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The impact assessment of thermal pollution on subtidal sessile assemblages: a case study from Mediterranean rocky reefs

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Abstract. Coastal power plants generally use seawater as cooling fluid, discharging heated waters into the sea after the cooling process. The ensuing increase in seawater temperature could affect the marine biota in the nearby areas, causing alterations at different level of biological organization, from individuals to populations and assemblages. In the Mediterranean Sea, few attempts have been made to assess the effects of this point sources of thermal pollution, especially on rocky habitats. Here, we investigated the putative impact of a thermal effluent from one of the largest European coal-fired coastal power plants on sessile assemblages of subtidal rocky reefs. Sessile assemblages on rocky substrates were photographically sampled at one location near the effluent (*I*), and at two control locations (*Cs*) virtually unaffected by thermal discharge. An asymmetrical after-control impact experimental design was employed to test the hypothesis that the thermal discharge significantly modified sessile assemblages at *I* if compared to *Cs*. We detected significant differences in assemblages at *I* versus *Cs*, indicating a clear effect of the effluent on assemblage structure. Such differences were mostly due to shift in dominance among macroalgae between *I* and *Cs*, which likely depended on different tolerance limits of species to increased seawater temperature and other sources of disturbance associated to the effluent, such as increased sedimentation rates and water turbidity. Our findings stressed the need for further investigations of the impact of thermal effluents on marine communities, considering the potential synergistic effects of climate change especially in the Mediterranean Sea.

Keywords: Asymmetrical design, climate change, coal-fired power plants, macrobenthos, thermal effluents

Introduction

There are about 6300 operating power plants worldwide relying on combustion of fossil fuels (coal, gas, and oil) or nuclear reactors (WPPD, World Power Plant Database, 2020). They are commonly located close to rivers, lakes, or in coastal zones, to exploit water for cooling their energy-generating units. In power plants positioned onshore, seawater at ambient temperature is withdrawn through intake structures, generally placed on the sea bottom near the coastline, pumped in the cooling system of the power plant and, after the cooling process, discharged at a higher temperature into the sea. Temperature ranges of discharged heated waters are often imposed by law, although legal limits may change with country. In Europe, for example, admitted limits for temperature of thermal effluents range between 7 and 12°C higher than the ambient value, if related to the increase of seawater temperature in the nearby marine water body, should not exceed 1-3 °C at a distance that varies from few tens of meters to 1 km from the outfall (Gaeta et al., 2020).

Since water temperature influences a number of biological processes in marine organisms, including metabolism, physiology, reproduction, behaviour (Sylvester, 1970;

Bamber, 1990; Sanford, 1999; Lukšienė et al., 2000; Jang et al., 2009; Harvey et al., 2013; Givan et al., 2018), thermal pollution from coastal power plants has raised wide concerns on potential effects on aquatic populations and communities (Bush et al., 1974; Walther et al., 2009). The effect of the discharge of heated waters may cause detrimental effects on marine biodiversity, leading to drastic changes in the structure of assemblages, and affecting all marine ecological compartments, including plankton (Choi et al., 2002; Lin et al., 2018), benthos (Barnett, 1972; Deabes, 2020), and fish (Teixeira et al., 2012; White & Wahl, 2020). For example, several studies found a significant reduction in abundance and diversity of phytoplankton and zooplankton (Lin et al., 2018), but also of macroinvertebrates (Teixeira et al., 2012; Deabes, 2020) and fish assemblages (Teixeira et al., 2009; 2012) in areas adjacent to thermal effluents. Mechanisms driving such changes are manifold, and often related to physiological chronic stress (Kim et al. 2017), or to the disruption of normal reproductive timing or growth processes (Barnett, 1972) induced by the increased water temperature, which in turn constrain the persistence of viable populations, especially those of stenothermal organisms (Bamber, 1990).

