
sul2	-0.58	-0.31	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
sul3	0.23	-0.52	-0.41	-0.63	-0.54	-0.58	0.39	-0.55	-0.54
sul4	0.54	-0.44	-0.41	0.26	1.06	1.33	-0.79	0.02	-0.54
acrA_1	-0.23	-0.75	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
acrA_2	-0.5	-0.52	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
acrB	-0.52	-0.46	-0.29	-0.63	-0.54	-0.06	-0.59	-0.4	-0.09
adeB	-0.49	-0.49	-0.41	-0.38	-0.54	-0.45	-0.79	-0.55	-0.45
adeF	0.25	-0.27	-0.17	-0.63	0.04	-0.33	-0.2	-0.55	-0.19
adeG	-0.38	-0.16	-0.41	-0.63	-0.54	-0.25	-0.54	-0.55	-0.54
adeL	-0.36	-0.39	-0.41	-0.63	-0.54	-0.18	-0.49	-0.55	-0.54
adeR	4.25	3.23	1.67	1.42	0.69	1.08	1.31	2.13	3.62
adeS	-0.01	-0.5	-0.41	-0.63	-0.54	0.17	-0.79	-0.55	-0.03
emrK	-0.52	-0.75	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
hp1181	-0.66	-0.69	0.75	-0.63	-0.54	0.04	0.14	-0.55	-0.33
marA	-0.69	-0.75	-0.41	-0.63	-0.54	-0.58	0.84	-0.55	-0.54
oqxA	-0.43	-0.45	0.25	-0.63	-0.54	-0.23	-0.79	-0.55	-0.54
oqxB	0.19	0.08	-0.04	-0.63	-0.54	-0.2	0.39	-0.4	-0.27
otr(A)	-0.4	-0.57	-0.21	-0.63	-0.54	-0.58	-0.47	-0.3	-0.4
otrC	0.38	0.27	-0.41	0.45	-0.54	0.19	-0.79	-0.55	-0.54
rpsJ	5.75	3.85	-0.41	1.81	2.4	-0.58	3.21	-0.55	1.26
tet(30)	-0.37	-0.45	-0.08	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
tet(35)	-0.34	-0.75	-0.41	-0.63	-0.54	0.16	-0.22	-0.55	-0.54
tet(38)	-0.67	-0.69	0.16	-0.06	0.14	-0.58	0.59	1.29	0.08
tet(39)	0.27	-0.6	-0.41	0.02	-0.54	-0.58	-0.79	-0.55	-0.54
tet(41)	-0.38	-0.29	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.31
tet(42)	-0.66	-0.75	0.19	-0.63	-0.54	-0.58	-0.79	-0.55	1.42
tet(48)	-0.5	1.27	-0.41	0.65	0.22	-0.25	-0.79	1.11	-0.08
tet(K)	-0.66	-0.56	-0.12	-0.08	0.79	-0.58	-0.56	-0.18	0.68
Tet(X4)	-0.47	-0.59	-0.41	-0.63	-0.54	-0.58	-0.25	0.3	0.92
tet(Z)	-0.29	-0.75	-0.41	-0.63	-0.54	-0.58	-0.79	-0.55	-0.54
tet36	-0.43	-0.14	-0.41	-0.63	-0.54	0.06	-0.63	-0.55	-0.54
tetA	1.74	0.94	0.1	0.86	-0.54	0.49	1.44	-0.55	-0.36
tetA(46)	0.16	0.55	1.83	2.45	0.79	2.28	1.37	2.05	0.11
tetA(58)	2.5	0.38	-0.41	0.84	2.13	5.8	0.42	1.38	0
tetA(60)	-0.27	0.44	-0.19	0.68	1.3	0.84	2.25	0.59	0.26
tetA(P)	-0.69	-0.61	0.82	-0.63	-0.54	0.07	0.2	-0.15	-0.09
tetB(46)	1.53	0.65	0.48	0.9	-0.01	2.01	1.9	0.02	1.08
tetB(60)	0.92	0.23	-0.41	1.55	-0.28	0.61	1.01	0.59	-0.06
tetB(P)	0.66	0.4	-0.01	-0.43	-0.31	-0.16	1.44	-0.05	0.03
tetM	-0.57	-0.7	0.39	-0.63	-0.54	-0.15	-0.3	-0.55	-0.25
tetO	-0.43	-0.66	-0.41	-0.43	-0.05	-0.58	-0.79	-0.55	-0.25
tetQ	-0.49	-0.38	-0.41	-0.63	-0.31	-0.16	-0.79	-0.05	-0.54
tetS	-0.69	-0.47	-0.41	0.55	0.88	-0.36	-0.3	0.22	0.63
tetT	0.15	0.17	0.97	1.5	-0.07	0.26	0.97	-0.05	-0.25
tetU	-0.69	0.38	-0.41	-0.63	-0.54	-0.58	0.2	-0.55	-0.54
ykkD	-0.69	-0.46	2.02	-0.63	-0.54	-0.58	0.2	-0.55	-0.54

