

Metabarcoding and metabolomics offer complementarity in deciphering marine eukaryotic biofouling community shifts

Running title: **Meta-omics reveal shifts in macrofouling communities**

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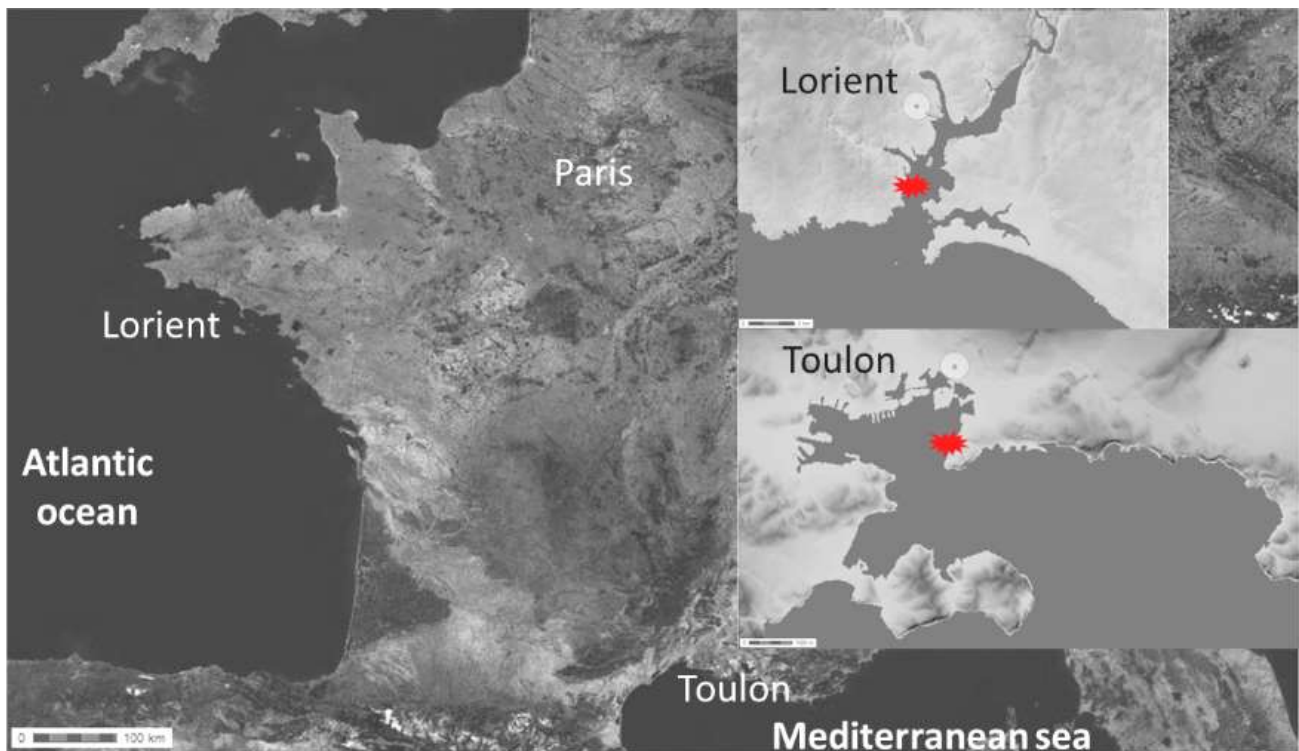
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Supplementary Figure S1:

Location of the two French sampling sites.



Supplementary Table S2:

Biocidal antifouling coatings composition (a) and associated wettability of the coatings (b)

(a)

Components	Nature of components	Weight percent (without biocide)	Weight percent (including biocide)
Néocryl B725	Binder	30.01	28.83
Butyl acetate	Solvent	11.67	13.33
Methoxypropanol	Solvent	23.33	26.67
Disperbyk 180	Dispersing agent	3.00	3.00
Aerosil 200	Thickener	1.00	1.00
Talc 0	Filler	9.66	6.72
CaCO₃	Filler	19.33	13.45
TiO₂	Pigment	2.00	2.00
CuPy or ZnPy or Zineb[®]	Biocide	0	5.00

(b)

θ (°)	Diiodomethane	Glycerol	Water
B725 (M)	47.8 ± 1.0	77.0 ± 0.8	79.0 ± 0.8
B725 + Zineb[®] (I)	48.6 ± 0.7	78.5 ± 0.9	69.4 ± 0.5
B725 + CuPy (C)	47.2 ± 1.4	77.2 ± 1.1	84.2 ± 0.3
B725 + ZnPy (Z)	44.5 ± 1.0	82.8 ± 2.1	87.6 ± 0.7

Supplementary Table S3:

Periods of immersion in 2012 at the four seasons for both sites

	Toulon	Lorient
Winter (Wi)	16 th February – 16 th March	1 st March – 2 nd April
Spring (Sp)	25 th April – 7 th June	2 nd April – 2 nd May
Summer (Su)	22 th June – 18 th July	17 th September – 28 th October
Autumn (Au)	15 th October – 13 th November	19 th November – 18 th December

Supplementary Table S4:

Mean values of temperature, pH, salinity, oxygen, nutrients and metals during the immersion periods at the Toulon and Lorient sites.