In most cases, the influence of thermal discharge on marine assemblages is limited within few hundreds of meters from the effluent (e.g., Bamber, 1990; Choi et al., 2002; Teixeira et al., 2009; Deabes, 2020). However, the effects of thermal pollution on marine species may be exacerbated by the increase of seawater temperature under future global warming scenarios (Keser et al., 2005; Coulter et al., 2014; Worthington et al., 2015). Coastal areas affected by thermal discharges could also provide suitable habitats for non-native species from warmer marine regions (Bamber, 1990; Baker et al., 2007; Wolf et al., 2014), and facilitate their recruitment and spread in the native assemblages, with potential ecological implications for the whole ecosystem (Walther et al., 2009). Intensified effects of thermal pollution may be envisaged in marine areas representing hot spots for climate change, particularly exposed to biological invasions and warming, such as the Mediterranean Sea (Rilov & Galil, 2009; Rivetti et al., 2014; Katsanevakis et al., 2014; Guarnieri et al. 2017; Yeruham et al., 2020). In this basin, more than 60 operating power plants using seawater as cooling fluid are located along the coast, and many other are under construction or projected (WPPD, 2020). Few attempts, however, have been done to understand the potential outcomes of thermal discharge on Mediterranean marine assemblages, and most of them centred on soft bottom infauna (e.g., Crema & Bonvicini Pagliai, 1980; Lardicci et al., 1999; Deabes, 2020), whereas even less case studies investigated the consequences of thermal pollution on hard bottoms and, however, they were focused on single taxa (e.g., Arieli et al., 2011).

In this study, we assessed the effects of thermal discharge from one of the largest coal-fired coastal power plants in Europe on Mediterranean rocky reefs. An asymmetrical experimental design contrasting the discharge area with unaffected control areas was structured to test whether the presence of the thermal effluent could cause changes in the structure and diversity of subtidal sessile assemblages.

Study area

The study area is located along the coast of SE Italy (S Adriatic, Mediterranean Sea), near the town of Brindisi, where a thermal effluent discharges the cooling waters from the coal-fired power plant 'Federico II' (Fig. 1). Along the coastline, sandy beaches alternate with silt-clay cliffs, whereas the sea bottom until 10 m depth is characterized by a mosaic of sandy habitats, *Posidonia oceanica* (L.) Delile, 1813 seagrass beds and rocky reefs, the latter being more common in the north sector of the study area. The power plant is one of the largest coal-fired energy plants in Europe, with a total installed capacity of 2640 MW. Seawater is used as cooling fluid for heat exchangers and, at the end of the production process, discharged into the sea through an 80-m width channel with 100 m^3/s flow (Gaeta et al., 2020). Seawater temperature of the effluent is estimated to be within 12 °C higher than the ambient value, and field measurements and effluent diffusion modelling showed that the increase in seawater temperature at 1 m depth varied from 0 to 4 °C with respect to the ambient values within 1 km radius from the effluent, depending on wind and wave direction (Gaeta et al., 2020).

Materials and methods

Sampling design and sample processing

Sampling was carried out in July 2015 at three locations 3-6 km far one each other, one putatively impacted by the thermal effluent, located at about 500 m north from the discharge channel (hereafter indicated as *I*), and two control locations (hereafter indicated as C1 and C2, and *Cs* altogether), to serve as unaffected reference conditions, positioned northward with respect to *I* (Fig. 1). The absence of large patches of rocky reefs in the southern sector of the study area, prevented positioning at least one control location on both sides of the effluent. C1 and C2 were randomly selected from a set of potential control locations with environmental conditions (exposure, type of rocky substrate, depth) comparable to those characterizing rocky reefs at *I* (Fig. 2). In each location, sampling was carried out at two sites (100-300 m apart) and, at each site, in three

