Continental Shelf Research
June 2019, Volume 181, Pages 34-49
https://doi.org/10.1016/j.csr.2019.04.014
https://archimer.ifremer.fr/doc/00494/60587/

Impact of wastewater treatment plant discharges on macroalgae and macrofauna assemblages of the intertidal rocky shore in the southeastern Bay of Biscay

Huguenin L. [1, 2, 3, *], Lalanne Y. [3], De Casamajor Marie-Noelle [4], Gorostiaga J.-M. [3], Quintano E. [3], Salerno M. [2], Monperrus M. [1, 2]

[1] CNRS/ UNIV PAU & PAYS ADOUR/ E2S UPPA, Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Materiaux – MIRA, UMR5254, Anglet, 64600, France
[2] UNIV PAU & PAYS ADOUR/ E2S UPPA, UFR Sciences et Techniques de la Côte Basque –1 Allée Parc Montaury, Anglet, 64600, France
[3] Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country UPV/EHU, PO Box 644, Bilbao, 48080, Spain
[4] IFREMER - Laboratoire Environnement Ressources Arcachon (FED 4155 MIRA) - 1 allée du parc Montaury, Anglet, 64600, France

* Corresponding author : L. Huguenin, email address : huguenin.laura@gmail.com

Abstract :

Rocky intertidal habitats are particularly vulnerable to anthropogenic pressures especially in areas with high urban concentrations such as southeastern Bay of Biscay. This research aims to establish an assessment of the potential impact of sewage discharges on intertidal rocky benthic assemblages on macroalgae and on macrofauna as required by the European Directives (Water Framework Directive - WFD and Marine Strategy Framework Directive -MSFD). The assemblages were sampled at five locations according to a control-impact design. A moderate detectable effect of discharges was highlighted on the assemblage structure by means of multivariate analyses but this was less evident using other biological and ecological metrics. Results would also suggest that benthic macroalgae constitute for the study area the best relevant biotic component to assess the effect of this pressure on the intertidal rocky platform habitats. Changes in the relative abundance of Ceramium spp., Corallina spp. and Halopteris scoparia were mainly responsible of the dissimilarities found. Finally, a pseudo-ecological quality ratio, based on the current WFD metrics, was also calculated for each site within locations (i.e. each distance from the outfall) to assess its sensitivity to this type of pressure. Results were conformed with those of the WFD monitoring because un- or less-impacted sites were ranked as “Good” contrary to the others ranked as “Moderate”. Thus, this work provides additional information for the MSFD and bridges deficiencies emphasized by Directives on the response of biological indicators to various pressures and the biocenosis of southeastern Bay of Biscay.

Highlights

► Detectable effects of discharges were highlighted on assemblage structure. ► Macroalgae constituted a relevant biotic component to study impact of WWTP discharges. ► 24 contributors responsible for differences (impacted *vs.* control) were identified. ► The pseudo-EQR ratio was sensitive to the WWTP pressure.

Keywords : bioindicators, impacts, pollution, sewage, Marine Strategy Framework Directive, benthic communities

Introduction

Rocky shore habitats constitute one of the most common environments in coastal areas (Coutinho et al., 2016). The intertidal zone is a very important part of the coastal ecosystem providing many services in terms of primary productivity, fisheries and tourism (Seitz et al., 2013). These areas are governed by particular environmental factors (e.g., hydrodynamics, tides, salinity and temperature gradients) (Ghilardi et al., 2008) but these coastal habitats are very vulnerable to anthropogenic pressures (e.g. waste waters, urban runoff, spilled chemicals, overexploitation, invasive species introduction, habitat fragmentation and destruction) (Becherucci et al., 2016; Crain et al., 2008).

Among those pressures, sewage discharges are responsible for nutrient enrichment, turbidity, increased sedimentation, decreased salinity (Azzurro et al., 2010; Terlizzi et al., 2005) and contamination (by heavy metals, priority and emerging contaminants) (Costanzo et al., 2001; Millennium Ecosystem Assessment -MEA, 2005). In this regard, the European Urban Waste Water Treatment Directive (91/271/EEC) was adopted to protect the water environment from harmful effects of wastewater discharges (urban and industrial). It constitutes a prerequisite for the achievement of the objectives within the Water Framework Directive (WFD; 2000/60/EC; EC, 2000) which aims to attain "good ecological status" of all water bodies by 2020. This obliges politicians to make additional efforts to increase connections between a given population and wastewater systems and to improve the running of sewage treatment plants. Monitoring networks also have to be implemented by scientists and environmental managers to understand benthic communities' response and to distinguish changes caused by anthropogenic impacts from natural variability (Veríssimo et al., 2013). Indeed, sewage discharges constitute an important stressor for marine communities in many intertidal systems around the world (Arévalo et al., 2007; Becherucci et al., 2016; Borowitzka, 1972; Littler and Murray, 1975; Liu et al., 2007; O'Connor, 2013; Vinagre et al., 2016a). Depending on their type, source and level, they may have direct or indirect effects on the environment (Borja et al., 2011). Some studies highlight negative effects such as the alteration of benthic composition and abundance patterns (Guidetti et al., 2003; Terlizzi et al., 2005, 2002). The consequences may be diverse: a biotic homogenization (Amaral et al., 2018) with a simplification of community structure through a decrease in macroalgae species richness and abundance (Borowitzka, 1972; Díez et al., 1999; Littler and Murray, 1975), a decrease of pollution-sensitive species (Scherner et al., 2013), an increase of pollution-tolerant opportunistic species abundance due to their high reproductive capacity (Amaral et al., 2018) and a shift from algal-dominated assemblages to invertebrate-dominated assemblages (Díez et al., 2012). Contaminants released into the environment may also thereafter be accumulated in biological tissues or cause harmful effects such as endocrine disruption, behavioral changes, energy metabolism disturbances and genetic responses (Macdonald et al., 2003). However, other studies did not find an effect of this stressor on species richness of rocky shores (Archambault et al., 2001; O'Connor, 2013).

Over the last decades, large investigations and survey methods have been developed to study benthic communities of intertidal rocky shores (e.g. Huguenin et al., 2018; Le Hir and Hily, 2005; Vinagre et al., 2016b, 2016a; Wells et al., 2007; Zhao et al., 2016) in different contexts such as global climate change prospects (Barange, 2003; Thompson et al., 2002) or ecological status assessment of water bodies (e.g., WFD) (Borja et al., 2013; Guinda et al., 2014). In addition, the study of environmental pollution through biotic diversity analyses has become of major importance because it gives precise information of the deleterious effects of contaminants (Borja et al., 2011). In this context, and as described by Echavarri-Erasun et al. (2007), effects of sewage discharges have already been studied on different environmental compartments (e.g. sediments, water body, trophic web, benthic and pelagic communities). Benthic communities are often used to assess marine pollutions because they reflect both previous and present conditions to which communities have been exposed (Reish, 1987).

Macroalgae constitute the primary food chain producers and the dominant group on rocky shore bottoms (Amaral et al., 2018). Because of their sedentary nature and the sensitivity of their components, they are known to be an accurate bioindicator (e.g., biochemical and physiological) of environmental changes (e.g. water quality of coastal waters for the WFD (Ar Gall et al., 2016; Borja et al., 2013; Gorostiaga and Diez, 1996). Their assessment is fundamental because their modification can also alter the trophic structures of other communities (e.g. grazers, carnivorous, scavengers) (Airoldi et al., 2008; Scherner et al., 2013; Schramm, 1999; Viaroli et al., 2008). Macrofauna has also to be considered, as it is requested by the Marine Strategy Framework Directive (MSFD; 2008/56/CE; EC, 2008). The use of mobile macrofauna as an indicator constitutes a "*snapshot in space and time*" because their community structure respond with short-term variability to environmental changes (Casamajor (de) and Lalanne, 2016; Davidson et al., 2004; Mieszkowska, 2015; Takada, 1999). Moreover, sessile species or slightly mobile species cannot redistribute themselves when faced with disturbances. They are then highly sensitive and constitute the first biological compartment impacted by environmental stressors (Maughan, 2001; Mieszkowska, 2015; Murray et al., 2006; Roberts et al., 1998). So, dispersion patterns of sessile macrofauna constitute more precise descriptors of population dynamics (e.g. recruitment and mortality), community structure, individual performance (e.g. physiology, morphology and behavior changes) in response to environmental changes (Mieszkowska, 2015). However, most studies are focused either on the survey of macroalgae or macrofauna assemblages independently (Anderlini and Wear, 1992; Cabral-Oliveira et al., 2014; Díez et al., 1999; Souza et al., 2013) and rarely together (Bishop et al., 2002; Echavarri-Erasun et al., 2007; Littler and Murray, 1975; López Gappa and Tablado, 1990; O'Connor, 2013; Terlizzi et al., 2002; Vinagre et al., 2016a).

The Basque coast ("Bay of Biscay" subregion) displays a set of environmental specificities: mesotidal conditions, with a magnitude between 1.85 and 3.85 m (Augris et al., 2009), energetic waves (Abadie et al., 2005), freshwater inputs caused by rainfall and a dense river system (Winckel et al., 2004), N-NW dominant winds**,** a specific coast orientation and geomorphology (cliffs, rocky platforms, boulder fields and semi-enclosed bays with sandy beaches) (Borja and Collins, 2004). In the western Basque coast (Spanish side), around 90% of the shore is constituted by rocky substrata (Borja and Collins, 2004) whereas in the eastern (French side) it is only 30% (Chust et al., 2009). All those parameters make this region a heritage area (Augris et al., 2009; Casamajor (de) and Lalanne, 2016) and justify the presence of specific communities in these remarkable habitats (Borja et al., 2004). Thus, rocky platforms constitute a habitat of European Community importance (High energy littoral rock; EUNIS A1-1). But, over the last decades, the French Basque coast has been subjected to urban sprawl and massive summer overcrowding (Le Treut, 2013) which explains the large number of WWTP (Wastewater Treatment Plant) outfalls along the coast.

Studies are scarce and local and are carried out only on the Spanish coastal area on macroalgae (Díez et al., 2013) and macrofauna (Bustamante et al., 2012) independently. This study therefore aims to offer a broader and integrated view on the potential impact of these discharges on intertidal rocky benthic assemblages (macroalgae and macrofauna) in the southeastern Bay of Biscay by comparing control and impacted locations and sites within locations (i.e. different distances from the outfalls). The general hypothesis is that if WWTP treatments are efficient, structural parameters of communities and results based on the WFD monitoring between impacted and control locations should be similar. This work also provides a framework for future monitoring allowing an assessment of benthic communities' changes related to WWTP mitigation measures. The interest in studying both benthic fauna and flora is also discussed in this context.

1. Methodology

1.1 Choice of the sampling design

To evaluate the impact of WWTP discharges, a control-impact design was chosen due to the absence of previous data of benthic assemblages in the impacted locations (before-after design) and models based on data characterizing the study area under reference conditions. This design is widely used to study an impact, a perturbation or a stressor on the environment (Murray et al., 2006) and allows temporal variation to be integrated. Impacted locations (with direct discharges from WWTP) and control locations (natural conditions) were thus chosen. Control locations were selected by expert judgment that is to say with similar features to WWTP locations: wave exposure (N-NW) and slight to moderate slope (<30°).

1.2 Study area and sampling locations

The study was conducted in the southeastern Bay of Biscay. The field sampling campaign took place on intertidal rocky platforms in French and Spanish coastal areas of the Basque coast. The sampling was carried out during spring tide periods and in a relatively short period, from March 2nd to July 27th 2017 (the same as used within the WFD). A total of five locations were selected (Fig. 1). Three locations were potentially impacted by Wastewater Treatment Plants (WWTP): 'WWTP 1' and 'WWTP 2' in France and 'WWTP 3' in Spain. General information of each WWTP were summarized in (Table 1). Two locations were considered as control: 'Control 1' in France and 'Control 2' in Spain.

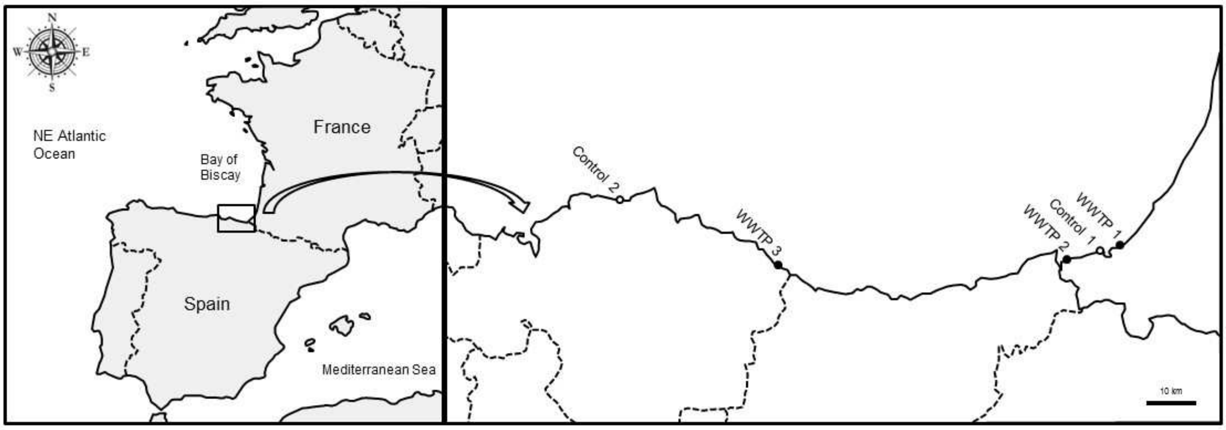

Fig. 1: Study area and locations: 'Control 1' (France) and 'Control 2' (Spain) (white points) and 'WWTP 1' (France), 'WWTP 2' (France) and 'WWTP 3' (Spain) constitute the impacted locations (black points).

Table 1: General WWTP features

	WWTP 1	**WWTP 2**	**WWTP 3**
Location	France	France	Spain
Population equivalent (PE)	78 217	45 000	27 500
Nominal flow (m^3/day)	10 450	7 350	5 930
Outfall location	Intertidal zone	Intertidal zone	Intertidal zone

1.3 Field data collection strategy

Each location was 200 m long and was represented by three sites in order to explore the spatial variability in a lower spatial scale. The selection of sites was done by means of a random stratified sampling design (i.e. sites were placed 100 m from each other and within them, the sampling was done randomly). Within impacted locations, sites corresponded to three distances from the outfall and were positioned on one side of the outfall, the first one being to a maximum of 10 m from the discharge. Sites within controls were established along the location maintaining the mentioned distances (Fig. 2). Within each site, two midlittoral zones were separately sampled: upper and lower midlittoral zones, characterized by algal-dominated communities described in the WFD "*Corallina* spp*. & Caulacanthus ustulatus*" and "*Halopteris scoparia & Gelidium* spp." (Ar Gall et al., 2016). In each site, a set of six randomly selected surfaces (33 x 33 cm quadrats) were positioned on comparable substrata (stable substrate and continuous bedrock) avoiding special microhabitats (crevices and pools) and separated by at least 1 m. The random sampling design ensures independence of errors and allows samples to be considered as replicates (Murray et al., 2006). In each quadrat, the percentage cover of macroalgae and sessile macrofauna (e.g. hexacorallia, mussels, barnacles, ascidiacea, etc.) was visually estimated and the abundance of mobile or slightly mobile macroafauna (e.g. gasteropods, crustaceans or limpets) was counted. This size quadrat is the same as those used for the WFD sampling and allows for direct comparison with others studies (Ar Gall et al., 2016; Casamajor (de) et al., 2016; Huguenin et al., 2018). Most organisms were identified *in situ* at species level to limit the sampling impact. When identification was impossible in the field (especially for small species), specimens were taken to the laboratory for further identification by taxonomic specialists. Due to the complex taxonomy of certain taxa, some organisms were identified at genus level (Huguenin et al., 2018).

