

## **Functional role of biofouling linked to aquaculture facilities in Mediterranean enclosed locations**

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### **Text S1: Environmental variables**

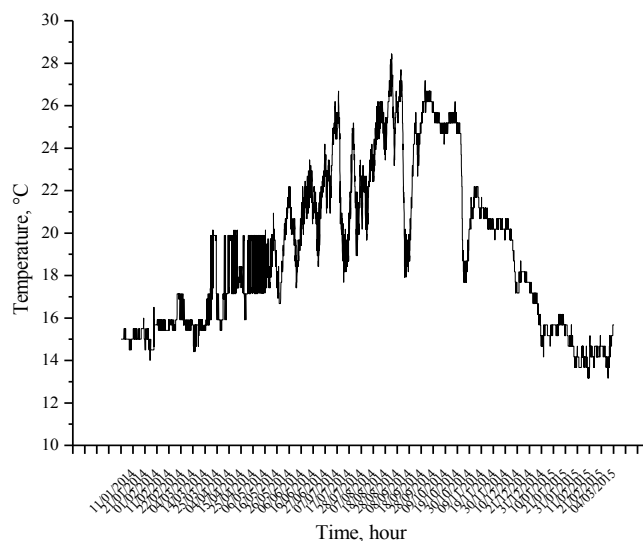
Water temperature, dissolved oxygen concentrations and trophic variables have been monitored throughout the period which this study was carried out. Specifically, temperature and Dissolved Oxygen (DO) were continuously monitored by mean of specific sensors located within the study site and set to record measurements at 1hour intervals; as showed in Figs S1-S2, minimum temperatures were recorded in February 2015 ranging between 12.5 - 13.1°C while the warmest value was 28.43°C which was reached in August 2014. Overall however results showed that temperatures of water masses surrounding cages were almost the same ranging between  $19.74 \pm 3.97$  °C (Fig. S1) and  $19.74 \pm 4.08$  °C (Fig. S2).

On the contrary, mean oxygen concentration slightly differed between the two cages resulting equal to  $7.36 \pm 1.39$  mg l<sup>-1</sup> (Fig. S3) e  $5.98 \pm 2.22$  mg l<sup>-1</sup> (Fig. S4) respectively; however in both locations maximum concentrations were recorded during the winter season with values higher than 9.50 mg l<sup>-1</sup>, while the lowest availability was measured during October-November 2014, when severe decreases of dissolved oxygen availability (about 90% less) occurred.

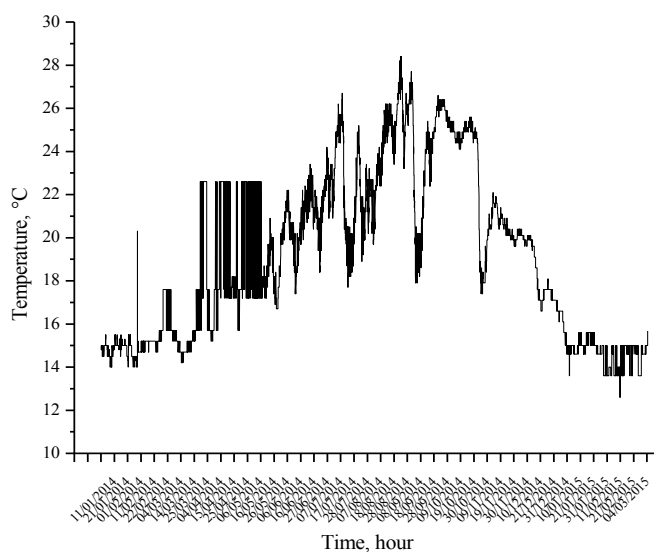
On a seasonal basis starting from the beginning of the present study, water samples have been collected in order to characterize the trophic conditions of the system. To this purpose, chlorophyll-a (CHL-a; µg l<sup>-1</sup>) determinations were carried out following the procedures described by Lorenzen & Jeffrey (1980) and Total Suspended Matter (TSM; mg l<sup>-1</sup>) was determined gravimetrically by desiccation (105°C, 24 h). The organic fraction (OSM; mg l<sup>-1</sup>) was determined by loss on ignition (450°C, 4 h) and the material remaining after combustion is reported as the inorganic suspended fraction (ISM; mg l<sup>-1</sup>).