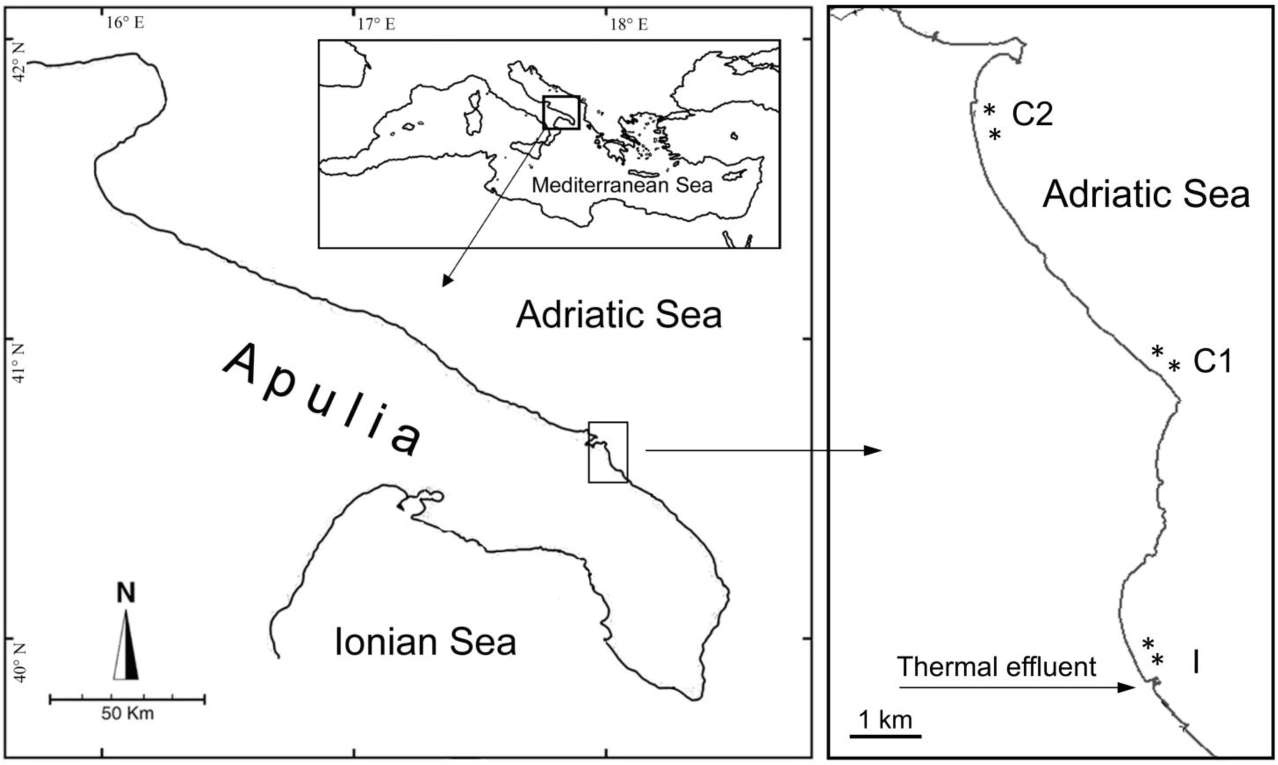

Fig. 1. Study area and position of the putatively impacted location (*I*) and control locations (C1 and C2). The position of sampling sites in each location were also indicated (*)

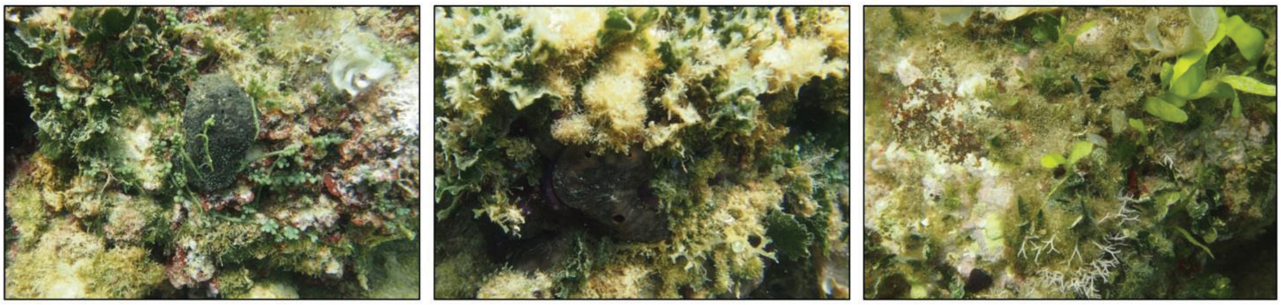

Fig. 2. Examples of macrobenthic assemblages characterizing C1 (left panel), C2 (central panel), and *I* (right panel)