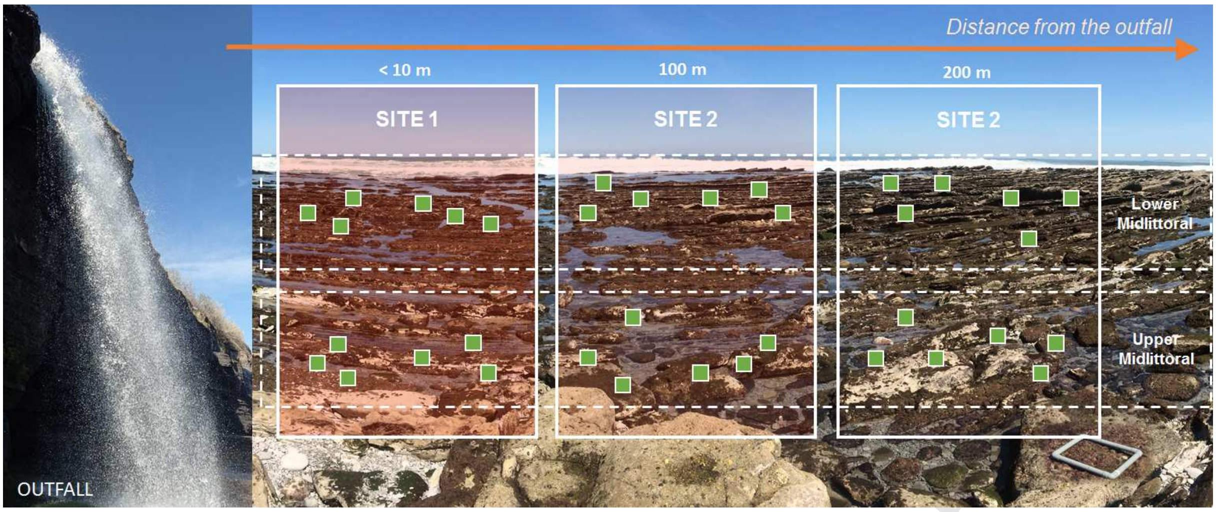

Fig. 2: Schematic layout of the sites in each location and midlittoral zone.

1.4 Statistical analyses

The variation on the species composition and abundance (community structure) was studied by means of PERMANOVA analysis (Permutational multivariate analysis of variance using distance matrices; with 999 permutations) with pairwise post hoc tests (Anderson, 2001) using the standardized data set with an *a priori* chosen significant level of $\alpha = 0.05$. For each midlittoral zone, statistical analyses were carried out separately for both areas (French and Spanish Basque coast), as a consequence of differences in the geomorphology (abrasion platforms *vs.* sloped platforms, respectively) and hydrodynamics (higher in the Spanish side). 'WWTP' within the French area were also studied separately as they have different features (Table 1). Therefore, each of the three 'WWTP' was compared with their corresponding 'Control'. Two factors were considered: (i) location (fixed, 2 levels) and (ii) sites (fixed and nested in location, 3 levels, representing increasing distances from the outfall in the case of impacted locations) with 6 random replicate samples. To avoid problems with unidentified species, analyses were conducted on aggregated data containing mixed taxonomic levels (species, genus, family, class). Data were standardized (e.g. each counting value, for one taxon, was divided by the maximum reached by this taxon in order to avoid differences in sampling units (percentage *vs.* abundance).

In order to explore the structure of benthic assemblages among locations (impacted and control) and within each location (different sites), a non-metric multi-dimensional scaling (nMDS) (after a distance matrix calculation) and a cluster analysis, based on Bray-Curtis dissimilarity, were conducted. These tools are useful in benthic marine community studies as they define the relative (dis)similarity between samples in multidimensional space (in two or more dimensional plots according to a number of reduced dimensions *k* defined by a degree of stress). nMDS does not use the absolute abundances of species in communities, but rather their rank distances (Clarke and Warwick, 2001; Murray et al., 2006)**.** To identify the important contributors to differences among assemblages**,** the SIMilarity PERcentage (SIMPER) analysis was used (Oksanen et al., 2013). It enables the identification of taxa which contribute (according to their abundance) to the dissimilarity between locations and sites within each midlittoral zone.

Apart from the community structure, the mean abundance and the total taxonomic richness were calculated for each ecological group (macroalgae, mobile and sessile macrofauna). The mean taxonomic richness (MTR) was also calculated for each sample for macrofauna, for macroalgae and for characteristic and opportunistic taxa, in order to calculate the characteristic/opportunistic MTR ratio. For the MTR of macrofauna, species were assigned to one of five Ecological Groups (EG I-V) according to their responses to natural and man-induced changes in water quality: the higher the group, the higher the tolerance to pollution (Borja et al., 2000). The classification of macroalgae into characteristic or opportunistic algae was done according to Ar Gall et al. (2016) for French locations and Juanes et al. (2008) for Spanish ones. The spatial variability of the mean taxonomic richness was studied by means of PERMANOVA analysis (Permutation analysis of variance; with 999 permutations) with pairwise post hoc tests (Anderson, 2001) using raw data considering the two factors and the design mentioned above.

The graphs and statistical analyses were undertaken using Excel v7® and R® software.

1.5 Ecological quality

The quality index, achieved using the "intertidal macroalgae" WFD protocol (Casamajor (de) et al., 2016), was calculated for each location to assess its sensitivity to the pressure (Table 2). The WFD protocol was based on the Spanish CFR index (Guinda et al., 2008) and it was firstly adapted to Brittany by Ar Gall and Le Duff (2007). Then, it was adapted to the Basque coast by Casamajor (de) et al.(2010) due to a greater number of warm water species, the absence of large fucoids and a lower number of algal belt on the Basque coast. It constitutes a simplified version of the CCO index (Cover Characteristic - Opportunistic species; Ar Gall et al., 2016). The final rating of the index used in this study (on 1 point) was based on the sum of three subindices: (i) the global cover of macroalgae communities [C] (rated on 0.40 points), (ii) the number of characteristic species [N] (rated on 0.30 points) and (iii) the cover of opportunistic species [O] (rated on 0.30 points) (Casamajor (de) et al., 2016). This quality index was also calculated for each distance from the outfall of impacted location. It was called "pseudo-index" because it was calculated on only 12 quadrats (6 per midlittoral zone) randomly sampled during the campaign, as opposed to 18 (9 per midlittoral zone) in the WFD protocol.

Table 2: Ecological quality according to the CFR index.

Score	Ecological quality
0.80 - 1	*Very good*
0.60 - 0.79	*Good*
0.40 - 0.59	*Moderate*
0.20 - 0.39	*Poor*
0 - 0.19	*Bad*

2. Results

2.1 Effects of WWTP discharges on the structure of intertidal rocky benthic assemblages

Taking into account the whole study area, benthic assemblages differed in relation to coastal stretch (French or Spanish) and locations (WWTP and control) for both midlittoral zones (Fig. 3; Supplementary materials 1).

The analyses showed significant differences between each WWTP and their respective control (PERMANOVA, $p<0.05$; Table 3). Furthermore, the analyses also detected significant variability at a lower scale (sites) in all three cases and for both midlittoral zones (Table 3). Post hoc pairwise comparisons revealed significant differences between almost of all the distances from the outfall within the French impacted locations ('WWTP 1' and 'WWTP 2') in both midlittoral zones (Table 4). In contrast, no such obvious differences were found in the control location ('Control 1') (only differences between sites at the upper level). In the Spanish area the variability among sites was slightly higher (at both midlittoral zones) in the control ('Control 2') than the impacted one ('WWTP 3') (Table 4). Differences at the site level were also supported by nMDS and dendrograms which also showed clear distinctions between them (Supplementary materials 2; Fig. 3).

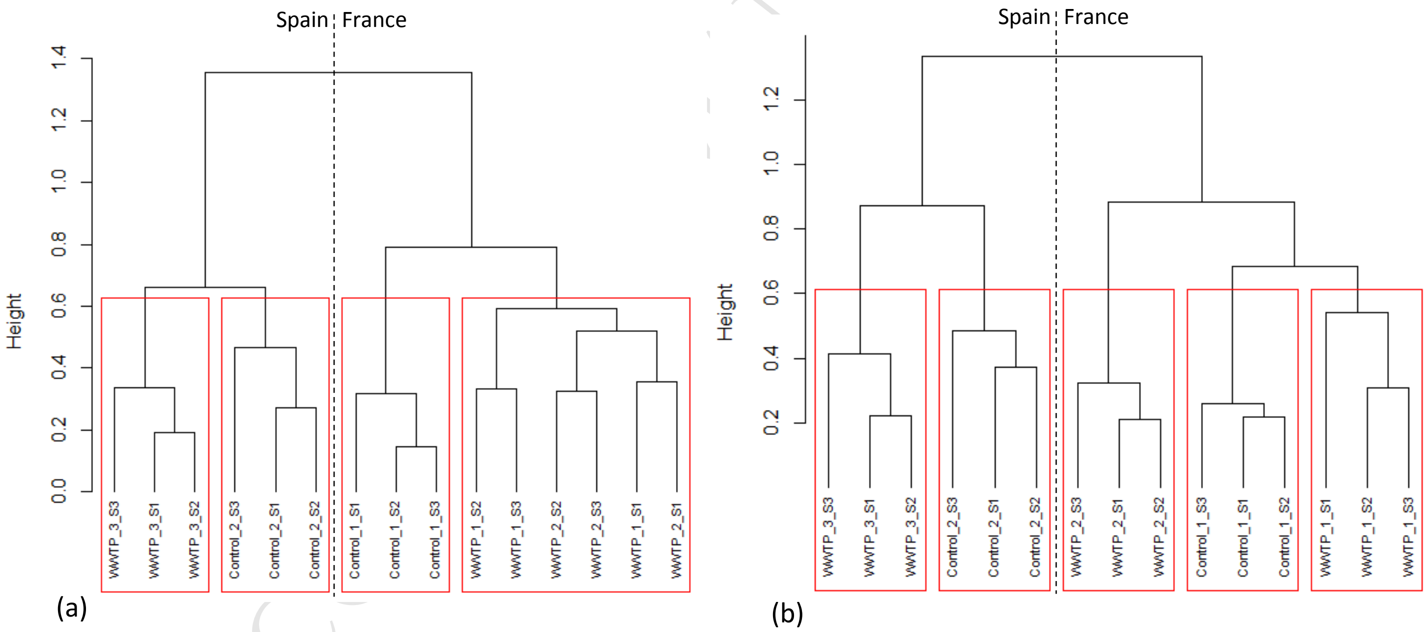

Fig. 3: Cluster analysis dendrograms computed on benthic taxon assemblages (macroalgae and macrofauna) in the upper midlittoral zone (Corallina spp. belt) (a) and the lower midlittoral zone (Halopteris scoparia belt) (b) of French and Spanish impacted ('WWTP 1', 'WWTP 2', 'WWTP 3') and control locations ('Control 1', 'Control 2').

Table 3: Summary of PERMANOVA results testing for effects of presence of sewage discharges on benthic assemblages between impacted and control locations ('WWTP 1'/'Control 1' (a), 'WWTP 2'/'Control 1' (b), 'WWTP 3'/'Control 2' (c)) in both midlittoral zones.

(a)

'WWTP 1'/'Control 1'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	2.93519	27.8767	0.19025	1.00E-04	***
Locations/Sites	4	0.59622	5.6626	0.15458	1.00E-04	***
Residuals	96	0.10529	0.65517			
Lower midlittoral zone						
Locations	1	2.3794	13.3604	0.11347	1.00E-04	***
Locations/Sites	4	0.50673	2.8453	0.09666	1.00E-04	***
Residuals	93	0.17809	0.78986			

(b)

'WWTP 2'/'Control 1'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	4.1775	34.2	0.19055	1.00E-04	***
Locations/Sites	4	0.7719	6.319	0.14084	1.00E-04	***
Residuals	120	0.1221	0.66861			
Lower midlittoral zone						
Locations	1	4.6721	23.5977	0.15924	1.00E-04	***
Locations/Sites	4	0.3758	1.8983	0.05124	0.0011	**
Residuals	117	0.198	0.78952			

(c)

'WWTP 3'/'Control 2'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	1.05628	8.1187	0.12313	1.00E-04	***
Locations/Sites	4	0.31941	2.4551	0.14893	2.00E-04	***
Residuals	48	0.1301	0.72795			
Lower midlittoral zone						
Locations	1	2.41452	17.8223	0.22095	1.00E-04	***
Locations/Sites	4	0.50256	3.7096	0.18396	1.00E-04	***
Residuals	48	0.13548	0.59509			

Table 4: Summary of pairwise post hoc results testing for effects of presence of sewage discharges on benthic assemblages between sites within each location ('WWTP 1' (a), 'WWTP 2' (b), 'WWTP 3' (c), 'Control 1' (d), 'Control 2' (e)) in both midlittoral zones.

(a)

Upper midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	**0.004**
Lower midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	**0.017**

(b)

Upper midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	**0.001**	-
	Site 3	**0.001**	**0.001**
Lower midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	0.479	-
	Site 3	**0.006**	**0.019**

(c)

Upper midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.284	-
	Site 3	**0.042**	0.112
Lower midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.327	-
	Site 3	**0.003**	**0.01**

(d)

Upper midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	0.2967
Lower midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	0.21	-
	Site 3	0.21	0.27

(e)

Upper midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	0.094	-
	Site 3	**0.015**	**0.033**
Lower midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	**0.002**	-
	Site 3	**0.002**	**0.002**

2.2 Identification of contributors to differences in assemblages structure

SIMPER analyses identified, per each midlittoral zone, taxa responsible for differences between impacted and control locations and between sites within each location. Very few macrofauna species/taxa appeared in the analyses, consequently only macroalgae taxa identified as significant contributors were listed in Supplementary materials 3.

In both areas and midlittoral zones, the global dissimilarity between impacted and control locations varied from 42% to 71.14% (Table 5). The highest dissimilarity was obtained between 'WWTP 2' vs. 'Control 1' with 53.81% in the upper zone and 71.14% in the lower zone. The lowest occurred between 'WWTP 3' vs. 'Control 2' with 42% and 52.54%, respectively. At a lower scale, the highest global dissimilarity always appeared between S1 vs. S3 (the furthest sites) within impacted locations. Within control locations, a global dissimilarity between sites was also observed but higher values were either between S1 vs. S2 or between S2 vs. S3.

Table 5: Summary of global dissimilarities between 2 groups from SIMPER analyses (i.e. between impacted and control locations and between sites within each location) for both midlittoral zones (upper and lower).