Analysis of variance (ANOVA; Underwood 1997) was carried out to test the differences in the trophic variables at varying seasons. CHL-a, TSM, OSM and ISM were treated as fixed factors, and three replicates were used for each variable. The assumption of homogeneity of variance was tested a priori by means of the Cochran's test, and when significant differences were observed, the appropriate means were compared using the Student-Newman-Keuls (SNK) tests (Underwood 1997). When no homogeneous variances were rendered with any type of transformation such as in the case of TSM and ISM, the significance level was set at 0.01 instead of 0.05, reducing the possibility of a Type I error (Ruiz et al. 2010, Sarà et al. 2013). The ANOVA was carried out by using the GMAV software (version 5.0) and as showed in Tab S1 and in Figs S5-S8, it returned significant differences for all variables tested. Specifically, CHL-a varied between 0.18 and 0.78 µg l<sup>-1</sup> during the first and the last date of sampling with the only exception represented by the autumn season when the

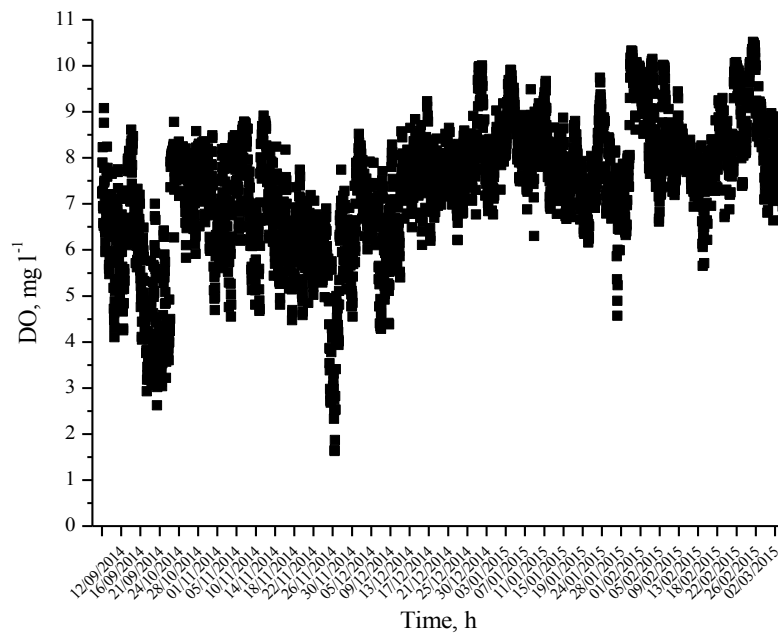
concentration showed a significant increase of over one order of magnitude (Fig. S5). Lastly TSM, OSM and ISM showed very similar patterns with the highest concentrations measured during Spring 2015 and corresponding respectively to  $21.21 \pm 0.001$  (Fig S6),  $3.31 \pm 0.119$  (Fig S7) e  $17.90 \pm 0.248 \text{ mg l}^{-1}$  (Fig S8)