sampling stations (10s m apart), in order to quantify the spatial variability of macrobenthic assemblages at a range of spatial scales. In each sampling station, sessile assemblages at approximately 5 m depth were sampled with $n = 10$ random photographic replicate samples of 16×23 cm each (Roberts et al., 1994), using a digital underwater camera Canon EOS 400D™ equipped with strobes, yielding a total of 180 photographic samples. Digital images were then analysed on the computer by superimposing a grid of 24 equally sized quadrats, to help estimating the % cover of sessile organisms in the photographic sample (Dethier et al., 1993). Vagile organisms were not considered in the analysis. A total of 53 taxa were found (Table 1). Most of them (~90%) were identified at the level of species or genus. In some cases (~10%) organisms were collapsed into morphological groups due to the intrinsic difficulties of taxonomic identification from images.

Statistical analysis

Distance-based permutational multivariable analysis of variance (PERMANOVA, Anderson 2001) was used to test

Table 1. List of taxa found in the study

Algae	*Acetabularia acetabulum* (Linnaeus) P.C. Silva, 1952	**Cnidaria**	*Aiptasia mutabilis* (Gravenhorst, 1831)
	Anadyomene stellata (Wulfen) C. Agardh, 1823		*Anemonia viridis* (Forsskål, 1775)
	Amphiroa rigida J.V. Lamouroux, 1816		*Balanophyllia (Balanophyllia) europaea* (Risso, 1826)
	Caulerpa cylindracea Sonder, 1845		*Cereus pedunculatus* (Pennant, 1777)
	Caulerpa prolifera (Forsskål) J.V. Lamouroux, 1809		*Cladocora caespitosa* (Linnaeus, 1767)
	Ceramium sp.		*Clavularia* sp.
	Cladophora sp.		*Eudendrium* sp.
	Corallina sp.		*Sertularella* sp.
	Dictyota spp.	**Annelida**	Serpulids
	Encrusting Corallines Rhodophytes (ECR)	**Mollusca**	*Arca noae* Linnaeus, 1758
	Filamentous Algae		*Dendropoma* sp.
	Flabellia petiolata (Turra) Nizamuddin, 1987		*Ostrea* sp.
	Halimeda tuna (J.Ellis & Solander) J.V.Lamouroux, 1816		*Rocellaria dubia* (Pennant, 1777)
	Jania sp.		*Vermetus arenarius* (Linnaeus, 1758)
	Laurencia complex	**Bryozoa**	*Calpensia nobilis* (Esper, 1796)
	Padina pavonica (Linnaeus) Thivy, 1960		*Schizobrachiella sanguinea* (Norman, 1868)
	Peyssonnelia spp.	**Tunicata**	*Aplidium* sp.
	Halopteris scoparia (Linnaeus) Sauvageau, 1904		*Botrylloides* sp.
	Tricleocarpa fragilis (Linnaeus) Huisman & R.A. Townsend, 1993		*Cystodytes dellechiajei* (Della Valle, 1877)
	Valonia macrophysa Kützing, 1843		*Didemnum* sp.
Porifera	*Aplysina aerophoba* (Nardo, 1833)		*Diplosoma listerianum* (Milne Edwards, 1841)
	Chondrilla nucula Nardo, 1847		*Microcosmus sabatieri* Roule, 1885
	Chondrosia reniformis Nardo, 1847		
	Cliona celata Grant, 1826		
	Cliona rhodensis Rützler & Bromley, 1981		
	Cliona viridis (Schmidt, 1862)		
	Crambe crambe (Schmidt, 1862)		
	Ircinia variabilis (Schmidt, 1862)		
	Petrosia (Petrosia) ficiformis (Poiret, 1789)		
	Phorbas fictitius (Bowerbank, 1866)		
	Sarcotragus spinosulus (Schmidt, 1862)		