		Global dissimilarity (%)	
		Upper midlittoral zone	Lower midlittoral zone
WWTP 1' vs. 'Control 1'		**51.12**	**63.58**
WWTP 2' vs. 'Control 1'		**53.81**	**71.14**
'WWTP 1'	S1 vs. S2	43.62	52.00
	S2 vs. S3	39.45	41.41
	S1 vs. S3	52.74	56.96
'WWTP 2'	S1 vs. S2	49.57	56.88
	S2 vs. S3	49.17	55.08
	S1 vs. S3	52.93	57.36
'Control 1'	S1 vs. S2	42.41	56.27
	S2 vs. S3	40.95	61.04
	S1 vs. S3	41.89	57.44
WWTP 3' vs. 'Control 2'		**42.00**	**52.54**
'WWTP 3'	S1 vs. S2	31.16	33.44
	S2 vs. S3	33.08	50.08
	S1 vs. S3	36.56	51.28
'Control 2'	S1 vs. S2	32.65	30.99
	S2 vs. S3	40.26	46.28
	S1 vs. S3	37.47	45.78

In the French area, among species/taxa identified in the upper midlittoral as significant contributors to the dissimilarity between impacted and control locations, only *Ceramium* spp. had a contribution higher than 10%, being more abundant in 'WWTP 1' than in 'Control 1' (2.07 vs. 0.38) (Supplementary materials 3). Furthermore, species such as *Laurencia obtusa* and *Osmundea pinnatifida* were more abundant in 'Control 1' than in 'WWTP 1' and 'WWTP 2', despite their contribution was lower than 10%. Actually, *Osmundea pinnatifida* was not present in 'WWTP 1'. In the lower midlittoral zone, the significant contributors (>10%) to the dissimilarity between impacted and control locations were *Halopteris scoparia* and *Ceramium* spp. (Supplementary materials 3). The former was more abundant in 'Control 1' than in 'WWTP1' (2.62 vs. 1.07) and 'WWTP 2' (2.62 vs. 0.15), whereas the latter showed higher values in 'WWTP 1' comparing to 'Control 1' (2.40 vs. 0.22). With a contribution below 10%, it should be highlighted the absent and the lower abundance of *Cystoseira tamariscifolia* in WWTP 1 and WWTP 2 (average abundance of 0.20), respectively, comparing to Control 1 (average abundance of 0.78). In the Spanish area, only *Corallina* spp. showed a contribution higher than 10% in the upper midlittoral zone (Supplementary materials 3). The abundance of this species was higher in 'WWTP 3' than in 'Control 2' (4.56 vs. 3.06). Furthermore, with a contribution below 10%, *Halopteris scoparia* showed slightly higher abundance in 'Control 2' comparing to 'WWTP 3' (0.50 vs. 0.03). Regarding the lower midlittoral zone, *Chondria coerulescens* was the species that contributed most (9.63%) to the dissimilarity between 'Control 2' and 'WWTP 3', being more abundant in the former location (1.44 vs. 0.03).

Focusing on the upper midlittoral zone and within French 'WWTP' locations, *Caulacanthus ustulatus* was a significant contributor (>10%) in 'WWTP1' (Supplementary materials 3). The abundance of this species was higher in Site 1 (close to the outfall), decreasing towards Site 3. Within 'WWTP 2', *Ceramium* spp., *Corallina* spp. and *Laurencia obtusa* showed contributions higher than 10% (Supplementary materials 3). The former two species showed higher abundances in Site 1, whereas the latter, absent in Site 1, increased from Site 2 to Site 3 (0.39 to 1.67). Within the French control location ('Control 1'), four significant contributors were detected: *Halopteris scoparia*, *Caulacanthus ustulatus*, *Enteromorpha* spp. and *Codium adharens* (Supplementary materials 3). It is remarkable the higher abundance of *Halopteris scoparia* in Site 3 (1.33) comparing to Site 1 (absent), two distant sites according to the cluster (Fig. 3). It is also noticeable the higher abundance of *Caulacanthus ustulatus* and the lower abundance of *Enteromorpha* spp. in Site 2 comparing to Site 1.

Regarding the lower midlittoral zone, four species had a contribution higher than 10% within 'WWTP 1' (Supplementary materials 3). Among them, it should be highlighted the increasing abundance of *Halopeteris scoparia* from Site 1 to Site 3 (away from the outfall). By contrast, *Ceramium* spp showed higher abundance (3.08) in Site 1. Within 'WWTP 2', there was no contributor higher than 10%, but the abundances of *Cystoseira tamariscifolia* and *Halopteris scoparia* were higher in Site 3 (0.56 and 0.28, respectively) (Supplementary materials 3). However, it should be noticed that *Cystoseira tamariscifolia* was also present, with a very low abundance, in Site 1 (the closest from the outfall), whereas it was not detected in Site 2. Within the French control ('Control 1') and for the same tidal level no significant contributor was detected. Looking at sites comparisons, the abundance of

Enteromorpha spp. decreased from Site 1 to Site 3 (from 0.38 to 0.05), whereas *Gelidium* spp. showed higher abundance in Site 3 comparing to Site 2 (Supplementary materials 3).

Regarding the upper midlittoral zone within Spanish 'WWTP 3', *Lithophyllum incrustans* was pointed as a significant contributor with lower abundance in Site 3 comparing to Site 1 (1.25 vs. 2.17) (Supplementary materials 3). By contrast, in the Spanish control location ('Control 2') three species with contributions higher than 10% were detected: *Chondria coerulescens*, *Corallina* spp. and *Halopteris scoparia* (Supplementary materials 3). The former two showed lower abundances in Site 3 comparing to Site 2, whereas the values of *Halopteris scoparia* were higher in Site 3 than in Site 1. In relation to the lower midlittoral zone within 'WWTP3', three significant contributors were recorded. Whilst *Codium adhaerens* increased from Site 1 to site 3, *Ceramium* spp. and *Corallina* spp. were higher in Site 1 (Supplementary materials 3). By contrast, within 'Control 2', four species had contribution higher than 10%. *Halopteris scoparia* showed the higher abundance in Site 2 (1.83), *Corallina* spp. decreased from Site 1 to Site 3 and *Cladostephus spongiosus* and *Codium adhaerens* were more abundant in Site 3 (Supplementary materials 3).

2.3 Effects of WWTP discharges on the diversity of intertidal rocky benthic assemblages

88 species/taxa were identified during the field campaigns: 59 macroalgae (38 Rhodophyta, 12 Ochrophyta and 9 Chlorophyta), 7 sessile and 22 mobile macrofauna (Table 6).

Table 6: List of species/taxa identified into quadrats in control locations ('Control 1' and 'Control 2') and in impacted locations ('WWTP 1', 'WWTP 2' and 'WWTP 3'). Mean abundances (ind./0.1m²) and total mean taxonomic richness for each location are shown. Macroalgae were classed into taxonomic groups (red, brown and green) and functional groups (characteristic, opportunistic) according to Ar Gall et al. (2016) for French locations and Juanes et al. (2008) for Spanish ones. Macroalgae were assigned to one of two Ecological Status Groups (ESG) according to morphological and functional characteristics (Ar Gall et al., 2016; Gaspar et al., 2012; Neto et al., 2012; Orfanidis et al., 2011, 2001;

Vinagre et al., 2016a). ESG I corresponded to late successional or perennial to annual taxa and ESG II to opportunistics or annual taxa. Macrofauna species were assigned to one of five Ecological Groups (EG I–V) according to their responses to natural and man-induced changes in water quality: the higher the group, the higher the tolerance to pollution (Borja et al., 2000). Significance codes: M: Macroalgae; ESG: Ecological Status Groups for macroalgae species; EG: Ecological groups for macrofauna; L = Lower midlittoral zone; U = Upper midlittoral zone. Sampling fluctuations were described by their standard deviation (SD).