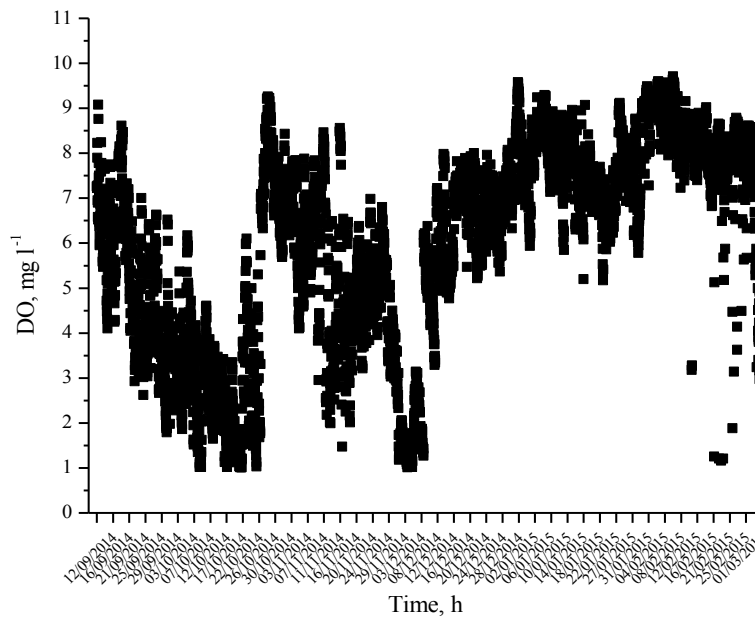
**Fig. S1** – Hourly water temperatures recorded within the harbour (Site 1) throughout the study period



**Fig. S2** – Hourly water temperatures recorded within the harbour (Site 2) throughout the study period



**Fig. S3** – Dissolved Oxygen (DO, mg l<sup>-1</sup>) records measured within the harbour (Site 1) throughout the study period



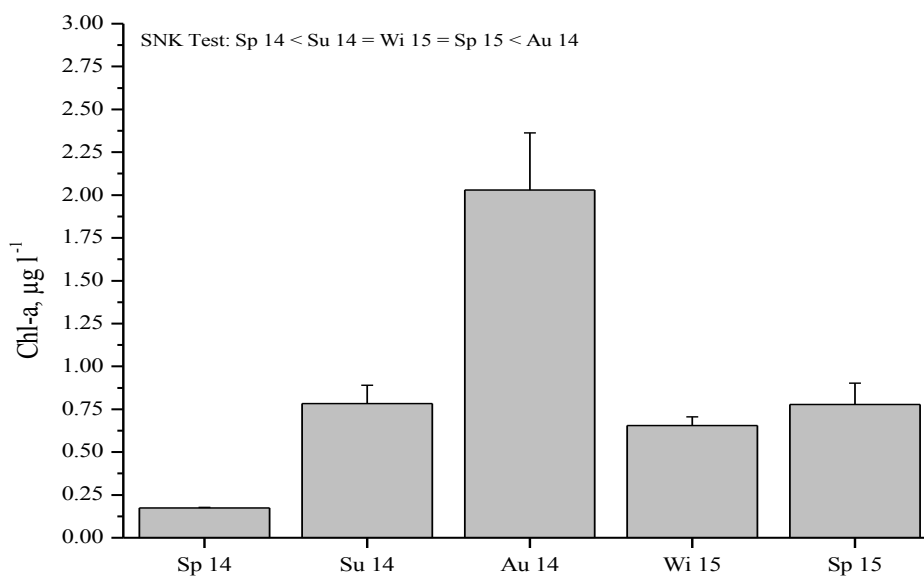
**Fig. S4** – Dissolved Oxygen (DO, mg l<sup>-1</sup>) records measured within the harbour (Site 2) throughout the study period

**Table S1** – ANOVA carried out on trophic variables at varying seasons; CHL-a = chlorophyll-a ( $\mu\text{g l}^{-1}$ ), TSM = Total Suspended Matter ( $\text{mg l}^{-1}$ ), OSM = Organic Suspended Matter ( $\text{mg l}^{-1}$ ), ISM = Inorganic Suspended Matter ( $\text{mg l}^{-1}$ ). SE = season, ns = not significant ( $p > 0.05$ );  $\zeta$  = no transformation and level of significance set to  $P < 0.01$