*data from technical report due to the dysfunction of the multiparameters probe at Lorient in autumn

	Temperature (°C)		pH		Salinity		O ₂ (%)		Zn	Cd	Pb	Cu	DOC	DIC	TN	Si(OH) ₄	PO ₄ ³⁻	NO ₃ ⁻
	MOY	SD	MOY	SD	MOY	SD	MOY	SD	(nM)	(nM)	(nM)	(nM)	(mg.L ⁻¹)	(mg.L ⁻¹)	(mg.L ⁻¹)	(μM)	(μM)	(μM)
TWi	13.08	0.59	8.03	0.06	38.7	0.07	89	2.6	66	0.11	1.1	24	0.90	27.2	0.05	0.63	0.08	1.7
TSp	16.90	1.31	8.20	0.02	38.1	0.18	104	3.4	73	0.10	1.4	24	0.98	26.7	0.05	0.61	0.04	1.8
TSu	21.95	1.86	7.95	0.04	38.9	0.18	103	4.9	67	0.12	1.7	26	1.01	28.2	0.13	3.0	0.05	1.7
TAu	17.30	1.35	8.61	0.14	37.8	0.93	82	3.1	93	0.13	1.7	23	0.99	30.1	0.14	5.8	0.04	7.5
LWi	10.84	1.07	8.35	0.05	31.0	1.6	117	5.5	74	0.15	0.11	24	1.81	16.2	1.34	48	0.15	122
LSp	11.87	0.50	8.27	0.05	32.3	1.4	104	4.8	73	0.17	0.18	41	2.37	17.9	1.09	17	0.10	54
LSu	15.81	0.93	8.30	0.09	30.6	4.1	95	4.0	86	0.11	0.18	24	2.38	24.3	0.70	32	0.09	21
LAu	11.9*		8.3*		31*		100*		63	0.13	0.24	36	3.22	17.5	2.33	67	0.10	122

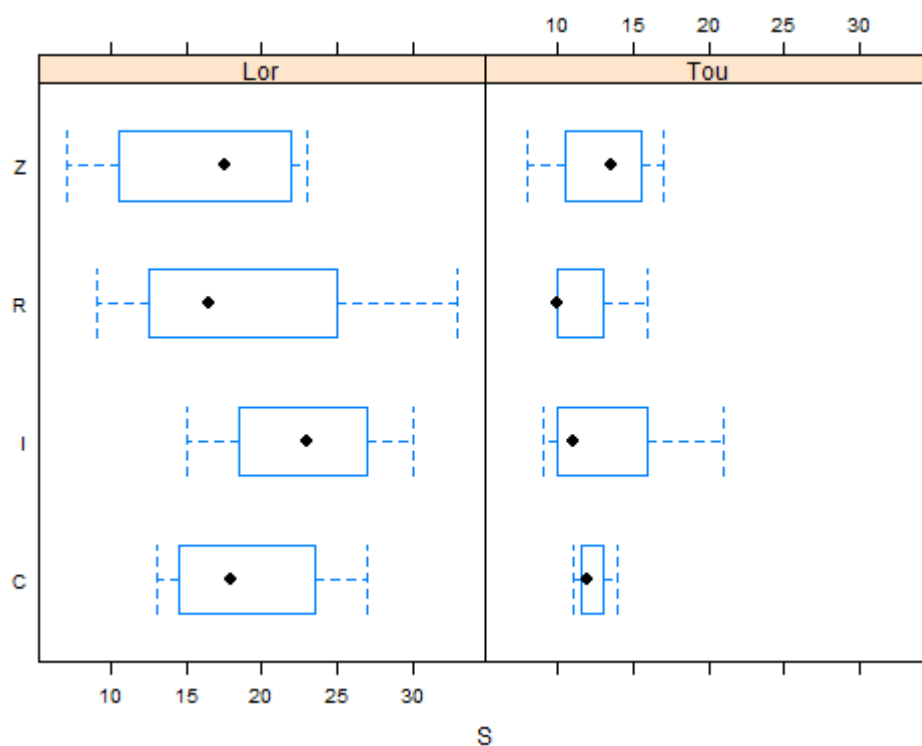
Rarefaction curves plotting the number of reads by the number of Operational Taxonomic Units (OTUs) for each of the samples from Toulon and Lorient locations.



Supplementary Figure S6:

Species richness (S) determined from the settlement panels by metabarcoding in two studied locations (Lorient and Toulon) for each of the applied AF treatments

(T = Toulon / Winter = Wi, Spring = Sp, Summer = Su, Autumn = Au / R = PVC, I = B725 + Zineb, C = B725 + Copper pyrithione, Z = B725 + Zinc pyrithione)



Supplementary Figure S7:

Relative abundance of Operational Taxonomic Units (OTUs) characteristic of the eukaryotic communities at Toulon and Lorient on the reference surface (R) during the four seasons: both non-specific and frequent (i.e., at least at three seasons at both sites) and site specific (i.e. at least at two seasons at one site without any presence at the other) OTUs.

T = Toulon, Winter = Wi, Spring = Sp, Summer = Su, Autumn = Au.