			Locations									
			France					Spain				
			Control 1	WWTP 1	WWTP 2			Control 2	WWTP 3			
Species/Taxa	Ecological group	Phylum				Characteristic	Opportunistic			Characteristic	Opportunistic	ESG / EG
Acrosorium spp.	M	Rhodophyta	**0.11** *(SD=0.31)*	**0.02** *(SD=0.13)*	**0.07** *(SD=0.30)*			-	-			II
Ahnfeltiopsis devoniensis	M	Rhodophyta	-	-	**0.06** *(SD=0.31)*			-	-			II
Antithamnionella sp.	M	Rhodophyta	-	-	-			**0.03** *(SD=0.17)*	**0.07** *(SD=0.26)*			
Antithamnion	M	Rhodophyta	-	-	-			-	**0.03** *(SD=0.17)*			
Asparagopsis/Falkenbergia	M	Rhodophyta	**0.33** *(SD=0.57)*	**0.80** *(SD=1.07)*	**1.21** *(SD=1.28)*	✓ (L)		**0.58** *(SD=0.50)*	**0.54** *(SD=0.67)*			II
Bonnemaisonia hamifera	M	Rhodophyta	**0.01** *(SD=0.17)*	-	**0.05** *(SD=0.25)*			-	-			
Caulacanthus ustulatus	M	Rhodophyta	**0.55** *(SD=0.81)*	**0.58** *(SD=1,08)*	**0.29** *(SD=0.64)*	✓ (L+U)		-	**0.06** *(SD=0.23)*	✓ (L+U)		I
Ceramium spp.	M	Rhodophyta	**0.28** *(SD=0.57)*	**2.23** *(SD=0.95)*	**0.75** *(SD=0.90)*		✓ (L+U)	**2.31** *(SD=0.58)*	**2.42** *(SD=1.20)*		✓ (L+U)	II
Champia parvula	M	Rhodophyta	**0.06** *(SD=0.26)*	-	**0.03** *(SD=0.17)*			**0.11** *(SD=0.40)*	**0.06** *(SD=0.23)*			II
Chondracanthus acicularis	M	Rhodophyta	**0.88** *(SD=0.85)*	**0.55** *(SD=0.81)*	**1.23** *(SD=0.93)*	✓ (U)		**0.06** *(SD=0.33)*	-			II
Chondria coerulescens	M	Rhodophyta	**0.33** *(SD=0.59)*	**0.60** *(SD=0.67)*	**1.00** *(SD=0.96)*	✓ (L+U)		**1.19** *(SD=0.75)*	**0.01** *(SD=0.12)*			II
Chylocladia verticillata	M	Rhodophyta	**0.04** *(SD=0.19)*	-	-			-	-			
Corallina spp.	M	Rhodophyta	**1.81** *(SD=0.98)*	**2.30** *(SD=0.77)*	**2.11** *(SD=1.40)*	✓ (L+U)		**3.14** *(SD=1.25)*	**4.17** *(SD=0.95)*	✓ (L+U)		I
Gastroclonium reflexum	M	Rhodophyta	**0.02** *(SD=0.14)*	**0.05** *(SD=0.22)*	**0.20** *(SD=0.49)*			**0.17** *(SD=0.38)*	**0.39** *(SD=0.49)*			II
Gelidium spp.	M	Rhodophyta	**0.28** *(SD=0.62)*	**0.12** *(SD=0.32)*	**1.19** *(SD=1.60)*	✓ (L)		**0.47** *(SD=0.61)*	**0.68** *(SD=0.69)*	✓ (L+U)		I
Gigartina spp.	M	Rhodophyta	**0.01** *(SD=0.12)*	**0.07** *(SD=0.25)*	**0.01** *(SD=0.10)*			-	-	✓ (L+U)		II
Gymnogongrus spp.	M	Rhodophyta	**0.09** *(SD=0.36)*	**0.07** *(SD=0.25)*	**0.22** *(SD=0.48)*			-	-			I
Halopitys incurva	M	Rhodophyta	**0.12** *(SD=0.42)*	-	-			**0.14** *(SD=0.42)*	-			
Cryptopleura ramosa	M	Rhodophyta	**0.01** *(SD=0.08)*	-	-			-	-			
Halurus equisetifolius	M	Rhodophyta	**0.37** *(SD=0.65)*	**0.15** *(SD=0.36)*	**0.10** *(SD=0.36)*	✓ (L)		**0.06** *(SD=0.23)*	-			II
Hypnea musciformis	M	Rhodophyta	**0.52** *(SD=0.97)*	**0.95** *(SD=1.05)*	**0.10** *(SD=0.30)*			-	-			II
Hypoglossum hypoglossoides	M	Rhodophyta	**0.03** *(SD=0.17)*	**0.03** *(SD=0.18)*	**0.07** *(SD=0.30)*			-	-			I
Jania rubens	M	Rhodophyta	**0.17** *(SD=0.48)*	**0.25** *(SD=0.51)*	**0.03** *(SD=0.17)*	✓ (L)		**0.56** *(SD=0.77)*	**0.10** *(SD=0.30)*			I
Laurencia obtusa	M	Rhodophyta	**0.92** *(SD=1.15)*	**0.67** *(SD=1)*	**0.69** *(SD=0.83)*			-	**0.21** *(SD=0.44)*	✓ (L+U)		II
Lithophyllum incrustans	M	Rhodophyta	**0.70** *(SD=0.87)*	**1.05** *(SD=0.75)*	**1.18** *(SD=0.77)*	✓ (L+U)		**2.17** *(SD=0.70)*	**1.64** *(SD=0.77)*			I
Mastocarpus /Petricelis	M	Rhodophyta	**0.22** *(SD=0.45)*	**0.07** *(SD=0.25)*	**0.19** *(SD=0.54)*			-	**0.01** *(SD=0.12)*			I
Mesophyllum lichenoides	M	Rhodophyta	**0.05** *(SD=0.25)*	**0.18** *(SD=0.50)*	**0.58** *(SD=0.84)*			-	-			
Nitophyllum punctatum	M	Rhodophyta	**0.13** *(SD=0.38)*	**0.02** *(SD=0.13)*	**0.29** *(SD=0.53)*			**0.03** *(SD=0.17)*	**0.03** *(SD=0.17)*			II
Ophidocladus spp.	M	Rhodophyta	**0.01** *(SD=0.12)*	-	-			-	**0.03** *(SD=0.17)*			
Osmundea pinnatifida	M	Rhodophyta	**0.32** *(SD=0.67)*	-	**0.03** *(SD=0.17)*			-	**0.14** *(SD=0.39)*			II
Peyssonnelia atropurpurea	M	Rhodophyta	**0.01** *(SD=0.08)*	-	**0.02** *(SD=0.14)*			-	**0.03***(SD=0.17)*			I
Phymatolithon lenormandii	M	Rhodophyta	**0.35** *(SD=0.60)*	**0.03** *(SD=0.18)*	**0.26** *(SD=0.50)*	✓ (U)		**0.03** *(SD=0.17)*	**0.14** *(SD=0.51)*			
Plocamium cartilagineum	M	Rhodophyta	**0.16** *(SD=0.47)*	**0.05** *(SD=0.22)*	**0.70** *(SD=0.96)*			-	-			I
Porphyra spp.	M	Rhodophyta	-	-	-			-	**0.01** *(SD=0.12)*			II
Rhodymenia pseudopalmata	M	Rhodophyta	**0.04** *(SD=0.20)*	-	**0.03** *(SD=0.21)*			-	-			
Scinaia furcellata	M	Rhodophyta	**0.01** *(SD=0.08)*	-	-			-	-			I
Tenarea tortuosa	M	Rhodophyta	-	-	**0.02** *(SD=0.14)*			-	-	✓ (L+U)		
Vertebrata fruticulosa	M	Rhodophyta	-	-	-		✓ (L+U)	**0.03** *(SD=0.17)*	-			II
Cladostephus spongiosus	M	Ochrophyta	-	-	-			**0.56** *(SD=0.88)*	-	✓ (L+U)		I
Colpomenia peregrina	M	Ochrophyta	**0.36** *(SD=0.48)*	**0.22** *(SD=0.42)*	**0.33** *(SD=0.55)*	✓ (L+U)		**0.03** *(SD=0.17)*	**0.58** *(SD=0.50)*			II
Cutleria adspersa	M	Ochrophyta	-	-	**0.11** *(SD=0.34)*			**0.06** *(SD=0.33)*	**0.29** *(SD=0.46)*			
Cutleria multifida	M	Ochrophyta	-	-	**0.01** (SD=0.10)			-	-			
Cystoseira tamariscifolia	M	Ochrophyta	**0.37** *(SD=0.89)*	-	**0.10** *(SD=0.39)*			-	-			I
Dictyota dichotoma	M	Ochrophyta	**0.05** *(SD=0.22)*	**0.02** *(SD=0.13)*	**0.23** *(SD=0.49)*	✓ (L)		-	-			II
Ectocarpales/Ectocarpus	M	Ochrophyta	**0.01** *(SD=12)*	-	**0.01** *(SD=0.10)*		✓ (L+U)	**0.14** *(SD=0.35)*	**0.22** *(SD=0.42)*		✓ (L+U)	II
Ralfsia verrucosa	M	Ochrophyta	-	-	-			**0.03** *(SD=0.17)*	**0.07** *(SD=0.26)*			I
Scytosiphon lomentaria	M	Ochrophyta	-	-	-			-	**0.08** *(SD=0.28)*			
Halopteris scoparia	M	Ochrophyta	**1.41** *(SD=1.66)*	**0.58** *(SD=1)*	**0.08** *(SD=0.39)*	✓ (L)		**0.69** *(SD=0.86)*	**0.01** *(SD=0.12)*	✓ (L+U)		II
Taonia sp.	M	Ochrophyta	**0.04** *(SD=0.20)*	**0.03** *(SD=0.18)*	**0.23** *(SD=0.49)*			**0.03** *(SD=0.17)*	**0.15** *(SD=0.36)*			
Zonardinia typus	M	Ochrophyta	-	-	**0.05** *(SD=0.29)*			-	-			
Chaetomorpha spp.	M	Chlorophyta	-	-	**0.01** *(SD=0.10)*			-	**0.13** *(SD=0.33)*		✓ (L+U)	
Cladophora spp.	M	Chlorophyta	**0.01** *(SD=0.12)*	-	**0.04** *(SD=0.19)*			-	**0.01** *(SD=0.12)*		✓ (L+U)	II
Codium adaerens	M	Chlorophyta	**0.33** *(SD=0.86)*	**0.32** *(SD=0.75)*	**0.30** *(SD=0.76)*	✓ (L)		**0.42** *(SD=0.77)*	**0.57** *(SD=0.85)*			II (spp.)
Codium decorticatum	M	Chlorophyta	-	-	-			-	**0.08** *(SD=0.28)*			II (spp.)
Codium fragile	M	Chlorophyta	-	**0.02** *(SD=0.13)*	-			**0.17** *(SD=0.38)*	**0.08** *(SD=0.28)*			II (spp.)
Derbesia tenuissima	M	Chlorophyta	-	-	**0.01** *(SD=0.10)*			-	-			
Enteromorpha spp.	M	Chlorophyta	**0.64** *(SD=0.83)*	**0.67** *(SD=0.68)*	**0.48** *(SD=0.73)*		✓ (L+U)	**0.53** *(SD=0.61)*	**0.33** *(SD=0.47)*		✓ (L+U)	II
Pterosiphonia spp.	M	Chlorophyta	**0.33** *(SD=0.78)*	**0.02** *(SD=0.13)*	**0.12** *(SD=0.43)*			**0.03** *(SD=0.17)*	-			II (*P. complanata*)
Ulva spp.	M	Chlorophyta	**1.55** *(SD=1)*	**1.47** *(SD=0.60)*	**1.11** *(SD=0.78)*		✓ (L+U)	**0.28** *(SD=0.51)*	**0.39** *(SD=0.52)*		✓ (L+U)	II
Macroalgae mean taxonomic richness			**8.79** *(SD=3.19)*	**8.38** *(SD=2.39)*	**9.90** *(SD=3.22)*			**7.97** *(SD=1.58)*	**7.88** *(SD=2.46)*			
Balanus sp.	SM	Crustacea	-	**0.05** *(SD=0.39)*	-			**1.72** *(SD=3.58)*	**0.53** *(SD=1.14)*			I
Chthamalus spp.	SM	Crustacea	**0.02** *(SD=0.25)*	-	-			**1.33** *(SD=1.82)*	**1.04** *(SD=1.44)*			I
Mytilus spp.	SM	Mollusca	**0.02** *(SD=0.25)*	**0.30** *(SD=0.91)*	**0.22** *(SD=0.79)*			**1.53** *(SD=2.67)*	**0.92** *(SD=2.11)*			III
Rocellaria dubia	SM	Mollusca	-	-	**0.03** *(SD=0.21)*			-	-			I
Serpula sp.	SM	Annelida	-	-	**0.02** *(SD=0.19)*			-	-			I
Spirobranchus spp.	SM	Annelida	-	-	-			-	**0.04** *(SD=0.35)*			II
Porifera	SM	Porifera	-	-	**0.28** (SD=1.56)			-	-			
Sessile macrofauna mean taxonomic richness			**0.01** *(SD=0.12)*	**0.12** *(SD=0.32)*	**0.16** *(SD=0.39)*			**1.06** *(SD=1.24)*	**0.79** *(SD=0.85)*			
Acanthochitona spp.	MM	Mollusca	-	**0.02** *(SD=0.13)*	**0.01** *(SD=0.10)*			-	-			I
Actinia equina	MM	Cnidaria	**0.01** *(SD=0.08)*	-	-			-	-			I
Actinothoe sphyrodeta	MM	Cnidaria	-	-	-			**0.03** *(SD=0.17)*	-			
Anemonia viridis	MM	Cnidaria	**0.01** *(SD=0.12)*	-	-			-	-			
Annelida	MM	Annelida	-	-	**0.02** *(SD=0.14)*			-	-			
Bittium reticulatum	MM	Mollusca	**0.02** *(SD=0.19)*	-	**0.07** *(SD=0.43)*			-	-			I
Cerithium spp.	MM	Mollusca	**0.02** *(SD=0.19)*	-	-			-	-			II
Chiton spp.	MM	Mollusca	**0.01** *(SD=0.08)*	-	**0.01** *(SD=0.10)*			-	-			II
Diodora gibberula	MM	Mollusca	-	-	**0.01** *(SD=0.10)*			-	-			
Eulalia viridis	MM	Annelida	-	**0.07** *(SD=0.25)*	**0.03** *(SD=0.17)*			**0.11** *(SD=0.40)*	**0.90** *(SD=2.46)*			II
Melarhaphe neritoides	MM	Mollusca	-	-	**0.14** *(SD=1.44)*			-	-			II
Ocenebra edwardsii	MM	Mollusca	**0.04** *(SD=0.22)*	-	**0.03** *(SD=0.17)*			-	-			II (sp.)
Pachygrapsus marmoratus	MM	Crustacea	**0.01** *(SD=0.12)*	-	-			-	-			II
Paguridae spp.	MM	Crustacea	**0.13** *(SD=0.58)*	**0.02** *(SD=0.13)*	**0.01** *(SD=0.10)*			-	-			II (*Pagurus sp.*)
Paracentrotus lividus	MM	Echinodermata	-	-	-			-	**0.10** *(SD=0.30)*			I
Patella spp.	MM	Mollusca	**0.12** *(SD=0.68)*	**0.12** *(SD=0.67)*	**0.03** *(SD=0.21)*			**3.08** *(SD=4.44)*	**3.81** *(SD=3.81)*			I
Porcellana platycheles	MM	Crustacea	-	-	-			-	**0.21** *(SD=1.10)*			I
Steromphala cineraria	MM	Mollusca	**0.02** *(SD=0.19)*	-	-			-	-			I
Steromphala pennanti	MM	Mollusca	**0.45** *(SD=1.32)*	**0.03** *(SD=0.18)*	**0.03** *(SD=0.17)*			-	-			I
Steromphala umbillicalis	MM	Mollusca	**0.16** *(SD=0.62)*	-	**0.02** *(SD=0.14)*			-	-			I
Stramonita haemastoma	MM	Mollusca	-	-	**0.01** *(SD=0.10)*			-	-			
Tritia incrassata	MM	Mollusca	-	-	**0.03** *(SD=0.21)*			-	-			II
Mobile macrofauna mean taxonomic richness			**0.52** *(SD=0.88)*	**0.18** *(SD=0.50)*	**0.25** *(SD=0.55)*			**0.67** *(SD=0.63)*	**1.15** *(SD=0.82)*			
Total mean taxonomic richness			**9.29** *(SD=3.26)*	**8.68** *(SD=2.46)*	**10.31** *(SD=3.26)*			**9.69** *(SD=2.29)*	**9.82** *(SD=2.98)*			

Mean taxonomic richness (MTR) per location, site and midlittoral zone showed a clear distinction between macroalgae and macrofauna taxa (Fig. 4, Fig. 5). Generally, macrofauna MTR were associated to low values and high standard deviations with higher values in Spanish part than in French part (Fig. 4, Supplementary materials 4). Regarding fauna MTR and within French part, univariate PERMANOVA analyses did not found significant differences between 'WWTP 1' and 'Control 1' for both midlittoral zones (Fig. 4, Supplementary materials 5). By contrast, 'WWTP 2' showed significantly lower MTR values than 'Control 1' (Fig. 4, Supplementary materials 5). Within the Spanish part, significant differences were found between 'WWTP 3' and 'Control 2' for both midlittoral zones and also at the scale of site (Fig. 4, Supplementary materials 5). Comparing the ecological groups, EG1 showed higher MTR values than EG2 and EG3 in most cases (Fig. 4).

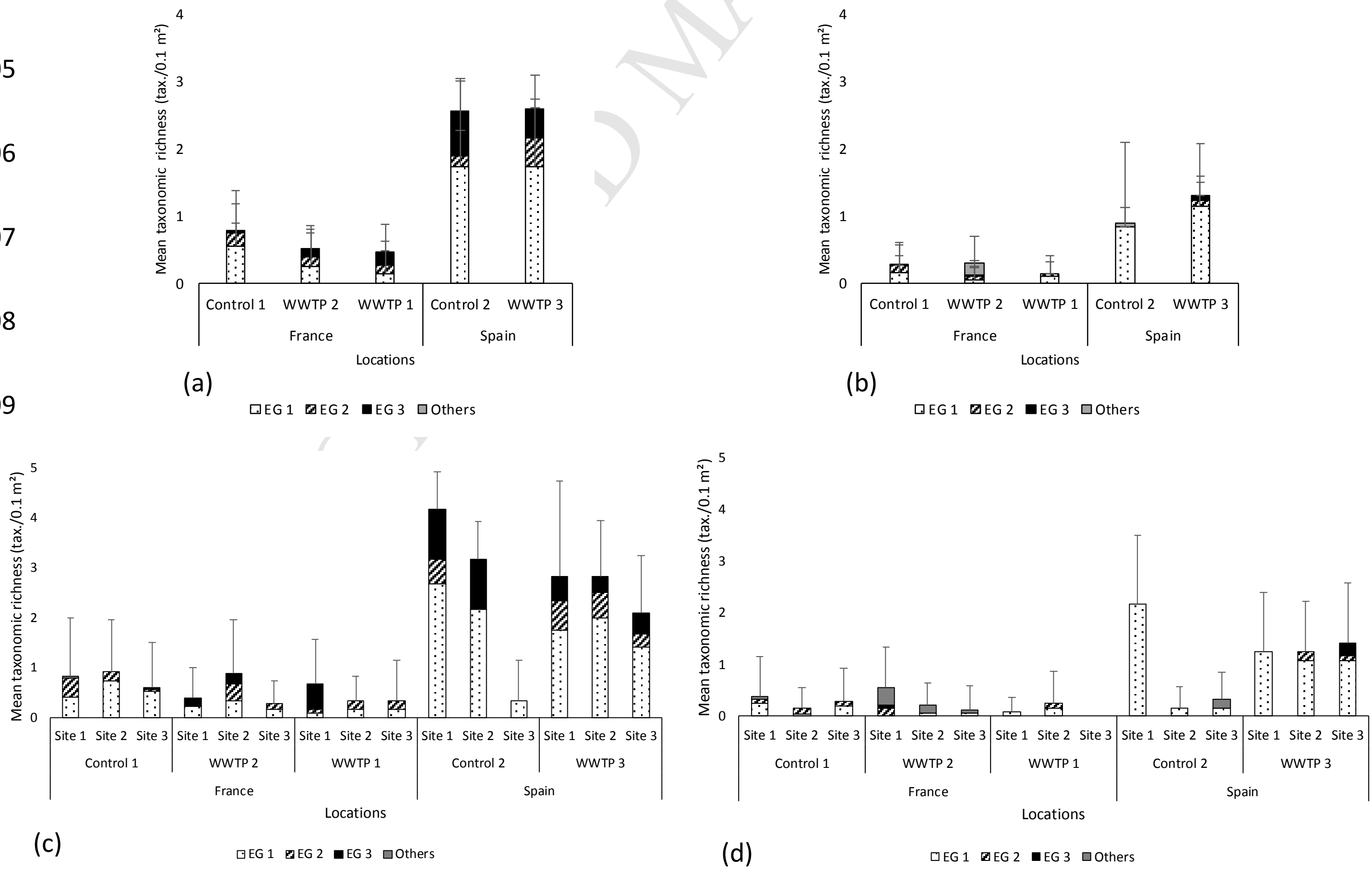

Fig. 4: Mean taxonomic richness of macrofauna in the upper (a, c) and lower midlittoral zones (b, d) for each impacted and control locations and site (i.e. each distance) within locations. Macrofauna species/taxa were *classed into ecological groups (EG1 in white with black points, EG2 hatched, EG3 in black and others in grey).*

In relation to macroalgae MTR, no difference was found between impacted and control in both countries except between 'WWTP 3' and 'Control 2' in the upper zone (Fig. 5; Supplementary materials 6). Within impacted locations, the only significant difference between sites occurred within 'WWTP 1' in the lower zone (Site 1 < Site 2 < Site 3) (PERMANOVA; p-value < 0.05). There were also significant differences within control locations in the lower zone (i.e. between Site 1 and 3 in 'Control 1' and between Site 2 and 3 in 'Control 2') (Supplementary materials 6).

Focusing on the ratio characteristic/opportunistic MTR, there were significant differences between impacted and control locations in both countries and midlittoral zones (except in the upper zone between 'WWTP 3' and 'Control 2') (PERMANOVA; p-value < 0.05; Supplementary materials 7). The ratio was always lower in impacted locations than in control with higher opportunistic MTR in impacted locations (except between 'WWTP 2' and 'Control 1' in the lower zone). This was not so obvious regarding the characteristic MTR. Indeed, it was higher in the control only between 'Control 1' and 'WWTP 1' in both midlittoral zones. At the site scale within impacted locations, there were only significant differences within 'WWTP 2' (i.e. between Site 1 and 3 in both zones and between Site 1 and 2 in the upper zone). In all three cases, the ratio was always lower in Site 1. The characteristic MTR was higher in furthest sites from the outfall (Sites 2 and 3) contrary to the opportunistic MTR which was higher in Site 1 in the upper zone. Within control locations, only 'Control 1' in the upper zone presented significant differences between sites (i.e. Site 1 significantly differed from Site 2 and Site 3).