Supplementary Methods

Standard and sample solutions

The extraction procedure was as follow: 250 mg dry weight (dw) of whole mussel powder were transferred into a centrifuge tube 10 µL of IS mixture and extracted twice with 1 mL of cold ACN:MeOH (50:50 v/v) mixture (10 µL of EDTA 25 mM and 0,25 g of $MgSO_4$ were added). The sample was vortexed for 2 min, cooled for 10 min at -20°C, centrifuged and the supernatant collected. The sample powder pellet underwent a second double extraction with 1 mL of a refrigerated Hexane:Acetone (50:50 v/v) solution, and the addition of 10 µL of EDTA 25 mM. The sample was vortexed for 2 min, cooled for 10 min at -20°C and centrifuged. The supernatants were collected and mixed with the ones obtained with ACN:MeOH (50:50 v/v) mixture. The extracted solution was vacuum dried, the oily residue was re-dissolved in 200 µL of CHAPS 0,6% (m/v) aqueous solution. The sample was vortexed for 1 min and centrifuged, the supernatant was collected. 100 µL of MeOH were added to the remaining residues, the sample was vortexed and centrifuged, the supernatant was collected and joined with the previously collected supernatant. This solution was centrifuged and 100 µL of the filtered (0.45 µm syringe filter) supernatant were injected in the LC-MS system.

LC-MS analysis

Liquid chromatography-mass spectrometry (LC-MS) was performed using a 2690 Alliance system (Waters, Milford, MA, USA) coupled to an ESI source and a triple quadruple mass spectrometer (Quattro-LC, Micromass) in the multiple reaction monitoring (MRM) acquisition mode. The analytical column was an Atlantis T3 (5 µm, 2.1 mm X 150 mm, Waters), the gradient elution was obtained with a mobile phase composed of 0.01% acetic acid in water (A) and 0,01% acetic acid in a solution of methanol and acetonitrile 65:35 (v/v) (B). Gradient elution was as follow: 10% of solvent B were held for 5 min, solvent B to 60% over 7 min, to 80% over 3 min and to 90% over 2 min, these conditions were held for 25 min. Finally mobile phase B was returned to its initial conditions over 10 min. The flow rate was 0.14 mL/min, the column temperature was 20°C and injection volume was of 5 µL.

The MS/MS detection was performed in positive mode (2500 V) and the spectra were acquired in multiple reaction monitoring (MRM) mode. Argon was the collision gas and nitrogen the nebulizer and heater gas. Nitrogen was used as nebulizer gas at 117 L/h flow rate and as desolvation gas at 622 L/h. Ion source block and desolvation temperatures were 120 °C and 180 °C, respectively. Capillary and cone voltages were 2,90 kV and 60 V, respectively.