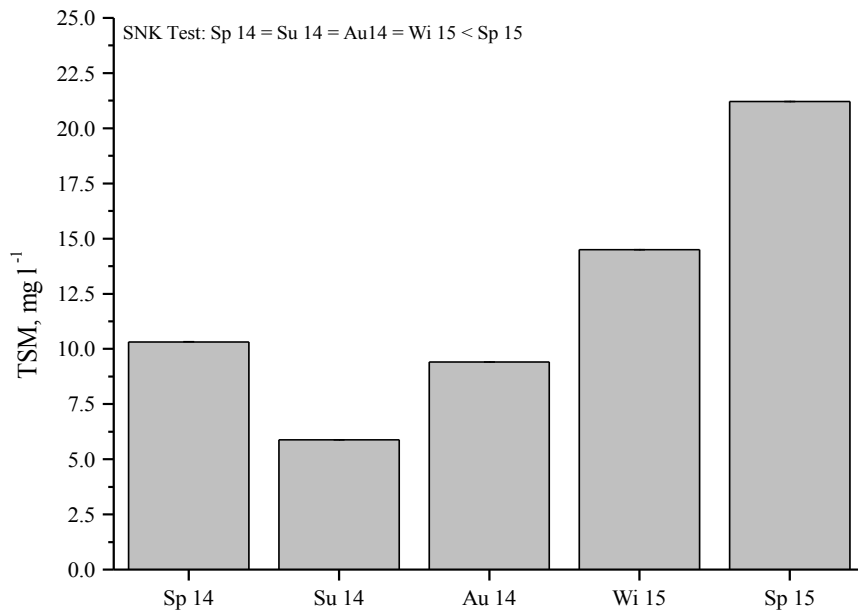
Source	DF	CHL-a			TSM		
		MS	F	P	MS	F	P
Season = SE	4	0.46	104.42	0.0000	137.82	13.69	0.0001
Residuals	15	0			10.07		
Cochran's C				$\text{Ln}(X+1)$			$\zeta$

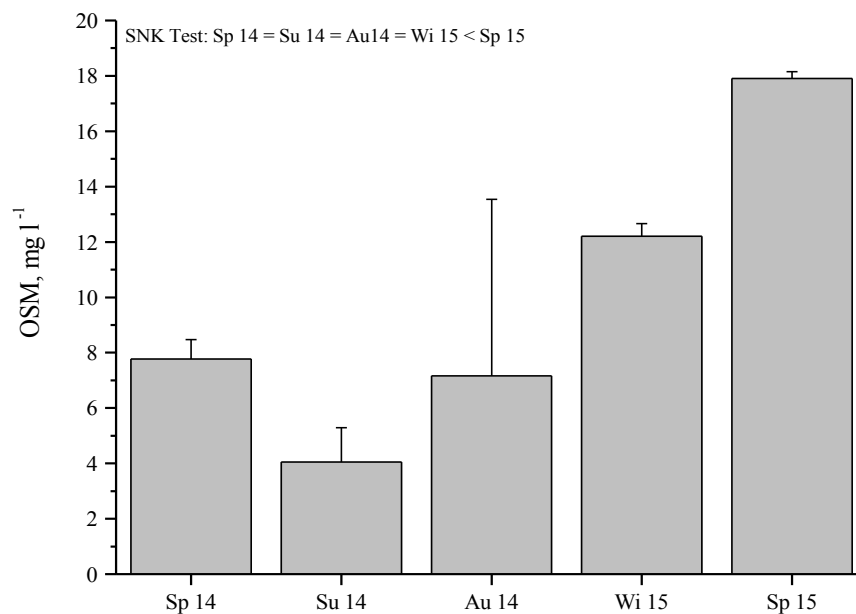
Source	DF	OSM			ISM		
		MS	F	P	MS	F	P
Season = SE	4	1.21	12.59	0.0001	115.52	13.47	0.0001
Residuals	15	0.1			8.58		
Cochran's C				ns			$\zeta$