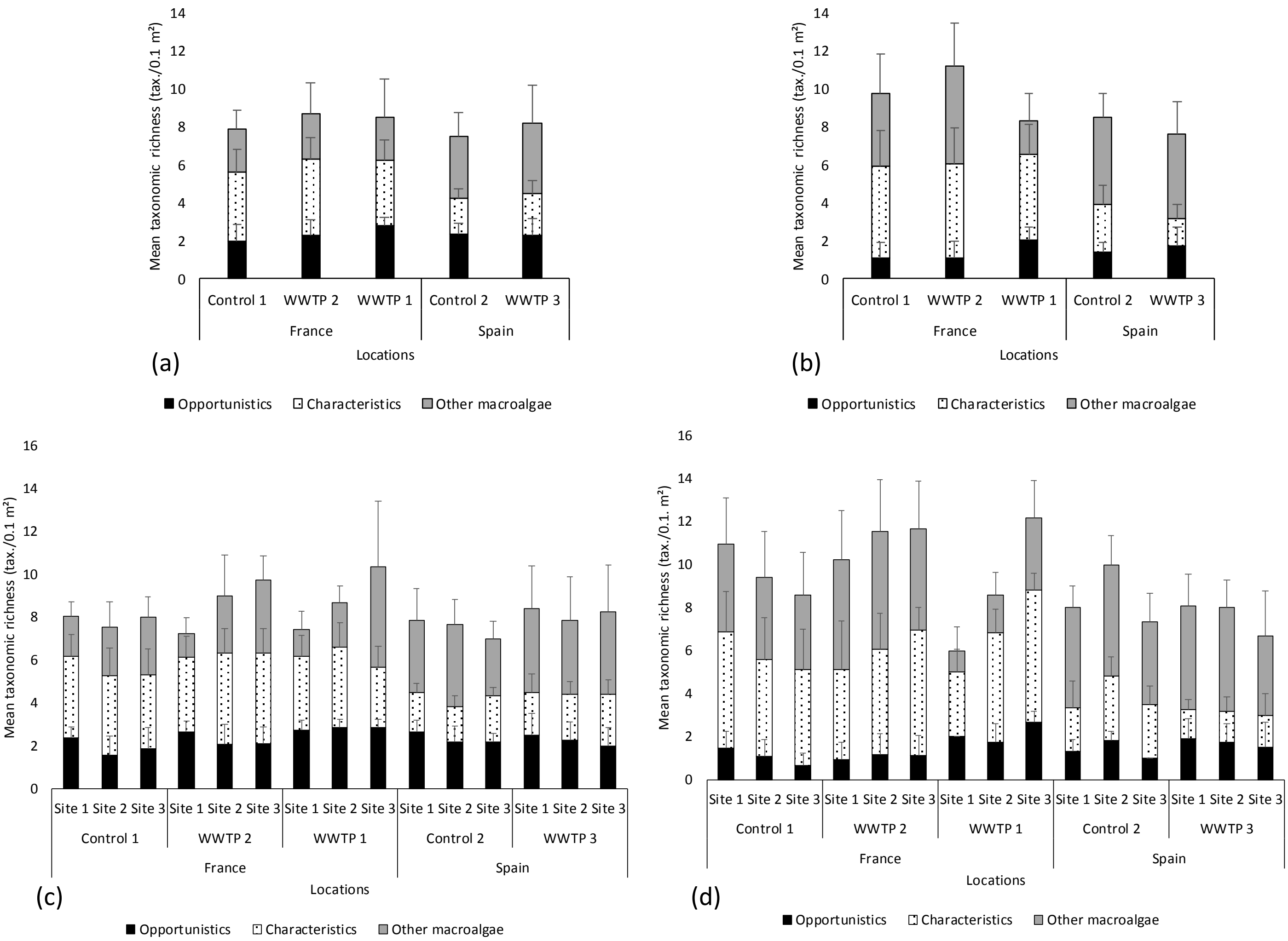

Fig. 5: Mean taxonomic richness of macroalgae of each impacted and control locations (a, b) and of each site (i.e. each distance) within locations (c, d) classed into functional groups (opportunistics in black, characteristics in white with black points and others in grey) according to Ar Gall & Le Duff (2016) for French locations and Juanes (2008) for Spanish ones for (a and c) upper and (b and d) lower midlittoral zones.

2.4 Ecological quality

The quality index was calculated, per location for controls, and per distance from the outfall (i.e. site) for impacted locations (Table 7). In France, sites from all locations were ranked as "*Good*" except the closest site from the outfall in 'WWTP 1'. In Spain, all final scores were ranked as "*Moderate*" in the impacted location and as "*Good*" in the control one.

Table 7: Metrics calculated using the Water Framework Directive (WFD) protocol for each control location and each distance of impacted locations.

		Control 1	WWTP 1			WWTP 2			Control 2	WWTP 3		
	Max. points		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3		Site 1	Site 2	Site 3
Global cover of macroalgae [C]	0.4	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306
Occurrence of characteristic species [N]	0.3	0.15	0.075	0.25	0.2	0.15	0.2	0.25	0.2	0.15	0.1	0.1
Total cover of opportunistic species [O]	0.3	0.2	0.075	0.1	0.15	0.2	0.15	0.2	0.15	0.125	0.15	0.1
Final score	1	0.656	0.456	0.656	0.656	0.656	0.656	0.756	0.656	0.581	0.556	0.506
Ecological quality		**Good**	**Moderate**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Moderate**	**Moderate**	**Moderate**

3. Discussion

The present study aimed to assess the effects of wastewater treatment plant (WWTP) discharges on rocky benthic intertidal assemblages (macroalgae and macroinvertebrates) of the French and Spanish Basque coast (southeastern Bay of Biscay). The results from this research show significant differences in the composition and abundances of taxa, including those sensitive to pollution, between the three studied WWTP and their respective controls for both midlittoral zones (upper and lower). Significant differences in the composition and abundance of assemblages were also found at a lower spatial scale (sites corresponding to the three distances from the outfalls). Regarding mean taxonomic richness, no evident differences were found, especially for macrofauna.

When detecting impacts due to pollution in the marine environment, the study of benthic communities provides several advantages, among which the bioindicator nature of some species (opportunists vs. sensitives) should be highlighted (Díez et al., 2009). In the present study, three macroalgae taxa (*Ceramium* spp., *Corallina* spp. and *Halopteris scoparia*) were identified as significant contributors (Ct (%) > 10) to the dissimilarity between the three WWTP and their respective controls. *Ceramium* spp., a corticated filamentous red alga that includes diverse opportunistic species tolerant to pollution (Díez et al., 1999; Juanes et al., 2008), showed higher abundance in WWTP locations for both midlittoral zones. In the upper midlittoral zone, *Corallina* spp. showed high abundances in both controls and WWTP locations being one of the most frequent macrophyte forming a distinctive belt in the intertidal zone at the southeastern Bay of Biscay (Gorostiaga et al., 2004). Nevertheless, its abundance was higher in WWTP comparing to the controls. This genus is considered as characteristic and is formed by articulated calcareous algae which show certain tolerance to moderated polluted environments (Díez et al., 1999; Díez et al., 2009; Gorostiaga et al., 2004; Mangialajo et al., 2008; Pellizzari et al., 2017). *Halopteris scoparia,* with a terete corticated thallus and considered as a characteristic species, was more abundant in the lower midlittoral of control locations. This species has been already reported in locations with good environmental conditions (Arévalo et al., 2007; Díez et al., 2012, 1999). Therefore, its lower abundance or even its absence (in Spanish WWTP location) could suggest an effect of discharges. Nevertheless, considering this species was also present in French WWTP locations, the impact of the discharges might not be considered as elevated.

Apart from these high contributors, other species appeared to be responsible for the difference between WWTP and control locations. For instance, in the upper midlittoral zone of French area, *Laurencia obtusa* and *Osmundea pinnatifida* were more abundant in the control location than in 'WWTP 1' and 'WWTP 2'. In fact, *Osmundea pinnatifida* was not present in 'WWTP 1'. These rhodophytes are typical intertidal species related to clean waters (Díez et al., 2009). In the lower midlittoral zone of the same area, the leathery species, *Cystoseira tamariscifolia,* also showed higher abundances in the control location comparing to 'WWTP 1' and 'WWTP 2'. It is well known the sensitiveness of the species of the genus *Cystoseira* to anthropogenic impact, and they are thus considered as indicators for good water quality in the European Directive (Duarte et al., 2018; García-Fernández and Bárbara, 2016; Valdazo et al., 2017). However, similar to that described for *Halopteris scoparia* above, *Laurencia obtusa* and *Cystoseira tamariscifolia* were also present (with lower abundances) in WWTP locations and, therefore, the potential impact of WWTP discharges might be considered as moderate.

WWTP and control locations also presented high variability in terms of taxa composition and abundance at the site scale (i.e. distance from the outfall) for both midlittoral zones and for French and Spanish areas. Within French WWTP locations, among the significant contributors (Ct (%) > 10), it should be highlighted the increase in the abundance of the sensitive species (*Halopteris scoparia)* and the decrease of *Caulacanthus ustulathus* from Site 1 (closest to the outfall) to Site 3 (furthest to the outfall) in 'WWTP 1'. The latter red macroalga was described as a more abundant species close the outfall in a study carried out in an inlet on the Basque coast (Díez et al., 2013). In 'WWTP 2', the abundance of *Ceramium* spp. and *Corallina* spp. decreased towards Site 3, whereas *Laurencia obtusa* increased. In this location *Cystoseira tamariscifolia* and *Halopteris scoparia* showed their highest values in Site 3 (the furthest one from the outfall). Within the Spanish WWTP location, similar trends were detected with some sensitive species being more abundant in Site 3 and opportunistic species in Site 1. For instance, *Chondria coerulescens,* a species related to high levels of sedimentation and tolerant to moderate pollution levels (Gorostiaga et al., 2004) decreased towards Site 3. Taking into account these trends of bioindicator macroalgae within WWTP locations, it might be deduced a gradient of the effect of the outfall on benthic intertidal assemblages. However, looking at Sites 1 and 3 within control locations, separated 200 m but in the absence of any gradient, results were somewhat similar. In this regard, sensitive species, such as *Cystoseira tamariscifolia* or *Halopteris scoparia,* dominated in Site 3, whereas opportunistic taxa, such as *Ceramium* spp and *Enteromorpha* spp., were more abundant in Site 1. Chlorophytes like the genus *Enteromorpha* are also common in non-polluted areas and their higher presence could be explained by the effect of other factors such as sediments accumulation (Littler et al., 1983) or grazing pressure (Hay, 1981). In relation to this taxa composition and abundance approach, it should be highlighted that some species were aggregated for the analysis at the genus level. This fact might have supposed a decrease of the bioindicator nature of some species. For example, two species from the same genus may have different sensitivity (e.g. *Gelidium pusillum* less sensitive to pollution than other species from this same genus such as *Gelidium corneum*) (Díez et al., 1999).

An environmental stress such as eutrophication or anthropogenic disturbances can result in a loss of richness (Amaral et al., 2018; Simboura and Zenetos, 2002). Therefore, the mean taxonomic richness (MTR) was assessed to detect changes caused by WWTP discharges because this metric could be also used as a criterion of ecological quality (Amaral et al., 2018; Simboura and Zenetos, 2002; Wells et al., 2007). However, using the macrofauna MTR, no detectable effect of WWTP discharges was highlighted due to very low values (<1 in France and <5 in Spain) and high variability compared to macroalgae (Fig. 4; Fig. 5) for which rocky platforms constitute a suitable habitat for their colonization (Guinda et al., 2014). Macrofauna settlement was not as favorable because the lack of canopy-forming macroalgae (Díez et al., 2014), the uniform geomorphology, high exposure to a strong hydrodynamic regime (Abadie et al., 2005) and the competitive advantage of the macroalgae in the lower levels of the intertidal zone (especially in the case of the caespitose vegetation). Therefore, it was only possible to highlight general trends, such as a higher macrofauna MTR in the Spanish side and in the upper midlittoral zone. Furthermore, results of macrofauna patterns would be probably quite different if outfalls were located in an intertidal boulder field providing hiding places for high macrofauna diversity (Bernard, 2012; Huguenin et al., 2018).

Macroalgae MTR appeared not to be really affected by discharges at the location scale. Indeed, no difference was highlighted between impacted and control locations (except in the upper zone in the Spanish side). However, the ratio between characteristic and opportunistic taxa was significantly affected in the three WWTP locations at both levels (one exception was the upper level of 'WWTP 3'). Between sites within impacted locations (i.e. between the three distances from the outfall), the only one significant MTR increase (from Site 1 to 3) was in 'WWTP 1' in the lower zone.

Thus, similarly to other works (Simboura and Zenetos, 2002; Vinagre et al., 2016a), our results show the difficulty to make accurate predictions of the effect of WWTP discharges on the MTR (especially on macrofauna). By contrast, multivariate analysis appeared as more appropriate because it allows to integrate all benthic assemblages (i.e. species composition and abundance of macroalgae as well as macrofauna). This may also be explained by the fact that the MTR does not consider the relative abundance of the species neither other relevant traits of the taxa (life cycle and morphology). Thus, only strong impacts could potentially influence the MTR. Some authors had already mentioned that such metrics are not universally relevant to study the effect of this type of disturbance (Harper and Hawksworth, 1994; Magurran, 2004) and that they could be often affected by sampling effort (Clarke and Warwick, 2001b). Average cover parameter could be thus probably more useful to detect impacts.

In this study, macroalgae and macrofauna communities were considered to assess potential effects of wastewater discharges as recommended by some studies (Archambault et al., 2001; Bishop et al., 2002; Underwood, 1996) and to fulfill European Directives requirements (WFD and MSFD). Indeed, these communities are playing a key role in water quality for the conservation status and functional aspects of the environment (Casamajor (de) et al., 2016). Vinagre et al. (2016a) even suggest that macrofauna might be considered as an indicator of disturbance in intertidal rocky shores as good as the macroalgae.

Using the quality index, all sites within 'WWTP 2' were ranked as *Good*, while all sites within the Spanish location 'WWTP 3' were ranked as *Moderate*. In 'WWTP 1', only the proximate site from the outfall was ranked as *Moderate*, while site 2 and 3 were ranked as *Good*. A study achieved in compliance with the WFD along the French Basque coast (Casamajor (de) et al., 2016) ranked two other locations (considered as not impacted and representative of the whole water body) as *Good* (with values between 0.706 and 0.732). This is entirely in line with indices calculated on 'Control 1' and sites away from the outfall on impacted locations, which seems to be less impacted and have a better ecological quality. Moreover, in Spain, the WWTP location was moderately impacted whatever the distance from the outfall. But, it is important to note that the ratio was calculated according to the list initially established for the French Basque coast (Ar Gall et al., 2016; Casamajor et al., 2010). A

Spanish list was anyway defined by Juanes et al. (2008) for the calculation of WFD metrics, but the number of opportunistic and characteristic species was much lower than the French one. Thus, scores assigned to each metric would have been not really significant and the ecological quality would have been underestimated. If we had wanted to calculate the Spanish CFR index (Guinda et al., 2008) with our data, this would not have been possible due to the differences of sampling designs (transects *vs.* random quadrats). In addition, despite the fact that the Basque coast has only two algal belts, it seemed preferable to stratify the protocol according to these two belts (quadrats in each belt) rather than to perform a transect covering the two belts.

4. Conclusion

The present work established the assessment of the potential impact of WWTP discharges on intertidal rocky benthic assemblages in the southeastern Bay of Biscay. Even if the importance to consider both communities was proved, it suggests that benthic macroalgae constitute the best relevant organisms to assess the effect of this pressure on the intertidal rocky platform habitat in the study area. The results from the present study do not evidence a clear impact of the WWTP discharges on the rocky benthic intertidal assemblages. Taking into account the presence of some sensitive taxa in WWTP locations and that a Good ecological status has been ranked in French WWTP locations, only the existence of a moderate impact associated with discharges could be concluded. In the Spanish side the ecological quality ratio offered lower values than those expected for the control and impacted locations. The use of the complementary metric "mean taxonomic richness" was not helpful to discriminate the potential impacts due to the absence of clear trends. Finally, multivariate analyses appeared thus to be more efficient than other biological and ecological metrics although certain difficulties emerge when discriminating between changes associated to natural variability and those caused by anthropic activity. For this reason, it is necessary to deepen on the bioindicator character of the different macroalgae. These results will enable several MSFD descriptors to be supported, such as “Biodiversity”, “Non-indigenous species”, “Eutrophication”, “Sea-floor integrity” and "Contaminants" whilst also bridging deficiencies emphasized by Directives on the response of biological indicators to various pressures and the biocenosis of the southeastern Bay of Biscay.

Acknowledgements

We gratefully acknowledge all taxonomic scientists who helped us to identify species: Alex Vanhaelen (IRSNB: Institute of Natural Sciences of Belgium); Jocelyne Martin (French Institute for exploitation of the Sea); Nicolas Lavesque and Benoît Gouilleux (UMR EPOC Bordeaux University); Alvaro Altuna of INSUB (San Sebastian). L. Huguenin is grateful to the French Ministry of Education & Research for her PhD Grant (Doctoral School ED 211, University of Pau and Pays Adour). The authors are grateful to the reviewer's valuable comments that improved the manuscript.

This work was supported by the Micropolit research program 'State and evolution of the quality of the South Atlantic coastal environment', co-financed by the European Union (European Regional Development Fund) and the "Agence de l'Eau Adour Garonne" (Adour Garonne Water Agency) grants.

Declarations of interest: none.

Bibliography

Abadie, S., Butel, R., Dupuis, H., Brière, C., 2005. Paramètres statistiques de la houle au large de la côte sud-aquitaine. Comptes Rendus Geosci. 337, 769–776.

Airoldi, L., Balata, D., Beck, M.W., 2008. The gray zone: relationships between habitat loss and marine diversity and their applications in conservation. J. Exp. Mar. Biol. Ecol. 366, 8–15.

Amaral, H.B.F., Reis, R.P., de Oliveira Figueiredo, M.A., de Gusmão-Pedrini, A., 2018. Decadal shifts in macroalgae assemblages in impacted urban lagoons in Brazil. Ecol. Indic. 85, 869–877.

Anderlini, V.C., Wear, R.G., 1992. The effect of sewage and natural seasonal disturbances on benthic macrofaunal communities in Fitzroy Bay, Wellington, New Zealand. Mar. Pollut. Bull. 24, 21–26.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Ar Gall, E., Le Duff, M., 2007. Protocole d'observation in situ et proposition de calcul d'un indice de qualité pour le suivi des macroalgues sur les estrans intertidaux rocheux dans le cadre DCE. Brest LEBHAM-IUEM–UBO 14.

Ar Gall, E., Le Duff, M., Sauriau, P.-G., de Casamajor, M.-N., Gevaert, F., Poisson, E., Hacquebart, P., Joncourt, Y., Barillé, A.-L., Buchet, R., 2016. Implementation of a new index to assess intertidal seaweed communities as bioindicators for the European Water Framework Directory. Ecol. Indic. 60, 162–173.

Archambault, P., Banwell, K., Underwood, A., 2001. Temporal variation in the structure of intertidal assemblages following the removal of sewage. Mar. Ecol. Prog. Ser. 222, 51–62.

Arévalo, R., Pinedo, S., Ballesteros, E., 2007. Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae. Mar. Pollut. Bull. 55, 104–113.

Augris, C., Caill-Milly, N., de Casamajor, M.-N., 2009. Atlas thématique de l'environnement marin du Pays basque et du sud des Landes. Editions Quae.

Azzurro, E., Matiddi, M., Fanelli, E., Guidetti, P., La Mesa, G., Scarpato, A., Axiak, V., 2010. Sewage pollution impact on Mediterranean rocky-reef fish assemblages. Mar. Environ. Res. 69, 390–397.

Barange, M., 2003. Ecosystem science and the sustainable management of marine resources: from Rio to Johannesburg. Front. Ecol. Environ. 1, 190–196.

Becherucci, M.E., Santiago, L., Benavides, H.R., Vallarino, E.A., 2016. Assessing sewage impact in a South-West Atlantic rocky shore intertidal algal community. Mar. Pollut. Bull. 106, 388–394.

Bernard, M., 2012. Les habitats rocheux intertidaux sous l'influence d'activités anthropiques: structure, dynamique et enjeux de conservation (Doctoral thesis). Université de Bretagne Occidentale, Laboratoire des Sciences de l'Environnement Marin et bureau d'études Hémisphère Sub.

Bishop, M., Underwood, A., Archambault, P., 2002. Sewage and environmental impacts on rocky shores: necessity of identifying relevant spatial scales. Mar. Ecol. Prog. Ser. 236, 121–128.

Borja, Á., Aguirrezabalaga, F., Martínez, J., Sola, J.C., García-Arberas, L., Gorostiaga, J.M., 2004. Benthic communities, biogeography and resources management. Oceanogr. Mar. Environ. Basque Ctry. Elsevier Oceanogr. Ser. 70, 455–492.

Borja, Á., Collins, M., 2004. Oceanography and marine environment in the Basque Country. Elsevier Oceanography Series 70, 616, Amsterdam.

Borja, Á., Elliott, M., Andersen, J.H., Cardoso, A.C., Carstensen, J., Ferreira, J.G., Heiskanen, A.-S., Marques, J.C., Neto, J.M., Teixeira, H., 2013. Good Environmental Status of marine ecosystems: What is it and how do we know when we have attained it? Mar. Pollut. Bull. 76, 16–27.

Borja, Á., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Mar. Pollut. Bull. 40, 1100–1114.

Borja, Á., Galparsoro, I., Irigoien, X., Iriondo, A., Menchaca, I., Muxika, I., Pascual, M., Quincoces, I., Revilla, M., Rodríguez, J.G., 2011. Implementation of the European Marine Strategy Framework Directive: a methodological approach for the assessment of environmental status, from the Basque Country (Bay of Biscay). Mar. Pollut. Bull. 62, 889–904.

Borowitzka, M., 1972. Intertidal algal species diversity and the effect of pollution. Mar. Freshw. Res. 23, 73–84.

Bustamante, M., Bevilacqua, S., Tajadura, J., Terlizzi, A., Saiz-Salinas, J.I., 2012. Detecting human mitigation intervention: Effects of sewage treatment upgrade on rocky macrofaunal assemblages. Mar. Environ. Res. 80, 27–37.

Cabral-Oliveira, J., Mendes, S., Maranhão, P., Pardal, M., 2014. Effects of sewage pollution on the structure of rocky shore macroinvertebrate assemblages. Hydrobiologia 726, 271–283.

Casamajor (de), Sanchez, F., Lissardy, M., 2010. Suivi DCE macroalgue intertidale pour la masse d'eau côte basque. HGS\LRHA\ANGLET\10-003. 33.

Casamajor (de), M.-N., Lalanne, Y., 2016. Intérêt biogéographique de la côte basque rocheuse. Bull. Société Zool. Fr. 141, 3–13.

Casamajor (de), M.-N., Lissardy, M., Sanchez, F., 2016. Suivi DCE du paramètre macroalgue intertidale. FRFC11 Côte basque-année 2015. RBE\HGS\LRHAQ\16-001, 40.

Cearreta, A., Irabien, M.J., Pascual, A., 2004. Human activities along the Basque coast during the last two centuries: geological perspective of recent anthropogenic impact on the coast and its environmental consequences. Oceanogr. Mar. Environ. Basque Ctry. Elsevier Oceanogr. Ser. 70, 27–50.

Chust, G., Borja, A., Liria, P., Galparsoro, I., Marcos, M., Caballero, A., Castro, R., 2009. Human impacts overwhelm the effects of sea-level rise on Basque coastal habitats (N Spain) between 1954 and 2004. Estuar. Coast. Shelf Sci. 84, 453–462.

Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. London, Primer-E. 320.

Costanzo, S., O'donohue, M., Dennison, W., Loneragan, N., Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 42, 149–156.

Coutinho, R., Yaginuma, L.E., Siviero, F., dos Santos, J.C.Q., López, M.S., Christofoletti, R.A., Berchez, F., Ghilardi-Lopes, N.P., Ferreira, C.E.L., Gonçalves, J.E.A., 2016. Studies on benthic communities of rocky shores on the Brazilian coast and climate change monitoring: status of knowledge and challenges. Braz. J. Oceanogr. 64, 27–36.

Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecol. Lett. 11, 1304–1315.

Davidson, I., Crook, A., Barnes, D., 2004. Macrobenthic migration and its influence on the intertidal diversity dynamics of a meso-tidal system. Mar. Biol. 145, 833–842.

Díez, I., Bustamante, M., Santolaria, A., Tajadura, J., Muguerza, N., Borja, A., Muxika, I., Saiz-Salinas, J., Gorostiaga, J., 2012. Development of a tool for assessing the ecological quality status of intertidal coastal rocky assemblages, within Atlantic Iberian coasts. Ecol. Indic. 12, 58–71.

Díez, I., Santolaria, A., Muguerza, N., Gorostiaga, J., 2014. Capacity for recovery of rocky subtidal assemblages following pollution abatement in a scenario of global change. Mar. Pollut. Bull. 86, 197–209.

Díez, I., Santolaria, A., Muguerza, N., Gorostiaga, J., 2013. Measuring restoration in intertidal macrophyte assemblages following sewage treatment upgrade. Mar. Environ. Res. 84, 31–42.

Díez, I., Santolaria, A., Secilla, A., Gorostiaga, J.M., 2009. Recovery stages over long-term monitoring of the intertidal vegetation in the 'Abra de Bilbao'area and on the adjacent coast (N. Spain). Eur. J. Phycol. 44, 1–14.

Díez, I., Secilla, A., Santolaria, A., Gorostiaga, J., 1999. Phytobenthic intertidal community structure along an environmental pollution gradient. Mar. Pollut. Bull. 38, 463–472.

Duarte, B., Martins, I., Rosa, R., Matos, A.R., Roleda, M., Reusch, T.B., Engelen, A.H., Serrão, E.A., Pearson, G.A., Marques, J.C., 2018. Climate change impacts on seagrass meadows and macroalgal forests: an integrative perspective on acclimation and adaptation potential.

EC, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action of marine environmental policy (Marine Strategy Framework Directive). European Commission.

EC, 2000. European Parliament and Council (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. European Commission.

Echavarri-Erasun, B., Juanes, J.A., García-Castrillo, G., Revilla, J.A., 2007. Medium-term responses of rocky bottoms to sewage discharges from a deepwater outfall in the NE Atlantic. Mar. Pollut. Bull. 54, 941–954.

García-Fernández, A., Bárbara, I., 2016. Studies of Cystoseira assemblages in Northern Atlantic Iberia. Presented at the Anales del Jardín Botánico de Madrid, p. 035.

Gaspar, R., Pereira, L., Neto, J.M., 2012. Ecological reference conditions and quality states of marine macroalgae sensu Water Framework Directive: An example from the intertidal rocky shores of the Portuguese coastal waters. Ecol. Indic. 19, 24–38.

Ghilardi, N.P., Pereira Filho, G.H., Berchez, F., 2008. Current knowledge status of the ecology of hard bottom benthic communities in Brazil and the need for new approaches. Oecologia Bras. 12, 2.

Gorostiaga, J., Diez, I., 1996. Changes in the sublittoral benthic marine macroalgae in the polluted area of Abra de Bilbao and proximal coast (Northern Spain). Mar. Ecol. Prog. Ser. 130, 157–167.

Gorostiaga, J., Díez, I., Pagola, S., Francés, G., Saiz, J., Borja, Á., 2004. Recovery of benthic communities in polluted systems. In, M. Collins & A. Borja (Eds.), Oceanography and Marine Environment of the Basque Country. Elsevier, 549-580.

Guidetti, P., Terlizzi, A., Fraschetti, S., Boero, F., 2003. Changes in Mediterranean rocky-reef fish assemblages exposed to sewage pollution. Mar. Ecol. Prog. Ser. 253, 269–278.

Guinda, X., Juanes, J.A., Puente, A., 2014. The Quality of Rocky Bottoms index (CFR): A validated method for the assessment of macroalgae according to the European Water Framework Directive. Mar. Environ. Res. 102, 3–10.

Guinda, X., Juanes, J.A., Puente, A., Revilla, J.A., 2008. Comparison of two methods for quality assessment of macroalgae assemblages, under different pollution types. Ecol. Indic. 8, 743–753.

Harper, J.L., Hawksworth, D.L., 1994. Biodiversity: measurement and estimation. Preface. Phil Trans R Soc Lond B 345, 5–12.

Hay, M.E., 1981. Herbivory, algal distribution, and the maintenance of between-habitat diversity on a tropical fringing reef. Am. Nat. 118, 520–540.

Huguenin, L., Lalanne, Y., Bru, N., Lissardy, M., D'Amico, F., Monperrus, M., de Casamajor, M.-N., 2018. Identifying benthic macrofaunal assemblages and indicator taxa of intertidal boulder fields in the south of the Bay of Biscay (northern Basque coast). A framework for future monitoring. Reg. Stud. Mar. Sci. 20, 13–22.

Juanes, J., Guinda, X., Puente, A., Revilla, J., 2008. Macroalgae, a suitable indicator of the ecological status of coastal rocky communities in the NE Atlantic. Ecol. Indic. 8, 351–359.

Le Hir, M., Hily, C., 2005. Macrofaunal diversity and habitat structure in intertidal boulder fields. Biodivers. Conserv. 14, 233–250.

Le Treut, H., 2013. Les impacts du changement climatique en Aquitaine : un état des lieux scientifique. Presses universitaires de Bordeaux. Coll/Dynamiques. 20-36.

Littler, M.M., Littler, D.S., Taylor, P.R., 1983. Evolutionary strategis in a tropical barrier reef system: functional-form groups of marine macroalgae 1. J. Phycol. 19, 229–237.

Littler, M.M., Murray, S.N., 1975. Impact of sewage on the distribution, abundance and community structure of rocky intertidal macro-organisms. Mar. Biol. 30, 277–291.

Liu, D., Bai, J., Song, S., Zhang, J., Sun, P., Li, Y., Han, G., 2007. The impact of sewage discharge on the macroalgae community in the Yellow Sea coastal area around Qingdao, China. Water Air Soil Pollut. Focus 7, 683.

López Gappa, J., Tablado, N.M., 1990. Influence of sewage pollution on a rocky intertidal community dominated by the mytilid Brachidontes. Mar. Ecol. Prog. Ser. 63, 163–175.