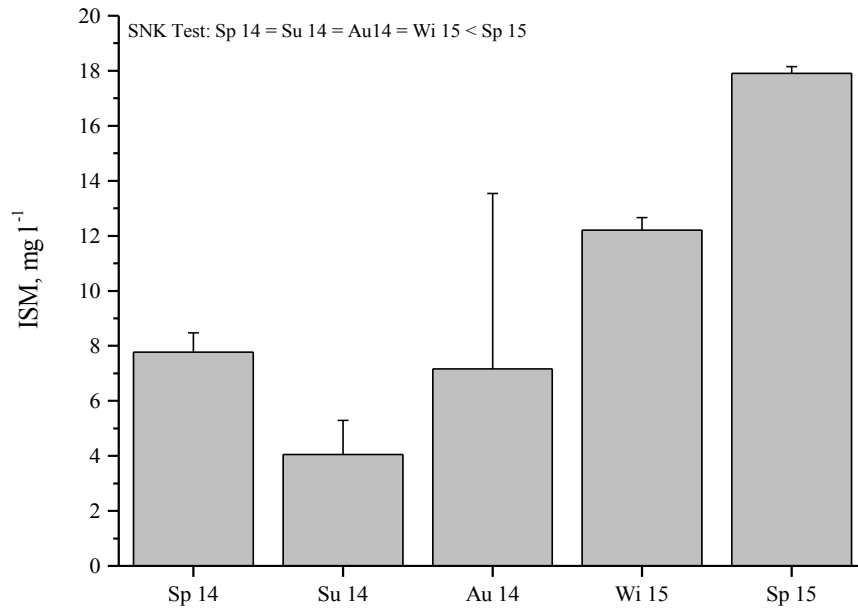
**Fig. S5** – Chlorophyll-a concentrations (CHL-a,  $\mu\text{g l}^{-1}$ ) at varying seasons; Sp 14 = Spring (March) 2014, Su 14 = Summer (June) 2014, Au 14 = Autumn (September) 2014, Wi 15 = Winter (December) 2015, Sp 15 = Spring (March) 2015



**Fig. S6** – Total Suspended Matter (TSM, mg l<sup>-1</sup>) at varying seasons; Sp 14 = Spring (March) 2014, Su 14 = Summer (June) 2014, Au 14 = Autumn (September) 2014, Wi 15 = Winter (December) 2015, Sp 15 = Spring (March)2015



**Fig. S7** - Organic Suspended Matter (OSM, mg l<sup>-1</sup>)at varying seasons; Sp 14 = Spring (March) 2014, Su 14 = Summer (June) 2014, Au 14 = Autumn (September) 2014, Wi 15 = Winter (December) 2015, Sp 15 = Spring (March) 2015



**Fig. S8** – Inorganic Suspended Matter (ISM, mg l<sup>-1</sup>) at varying seasons (ISM, mg l<sup>-1</sup>); Sp 14 = Spring (March) 2014, Su 14 = Summer (June) 2014, Au 14 = Autumn (September) 2014, Wi 15 = Winter (December) 2015, Sp 15 = Spring (March) 2015

### Text S2: Benthic fauna

In Table S2, we listed the animal species found on the panels throughout period during which the study was performed. On the right side are reported the Non-Indigenous Species (NIS) recorded in the Mediterranean region according to 1) the pan-European inventory of NIS provided by the European Alien Species Information Network (EASIN – Katsanevakis et al. 2012), 2) AquaNIS (<http://www.corpi.ku.lt/databases/index.php/aquanis>; accessed on 26-10-2018) and 3) Zenetos et al. (2010). Both platforms and the inventory realized by Zenetos and colleagues enable an easy access to data on NIS and species which might be considered as NIS, *i.e.* cryptogenic species (Olenin et al 2014; Tsiamis et al. 2018).

**Table S2** – List of species found on experimental panels throughout the study period; abbreviations: *ANT* = ANTHOZOA, *ASC* = ASCIDIACEA, *BIV* = BIVALVIA, *DEM* = DEMOSPONGIAE, *GAS* = GASTROPODA, *GYM* = GYMNOLEAEMATA, *HEX* = HEXANAUPLIA, *HYD* = HYDROZOA, *MAL* = MALACOSTRACA, *OPH* = OPHIUROIDEA, *POL* = POLYCHAETA, *PYC* = PYCNOGONIDA; 1 = AQUANIS information system, 2 = EASIN platform, 3 = Zenetos et al 2010