Macdonald, R.W., Morton, B., Johannessen, S.C., 2003. A review of marine environmental contaminant issues in the North Pacific: The dangers and how to identify them. Environ. Rev. 11, 103–139.

Magurran, A.E., 2004. Measuring Biological Diversity, 1st edn. Willey-Blackwell, Oxford, UK. ed.

Maughan, B.C., 2001. The effects of sedimentation and light on recruitment and development of a temperate, subtidal, epifaunal community. J. Exp. Mar. Biol. Ecol. 256, 59–71.

Mieszkowska, N., 2015. Intertidal Indicators of Climate and Global Change, in: Climate Change, Second Edition: Observed Impacts on Planet Earth. Elsevier, pp. 632, 213–229.

Millennium Ecosystem Assessment (MEA), 2005. Ecosystems and human well-being: wetlands and water. Island Press. Washington, DC: World Resources Institute.

Murray, S.N., Ambrose, R., Dethier, M.N., 2006. Monitoring rocky shores. University of California Press. 220.

Neto, J.M., Gaspar, R., Pereira, L., Marques, J.C., 2012. Marine Macroalgae Assessment Tool (MarMAT) for intertidal rocky shores. Quality assessment under the scope of the European Water Framework Directive. Ecol. Indic. 19, 39–47.

O'Connor, N., 2013. Impacts of sewage outfalls on rocky shores: Incorporating scale, biotic assemblage structure and variability into monitoring tools. Ecol. Indic. 29, 501–509.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' Community Ecol. Package Version 2.

Orfanidis, S., Panayotidis, P., Stamatis, N., 2001. Ecological evaluation of transitional and coastal waters: A marine benthic macrophytes-based model. Mediterr Mar Sci 2, 45–65.

Orfanidis, S., Panayotidis, P., Ugland, K., 2011. Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. Mediterr. Mar. Sci. 12, 199–232.

Reish, D.J., 1987. The use of benthic communities in marine environmental assessment. Mem. V Simp. Biol. Mar. University Auton. Baja Calif. Sur. La Paz. Mexico. 123-125.

Roberts, M.S., Smart, P.L., Baker, A., 1998. Annual trace element variations in a Holocene speleothem. Earth Planet. Sci. Lett. 154, 237–246.

Scherner, F., Horta, P.A., de Oliveira, E.C., Simonassi, J.C., Hall-Spencer, J.M., Chow, F., Nunes, J.M.C., Pereira, S.M.B., 2013. Coastal urbanization leads to remarkable seaweed species loss and community shifts along the SW Atlantic. Mar. Pollut. Bull. 76, 106–115.

Schramm, W., 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. Presented at the Sixteenth International Seaweed Symposium, Springer, pp. 583–592.

Seitz, R.D., Wennhage, H., Bergström, U., Lipcius, R.N., Ysebaert, T., 2013. Ecological value of coastal habitats for commercially and ecologically important species. ICES J. Mar. Sci. 71, 648–665.

Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterr. Mar. Sci. 3, 77–111.

Souza, F., Brauko, K., Lana, P., Muniz, P., Camargo, M., 2013. The effect of urban sewage on benthic macrofauna: a multiple spatial scale approach. Mar. Pollut. Bull. 67, 234–240.

Takada, Y., 1999. Influence of shade and number of boulder layers on mobile organisms on a warm temperate boulder shore. Mar. Ecol. Prog. Ser. 189, 171–179.

Terlizzi, A., Benedetti-Cecchi, L., Bevilacqua, S., Fraschetti, S., Guidetti, P., Anderson, M.J., 2005. Multivariate and univariate asymmetrical analyses in environmental impact assessment: a case study of Mediterranean subtidal sessile assemblages. Mar. Ecol. Prog. Ser. 289, 27–42.

Terlizzi, A., Fraschetti, S., Guidetti, P., Boero, F., 2002. The effects of sewage discharge on shallow hard substrate sessile assemblages. Mar. Pollut. Bull. 44, 544–550.

Thompson, R., Crowe, T., Hawkins, S., 2002. Rocky intertidal communities: past environmental changes, present status and predictions for the next 25 years. Environ. Conserv. 29, 168–191.

Underwood, A., 1996. Detection, interpretation, prediction and management of environmental disturbances: some roles for experimental marine ecology. J. Exp. Mar. Biol. Ecol. 200, 1–27.

Valdazo, J., Viera-Rodríguez, M.A., Espino, F., Haroun, R., Tuya, F., 2017. Massive decline of Cystoseira abies-marina forests in Gran Canaria Island (Canary Islands, eastern Atlantic). Sci. Mar. 81, 499–507.

Veríssimo, H., Lane, M., Patrício, J., Gamito, S., Marques, J.C., 2013. Trends in water quality and subtidal benthic communities in a temperate estuary: Is the response to restoration efforts hidden by climate variability and the Estuarine Quality Paradox? Ecol. Indic. 24, 56–67.

Viaroli, P., Bartoli, M., Giordani, G., Naldi, M., Orfanidis, S., Zaldivar, J.M., 2008. Community shifts, alternative stable states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview. Aquat. Conserv. Mar. Freshw. Ecosyst. 18, S105–S117.

Vinagre, P.A., Pais-Costa, A.J., Gaspar, R., Borja, Á., Marques, J.C., Neto, J.M., 2016a. Response of macroalgae and macroinvertebrates to anthropogenic disturbance gradients in rocky shores. Ecol. Indic. 61, 850–864.

Vinagre, P.A., Pais-Costa, A.J., Hawkins, S.J., Marques, J.C., Neto, J.M., 2016b. Ability of invertebrate indices to assess ecological condition on intertidal rocky shores. Ecol. Indic. 70, 255–268.

Wells, E., Wilkinson, M., Wood, P., Scanlan, C., 2007. The use of macroalgal species richness and composition on intertidal rocky seashores in the assessment of ecological quality under the European Water Framework Directive. Mar. Pollut. Bull. 55, 151–161.

Winckel, A., Petitjean, J., Borie, M., Mallet, C., Aubié, S., 2004. Etat des connaissances hydrologiques et hydrogéologiques de la côte basque. BRGM/RP-53372-FR, 113p.

Zhao, Q., Li, R., Ji, M., Ren, Z.J., 2016. Organic content influences sediment microbial fuel cell performance and community structure. Bioresour. Technol. 220, 549–556.

Table 1: General WWTP features

	'WWTP 1'	'WWTP 2'	'WWTP 3'
Location	France	France	Spain
Population equivalent (PE)	78 217	45 000	27 500
Nominal flow (m^3/day)	10 450	7 350	5 930
Outfall location	Intertidal zone	Intertidal zone	Intertidal zone

Table 2: Ecological quality according to the CFR index

Score	Ecological quality
0.80 - 1	*Very good*
0.60 - 0.79	*Good*
0.40 - 0.59	*Moderate*
0.20 - 0.39	*Poor*
0 - 0.19	*Bad*

Table 3: Summary of PERMANOVA results testing for effects of presence of sewage discharges on benthic assemblages between impacted and control locations ('WWTP 1'/'Control 1' (a), 'WWTP 2'/'Control 1' (b), 'WWTP 3'/'Control 2' (c)) in both midlittoral zones

(a)

'WWTP 1'/'Control 1'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	2.93519	27.8767	0.19025	1.00E-04	***
Locations/Sites	4	0.59622	5.6626	0.15458	1.00E-04	***
Residuals	96	0.10529	0.65517			
Lower midlittoral zone						
Locations	1	2.3794	13.3604	0.11347	1.00E-04	***
Locations/Sites	4	0.50673	2.8453	0.09666	1.00E-04	***
Residuals	93	0.17809	0.78986			

(b)

'WWTP 2'/'Control 1'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	4.1775	34.2	0.19055	1.00E-04	***
Locations/Sites	4	0.7719	6.319	0.14084	1.00E-04	***
Residuals	120	0.1221	0.66861			
Lower midlittoral zone						
Locations	1	4.6721	23.5977	0.15924	1.00E-04	***
Locations/Sites	4	0.3758	1.8983	0.05124	0.0011	**
Residuals	117	0.198	0.78952			

(c)

'WWTP 3'/'Control 2'	Df	MeanSqs	F.Model	R2	Pr(>F)	Significance
Upper midlittoral zone						
Locations	1	1.05628	8.1187	0.12313	1.00E-04	***
Locations/Sites	4	0.31941	2.4551	0.14893	2.00E-04	***
Residuals	48	0.1301	0.72795			
Lower midlittoral zone						
Locations	1	2.41452	17.8223	0.22095	1.00E-04	***
Locations/Sites	4	0.50256	3.7096	0.18396	1.00E-04	***
Residuals	48	0.13548	0.59509			

Table 4: Summary of pairwise post hoc results testing for effects of presence of sewage discharges on benthic assemblages between sites within each location ('WWTP 1' (a), 'WWTP 2' (b), 'WWTP 3' (c), 'Control 1' (d), 'Control 2' (e)) in both midlittoral zones.

(a)

Upper midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	**0.004**
Lower midlittoral zone		WWTP 1	
		Site 1	Site 2
WWTP 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	**0.017**

(b)

Upper midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	**0.001**	-
	Site 3	**0.001**	**0.001**
Lower midlittoral zone		WWTP 2	
		Site 1	Site 2
WWTP 2	Site 2	0.479	-
	Site 3	**0.006**	**0.019**

(c)

Upper midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.284	-
	Site 3	**0.042**	0.112
Lower midlittoral zone		WWTP 3	
		Site 1	Site 2
WWTP 3	Site 2	0.327	-
	Site 3	**0.003**	**0.01**

(d)

Upper midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	**0.0015**	-
	Site 3	**0.0015**	0.2967

Lower midlittoral zone		Control 1	
		Site 1	Site 2
Control 1	Site 2	0.21	-
	Site 3	0.21	0.27

(e)

Upper midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	0.094	-
	Site 3	**0.015**	**0.033**

Lower midlittoral zone		Control 2	
		Site 1	Site 2
Control 2	Site 2	**0.002**	-
	Site 3	**0.002**	**0.002**

Table 5: Summary of global dissimilarities between 2 groups from SIMPER analyses (i.e. between impacted and control locations and between sites within each location) for both midlittoral zones (upper and lower)

		Global dissimilarity (%)	
		Upper midlittoral zone	Lower midlittoral zone
	WWTP 1' vs. 'Control 1'	**51.12**	**63.58**
	WWTP 2' vs. 'Control 1'	**53.81**	**71.14**
'WWTP 1'	S1 vs. S2	43.62	52.00
	S2 vs. S3	39.45	41.41
	S1 vs. S3	52.74	56.96
'WWTP 2'	S1 vs. S2	49.57	56.88
	S2 vs. S3	49.17	55.08
	S1 vs. S3	52.93	57.36
'Control 1'	S1 vs. S2	42.41	56.27
	S2 vs. S3	40.95	61.04
	S1 vs. S3	41.89	57.44
	WWTP 3' vs. 'Control 2'	**42.00**	**52.54**
'WWTP 3'	S1 vs. S2	31.16	33.44
	S2 vs. S3	33.08	50.08
	S1 vs. S3	36.56	51.28
'Control 2'	S1 vs. S2	32.65	30.99
	S2 vs. S3	40.26	46.28
	S1 vs. S3	37.47	45.78

Table 6: List of species/taxa identified into quadrats in control locations ('Control 1' and 'Control 2') and in impacted locations ('WWTP 1', 'WWTP 2' and 'WWTP 3'). Mean abundances (ind./0.1m²) and total mean taxonomic richness for each location are shown. Macroalgae were classed into taxonomic groups (red, brown and green) and functional groups (characteristic, opportunistic) according to Ar Gall et al. (2016) for French locations and Juanes et al. (2008) for Spanish ones. Macroalgae were assigned to one of two Ecological Status Groups (ESG) according to morphological and functional characteristics (Ar Gall et al., 2016; Gaspar et al., 2012; Neto et al., 2012; Orfanidis et al., 2011, 2001; Vinagre et al., 2016a). ESG I corresponded to late successional or perennial to annual taxa and ESG II to opportunistics or annual taxa. Macrofauna species were assigned to one of five Ecological Groups (EG I–V) according to their responses to natural and man-induced changes in water quality: the higher the group, the higher the tolerance to pollution (Borja et al., 2000). Significance codes: M: Macroalgae; ESG: Ecological Status Groups for macroalgae species; EG: Ecological groups for macrofauna; L = Lower midlittoral zone; U = Upper midlittoral zone. Sampling fluctuations were described by their standard deviation (SD)

Species/Taxa	Ecological group	Phylum	Locations: France 'Control 1'	France 'WWTP 1'	France 'WWTP 2'	France Characteristic	France Opportunistic	Spain 'Control 2'	Spain 'WWTP 3'	Spain Characteristic	Spain Opportunistic	ESG / EG
Acrosorium spp.	M	Rhodophyta	**0.11** *(SD=0.31)*	**0.02** *(SD=0.13)*	**0.07** *(SD=0.30)*			-	-			**II**
Ahnfeltiopsis devoniensis	M	Rhodophyta	-	-	**0.06** *(SD=0.31)*			-	-			**II**
Antithamnionella sp.	M	Rhodophyta	-	-	-			**0.03** *(SD=0.17)*	**0.07** *(SD=0.26)*			
Antithamnion	M	Rhodophyta	-	-	-			-	**0.03** *(SD=0.17)*			
Asparagopsis/Falkenbergia	M	Rhodophyta	**0.33** *(SD=0.57)*	**0.80** *(SD=1.07)*	**1.21** *(SD=1.28)*	✓ (L)		**0.58** *(SD=0.50)*	**0.54** *(SD=0.67)*			**II**
Bonnemaisonia hamifera	M	Rhodophyta	**0.01** *(SD=0.17)*	-	**0.05** *(SD=0.25)*			-	-			
Caulacanthus ustulatus	M	Rhodophyta	**0.55** *(SD=0.81)*	**0.58** *(SD=1,08)*	**0.29** *(SD=0.64)*	✓ (L+U)		-	**0.06** *(SD=0.23)*	✓ (L+U)		**I**
Ceramium spp.	M	Rhodophyta	**0.28** *(SD=0.57)*	**2.23** *(SD=0.95)*	**0.75** *(SD=0.90)*		✓ (L+U)	**2.31** *(SD=0.58)*	**2.42** *(SD=1.20)*		✓ (L+U)	**II**
Champia parvula	M	Rhodophyta	**0.06** *(SD=0.26)*	-	**0.03** *(SD=0.17)*			**0.11** *(SD=0.40)*	**0.06** *(SD=0.23)*			**II**
Chondracanthus acicularis	M	Rhodophyta	**0.88** *(SD=0.85)*	**0.55** *(SD=0.81)*	**1.23** *(SD=0.93)*	✓ (U)		**0.06** *(SD=0.33)*	-			**II**
Chondria coerulescens	M	Rhodophyta	**0.33** *(SD=0.59)*	**0.60** *(SD=0.67)*	**1.00** *(SD=0.96)*	✓ (L+U)		**1.19** *(SD=0.75)*	**0.01** *(SD=0.12)*			**II**
Chylocladia verticillata	M	Rhodophyta	**0.04** *(SD=0.19)*	-	-			-	-			
Corallina spp.	M	Rhodophyta	**1.81** *(SD=0.98)*	**2.30** *(SD=0.77)*	**2.11** *(SD=1.40)*	✓ (L+U)		**3.14** *(SD=1.25)*	**4.17** *(SD=0.95)*	✓ (L+U)		**I**
Gastroclonium reflexum	M	Rhodophyta	**0.02** *(SD=0.14)*	**0.05** *(SD=0.22)*	**0.20** *(SD=0.49)*			**0.17** *(SD=0.38)*	**0.39** *(SD=0.49)*			**II**
Gelidium spp.	M	Rhodophyta	**0.28** *(SD=0.62)*	**0.12** *(SD=0.32)*	**1.19** *(SD=1.60)*	✓ (L)		**0.47** *(SD=0.61)*	**0.68** *(SD=0.69)*	✓ (L+U)		**I**
Gigartina spp.	M	Rhodophyta	**0.01** *(SD=0.12)*	**0.07** *(SD=0.25)*	**0.01** *(SD=0.10)*			-	-	✓ (L+U)		**II**
Gymnogongrus spp.	M	Rhodophyta	**0.09** *(SD=0.36)*	**0.07** *(SD=0.25)*	**0.22** *(SD=0.48)*			-	-			**I**
Halopitys incurva	M	Rhodophyta	**0.12** *(SD=0.42)*	-	-			**0.14** *(SD=0.42)*	-			
Cryptopleura ramosa	M	Rhodophyta	**0.01** *(SD=0.08)*	-	-			-	-			
Halurus equisetifolius	M	Rhodophyta	**0.37** *(SD=0.65)*	**0.15** *(SD=0.36)*	**0.10** *(SD=0.36)*	✓ (L)		**0.06** *(SD=0.23)*	-			**II**
Hypnea musciformis	M	Rhodophyta	**0.52** *(SD=0.97)*	**0.95** *(SD=1.05)*	**0.10** *(SD=0.30)*			-	-			**II**
Hypoglossum hypoglossoides	M	Rhodophyta	**0.03** *(SD=0.17)*	**0.03** *(SD=0.18)*	**0.07** *(SD=0.30)*			-	-			**I**
Jania rubens	M	Rhodophyta	**0.17** *(SD=0.48)*	**0.25** *(SD=0.51)*	**0.03** *(SD=0.17)*	✓ (L)		**0.56** *(SD=0.77)*	**0.10** *(SD=0.30)*			**I**
Laurencia obtusa	M	Rhodophyta	**0.92** *(SD=1.15)*	**0.67** *(SD=1)*	**0.69** *(SD=0.83)*			-	**0.21** *(SD=0.44)*	✓ (L+U)		**II**
Lithophyllum incrustans	M	Rhodophyta	**0.70** *(SD=0.87)*	**1.05** *(SD=0.75)*	**1.18** *(SD=0.77)*	✓ (L+U)		**2.17** *(SD=0.70)*	**1.64** *(SD=0.77)*			**I**
Mastocarpus /Petricelis	M	Rhodophyta	**0.22** *(SD=0.45)*	**0.07** *(SD=0.25)*	**0.19** *(SD=0.54)*			-	**0.01** *(SD=0.12)*			**I**
Mesophyllum lichenoides	M	Rhodophyta	**0.05** *(SD=0.25)*	**0.18** *(SD=0.50)*	**0.58** *(SD=0.84)*			-	-			
Nitophyllum punctatum	M	Rhodophyta	**0.13** *(SD=0.38)*	**0.02** *(SD=0.13)*	**0.29** *(SD=0.53)*			**0.03** *(SD=0.17)*	**0.03** *(SD=0.17)*			**II**
Ophidocladus spp.	M	Rhodophyta	**0.01** *(SD=0.12)*	-	-			-	**0.03** *(SD=0.17)*			
Osmundea pinnatifida	M	Rhodophyta	**0.32** *(SD=0.67)*	-	**0.03** *(SD=0.17)*			-	**0.14** *(SD=0.39)*			**II**
Peyssonnelia atropurpurea	M	Rhodophyta	**0.01** *(SD=0.08)*	-	**0.02** *(SD=0.14)*			-	**0.03***(SD=0.17)*			**I**
Phymatolithon lenormandii	M	Rhodophyta	**0.35** *(SD=0.60)*	**0.03** *(SD=0.18)*	**0.26** *(SD=0.50)*	✓ (U)		**0.03** *(SD=0.17)*	**0.14** *(SD=0.51)*			
Plocamium cartilagineum	M	Rhodophyta	**0.16** *(SD=0.47)*	**0.05** *(SD=0.22)*	**0.70** *(SD=0.96)*			-	-			**I**
Porphyra spp.	M	Rhodophyta	-	-	-			-	**0.01** *(SD=0.12)*			**II**
Rhodymenia pseudopalmata	M	Rhodophyta	**0.04** *(SD=0.20)*	-	**0.03** *(SD=0.21)*			-	-			

Scinaia furcellata	M	Rhodophyta	**0.01** *(SD=0.08)*	-	-			-	-			**I**
Tenarea tortuosa	M	Rhodophyta	-	-	**0.02** *(SD=0.14)*			-	-	✓ (L+U)		
Vertebrata fruticulosa	M	Rhodophyta	-	-	-		✓ (L+U)	**0.03** *(SD=0.17)*	-			**II**
Cladostephus spongiosus	M	Ochrophyta	-	-	-			**0.56** *(SD=0.88)*	-	✓ (L+U)		**I**
Colpomenia peregrina	M	Ochrophyta	**0.36** *(SD=0.48)*	**0.22** *(SD=0.42)*	**0.33** *(SD=0.55)*	✓ (L+U)		**0.03** *(SD=0.17)*	**0.58** *(SD=0.50)*			**II**
Cutleria adspersa	M	Ochrophyta	-	-	**0.11** *(SD=0.34)*			**0.06** *(SD=0.33)*	**0.29** *(SD=0.46)*			
Cutleria multifida	M	Ochrophyta	-	-	**0.01** *(SD=0.10)*			-	-			
Cystoseira tamariscifolia	M	Ochrophyta	**0.37** *(SD=0.89)*	-	**0.10** *(SD=0.39)*			-	-			**I**
Dictyota dichotoma	M	Ochrophyta	**0.05** *(SD=0.22)*	**0.02** *(SD=0.13)*	**0.23** *(SD=0.49)*	✓ (L)		-	-			**II**
Ectocarpales/Ectocarpus	M	Ochrophyta	**0.01** *(SD=12)*	-	**0.01** *(SD=0.10)*		✓ (L+U)	**0.14** *(SD=0.35)*	**0.22** *(SD=0.42)*		✓ (L+U)	**II**
Ralfsia verrucosa	M	Ochrophyta	-	-	-			**0.03** *(SD=0.17)*	**0.07** *(SD=0.26)*			**I**
Scytosiphon lomentaria	M	Ochrophyta	-	-	-			-	**0.08** *(SD=0.28)*			
Halopteris scoparia	M	Ochrophyta	**1.41** *(SD=1.66)*	**0.58** *(SD=1)*	**0.08** *(SD=0.39)*	✓ (L)		**0.69** *(SD=0.86)*	**0.01** *(SD=0.12)*	✓ (L+U)		**II**
Taonia sp.	M	Ochrophyta	**0.04** *(SD=0.20)*	**0.03** *(SD=0.18)*	**0.23** *(SD=0.49)*			**0.03** *(SD=0.17)*	**0.15** *(SD=0.36)*			
Zonardinia typus	M	Ochrophyta	-	-	**0.05** *(SD=0.29)*			-	-			
Chaetomorpha spp.	M	Chlorophyta	-	-	**0.01** *(SD=0.10)*			-	**0.13** *(SD=0.33)*		✓ (L+U)	
Cladophora spp.	M	Chlorophyta	**0.01** *(SD=0.12)*	-	**0.04** *(SD=0.19)*			-	**0.01** *(SD=0.12)*		✓ (L+U)	**II**
Codium adaerens	M	Chlorophyta	**0.33** *(SD=0.86)*	**0.32** *(SD=0.75)*	**0.30** *(SD=0.76)*	✓ (L)		**0.42** *(SD=0.77)*	**0.57** *(SD=0.85)*			**II (spp.)**
Codium decorticatum	M	Chlorophyta	-	-	-			-	**0.08** *(SD=0.28)*			**II (spp.)**
Codium fragile	M	Chlorophyta	-	**0.02** *(SD=0.13)*	-			**0.17** *(SD=0.38)*	**0.08** *(SD=0.28)*			**II (spp.)**
Derbesia tenuissima	M	Chlorophyta	-	-	**0.01** *(SD=0.10)*			-	-			
Enteromorpha spp.	M	Chlorophyta	**0.64** *(SD=0.83)*	**0.67** *(SD=0.68)*	**0.48** *(SD=0.73)*		✓ (L+U)	**0.53** *(SD=0.61)*	**0.33** *(SD=0.47)*		✓ (L+U)	**II**
Pterosiphonia spp.	M	Chlorophyta	**0.33** *(SD=0.78)*	**0.02** *(SD=0.13)*	**0.12** *(SD=0.43)*			**0.03** *(SD=0.17)*	-			**II (*P. complanata*)**
Ulva spp.	M	Chlorophyta	**1.55** *(SD=1)*	**1.47** *(SD=0.60)*	**1.11** *(SD=0.78)*		✓ (L+U)	**0.28** *(SD=0.51)*	**0.39** *(SD=0.52)*		✓ (L+U)	**II**
Macroalgae mean taxonomic richness			**8.79** *(SD=3.19)*	**8.38** *(SD=2.39)*	**9.90** *(SD=3.22)*			**7.97** *(SD=1.58)*	**7.88** *(SD=2.46)*			
Balanus sp.	SM	Crustacea	-	**0.05** *(SD=0.39)*	-			**1.72** *(SD=3.58)*	**0.53** *(SD=1.14)*			**I**
Chthamalus spp.	SM	Crustacea	**0.02** *(SD=0.25)*	-	-			**1.33** *(SD=1.82)*	**1.04** *(SD=1.44)*			**I**
Mytilus spp.	SM	Mollusca	**0.02** *(SD=0.25)*	**0.30** *(SD=0.91)*	**0.22** *(SD=0.79)*			**1.53** *(SD=2.67)*	**0.92** *(SD=2.11)*			**III**
Rocellaria dubia	SM	Mollusca	-	-	**0.03** *(SD=0.21)*			-	-			**I**
Serpula sp.	SM	Annelida	-	-	**0.02** *(SD=0.19)*			-	-			**I**
Spirobranchus spp.	SM	Annelida	-	-	-			-	**0.04** *(SD=0.35)*			**II**
Porifera	SM	Porifera	-	-	**0.28** (SD=1.56)			-	-			
Sessile macrofauna mean taxonomic richness			**0.01** *(SD=0.12)*	**0.12** *(SD=0.32)*	**0.16** *(SD=0.39)*			**1.06** *(SD=1.24)*	**0.79** *(SD=0.85)*			
Acanthochitona spp.	MM	Mollusca	-	**0.02** *(SD=0.13)*	**0.01** *(SD=0.10)*			-	-			**I**
Actinia equina	MM	Cnidaria	**0.01** *(SD=0.08)*	-	-			-	-			**I**
Actinothoe sphyrodeta	MM	Cnidaria	-	-	-			**0.03** *(SD=0.17)*	-			
Anemonia viridis	MM	Cnidaria	**0.01** *(SD=0.12)*	-	-			-	-			
Annelida	MM	Annelida	-	-	**0.02** *(SD=0.14)*			-	-			
Bittium reticulatum	MM	Mollusca	**0.02** *(SD=0.19)*	-	**0.07** *(SD=0.43)*			-	-			**I**

Cerithium spp.	MM	Mollusca	**0.02** *(SD=0.19)*	-	-	-	-	**II**
Chiton spp.	MM	Mollusca	**0.01** *(SD=0.08)*	-	**0.01** *(SD=0.10)*	-	-	**II**
Diodora gibberula	MM	Mollusca	-	-	**0.01** *(SD=0.10)*	-	-	
Eulalia viridis	MM	Annelida	-	**0.07** *(SD=0.25)*	**0.03** *(SD=0.17)*	**0.11** *(SD=0.40)*	**0.90** *(SD=2.46)*	**II**
Melarhaphe neritoides	MM	Mollusca	-	-	**0.14** *(SD=1.44)*	-	-	**II**
Ocenebra edwardsii	MM	Mollusca	**0.04** *(SD=0.22)*	-	**0.03** *(SD=0.17)*	-	-	**II (sp.)**
Pachygrapsus marmoratus	MM	Crustacea	**0.01** *(SD=0.12)*	-	-	-	-	**II**
Paguridae spp.	MM	Crustacea	**0.13** *(SD=0.58)*	**0.02** *(SD=0.13)*	**0.01** *(SD=0.10)*	-	-	**II (*Pagurus sp.*)**
Paracentrotus lividus	MM	Echinodermata	-	-	-	-	**0.10** *(SD=0.30)*	**I**
Patella spp.	MM	Mollusca	**0.12** *(SD=0.68)*	**0.12** *(SD=0.67)*	**0.03** *(SD=0.21)*	**3.08** *(SD=4.44)*	**3.81** *(SD=3.81)*	**I**
Porcellana platycheles	MM	Crustacea	-	-	-	-	**0.21** *(SD=1.10)*	**I**
Steromphala cineraria	MM	Mollusca	**0.02** *(SD=0.19)*	-	-	-	-	**I**
Steromphala pennanti	MM	Mollusca	**0.45** *(SD=1.32)*	**0.03** *(SD=0.18)*	**0.03** *(SD=0.17)*	-	-	**I**
Steromphala umbillicalis	MM	Mollusca	**0.16** *(SD=0.62)*	-	**0.02** *(SD=0.14)*	-	-	**I**
Stramonita haemastoma	MM	Mollusca	-	-	**0.01** *(SD=0.10)*	-	-	
Tritia incrassata	MM	Mollusca	-	-	**0.03** *(SD=0.21)*	-	-	**II**
Mobile macrofauna mean taxonomic richness			**0.52** *(SD=0.88)*	**0.18** *(SD=0.50)*	**0.25** *(SD=0.55)*	**0.67** *(SD=0.63)*	**1.15** *(SD=0.82)*	
Total mean taxonomic richness			**9.29** *(SD=3.26)*	**8.68** *(SD=2.46)*	**10.31** *(SD=3.26)*	**9.69** *(SD=2.29)*	**9.82** *(SD=2.98)*	

Table 7: Metrics calculated using the Water Framework Directive (WFD) protocol for each control location and each distance of impacted locations

		'Control 1'	'WWTP 1'			'WWTP 2'			'Control 2'	'WWTP 3'		
	Max. points		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3		Site 1	Site 2	Site 3
Global cover of macroalgae [C]	0.4	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306
Occurrence of characteristic species [N]	0.3	0.15	0.075	0.25	0.2	0.15	0.2	0.25	0.2	0.15	0.1	0.1
Total cover of opportunistic species [O]	0.3	0.2	0.075	0.1	0.15	0.2	0.15	0.2	0.15	0.125	0.15	0.1
Final score	1	0.656	0.456	0.656	0.656	0.656	0.656	0.756	0.656	0.581	0.556	0.506
Ecological quality		**Good**	**Moderate**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Moderate**	**Moderate**	**Moderate**

Highlights:

- Detectable effects of discharges were highlighted on assemblage structure
- Macroalgae constituted a relevant biotic component to study impact of WWTP discharges
- 24 contributors responsible for differences (impacted *vs.* control) were identified
- The pseudo-EQR ratio was sensitive to the WWTP pressure