Species	CLASS	NIS
<i>Actinia</i> sp.	<i>ANT</i>	
<i>Anadara inaequalvis</i> Bruguière, 1789	<i>BIV</i>	
<i>Anomia ephippium</i> Linnaeus, 1758	<i>BIV</i>	
<i>Apocorophium acutum</i> (Chevreux, 1908)	<i>MAL</i>	
<i>Balanus trigonus</i> Darwin, 1854	<i>HEX</i>	1,2,3

<i>Bittium (Bittium) reticulatum</i> (da Costa, 1778)	GAS	
<i>Branchiomma bombyx</i> (Dalyell, 1853)	POL	
<i>Caprella equilibra</i> Say, 1818	MAL	
<i>Caprella dilatata</i> Krøyer, 1843	MAL	
<i>Caprella scaura</i> Templeton, 1836	MAL	1,2,3
<i>Caprella</i> sp.	MAL	
<i>Chondrochelia savignyi</i> (Kroyer, 1842)	MAL	
<i>Cryptosula</i> sp.	GYM	
<i>Elasmopus rapax</i> Costa, 1853	MAL	
<i>Erichthonius brasiliensis</i> (Dana, 1853)	MAL	
<i>Erichthonius punctatus</i> (Spence Bate, 1857)	MAL	
<i>Erichthonius</i> sp.	MAL	
<i>Gammaropsis maculata</i> (Johnston, 1828)	MAL	
<i>Halichondria</i> sp.	DEM	
<i>Hydroides dianthus</i> (Verrill, 1873)	POL	1,2,3
<i>Hydroides dirampha</i> Mörch, 1863	POL	1,2,3
<i>Hydroides elegans</i> Haswell, 1883	POL	1,2
<i>Hydroides gracilis</i> (Bush, 1905)	POL	
<i>Hydroides</i> sp.	POL	
<i>Jassa marmorata</i> Holmes, 1905	MAL	2
<i>Jujubinus striatus</i> (Linnaeus, 1758)	GAS	
<i>Lepidonotus clava</i> (Montagu, 1808)	POL	
<i>Leucothoe spinicarpa</i> (Abildgaard, 1789)	MAL	
<i>Microprotopus maculatus</i> Norman, 1867	MAL	
<i>Monocorophium acherusicum</i> (Costa, 1853)	MAL	
<i>Musculus discors</i> (Linnaeus, 1767)	BIV	
<i>Mytilaster minimus</i> (Poli, 1795)	BIV	
<i>Mytilus galloprovincialis</i> Lamarck, 1819	BIV	
<i>Nereis pelagica</i> Linnaeus, 1758	POL	
<i>Nereis zonata</i> Malmgren, 1867	POL	
<i>Ophiothrix fragilis</i> (Abildgaard in O.F. Müller, 1789)	OPH	
<i>Orchomene humilis</i> (Costa, 1853)	MAL	
<i>Perinereis cultrifera</i> (Grube, 1840)	POL	
<i>Phyllodoce mucosa</i> Örsted, 1843	POL	
<i>Pilumnus hirtellus</i> (Linnaeus, 1761)	MAL	
<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)	POL	
<i>Plumularia</i> sp.	HYD	
<i>Pododesmus squama</i> (Gmelin, 1791)	BIV	
<i>Pycnogonida</i>	PYC	
<i>Quadrimaera inaequipes</i> (A. Costa in Hope, 1851)	MAL	
<i>Sertularella</i> sp.	HYD	
<i>Spirobranchus lamarcki</i> (Quatrefages, 1866)	POL	
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)	POL	
<i>Stenothoe tergestina</i> (Nebeski, 1881)	MAL	
<i>Stenothoe valida</i> Dana, 1852	MAL	
<i>Styela plicata</i> (Lesueur, 1823)	ASC	1,2
<i>Syllis gracilis</i> Grube, 1840	POL	
<i>Syllis prolifera</i> Krohn, 1852	POL	
<i>Tanais dulongii</i> (Audouin, 1826)	MAL	

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