for differences between *I* and *Cs*. The analysis was based on Bray–Curtis dissimilarities (Bray & Curtis, 1957) on untransformed data, and each term in the analysis was tested by 4999 random permutations of appropriate units. The design for the analysis comprised three factors: Location [Lo], random, with three levels, one impacted and two controls; Site [Si(Lo)], two levels, random, nested in Lo; and Station [St(Si(Lo))], three levels, random, nested in Si(Lo), with $n = 10$ replicates. Due to the presence of a single putatively impacted location, the design was asymmetrical (Underwood, 1994). Thus, the location term was partitioned into two portions: the 1-d.f. fixed contrast of *I*-vs-*Cs* and the variability between *Cs*. The same partitioning was also done for all the other terms in the analysis (e.g., the term Si(Lo) was partitioned into the two terms S(*I*) and S(*Cs*)). Finally, the residual variation was split in two portion, Res *I* and Res *Cs*, which is the variability for observations at *I* and *Cs* respectively. Full details for variance partitioning and the choice of appropriate denominators for *F* test in asymmetrical designs are provided in Terlizzi et al. (2005).

Non-metric multidimensional scaling ordination (nMDS) of centroids of stations based on the Bray-Curtis dissimilarity matrix was used to depict multivariate patterns of assemblages. Similarity Percentage analysis (SIMPER; Clarke, 1993) was also performed to evaluate the percentage contribution of variables to Bray–Curtis dissimilarities of *I*-vs-*Cs*, allowing identifying taxa most contributing to differentiate assemblages at *I*-vs-*Cs*. Only variables whose contribution to dissimilarity was ≥1% were selected.

Analysis of variance (ANOVA) was done to test for differences in the mean total percentage cover and mean number of taxa between *I*-vs-*Cs*. The design for the analysis was the same as for PERMANOVA, asymmetrical, and thus implying the same variance partitioning. Prior to analysis, Cochran's *C*-test was performed to check the assumption of variance homogeneity (Underwood, 1997) and data were transformed to remove heteroscedasticity, if required.

Due to the relatively small number of degrees of freedom for some of the tests, and in consideration of the precautionary principle in environmental impact assessment, $\alpha = 0.10$ was fixed a priori as a decision criterion for interpreting statistical results in both multivariate and univariate analyses (Gray, 1990; Fairweather, 1991).

Analysis was performed using the software package PRIMER v7 (Clarke & Gorley, 2015), including the add-on package PERMANOVA$^+$ (Anderson et al., 2008).

Results

Table 2. Results of asymmetrical PERMANOVA testing for differences between assemblages at the impacted location versus control locations based on Bray–Curtis dissimilarities (data untransformed) of multivariate data (53 taxa). Each test was performed using 4999 permutations of appropriate units. *P*-values given in italics were obtained using 4999 Monte Carlo samples from the asymptotic permutation distribution. The term used for the denominator mean square in each case is given in column $\mathrm{MS}_{\mathrm{DEN}}$

Source of variation	**d.f.**	**SS**	**MS**	**Pseudo-*F***	***P***	**$\mathrm{MS}_{\mathrm{DEN}}$**
Lo	2	92934.5	46467.3	6.275	*0.000*	Si(Lo)
I-vs-*Cs*	1	69444.0	69444.0	2.956	*0.092*	Cs
Cs	1	23490.5	23490.5	3.311	*0.042*	Si(*Cs*)
Si(Lo)	3	22216.1	7405.4	2.736	0.001	St(Si(Lo))
Si(*I*)	1	8026.1	8026.1	3.406	0.009	St(Si(*I*))
Si(*Cs*)	2	14190.0	7095.0	2.462	0.018	St(Si(*Cs*))
St(Si(Lo))	12	32479.3	2706.6	2.457	0.000	Res
St(Si(*I*))	4	9427.1	2356.775	1.600	0.031	Res *I*
St(Si(*Cs*))	8	23052.2	2881.5	3.146	0.000	Res *Cs*
Res	162	178440.2	1101.5			
Res *I*	54	79525.3	1472.7			
Res *Cs*	108	98914.9	915.9			
Total	179	326070.1				

Multivariate analysis detected a significant effect of the thermal effluent in modifying the structure of sessile assemblages at the impact *I*-vs-*Cs* (Table 2). This was clearly depicted in the nMDS plot, where the site centroids of *I* set apart from those of the two control locations that, instead, clustered together in the multivariate space (Fig. 3).

Twelve taxa contributed to explain >90% of dissimilarity between assemblages in *I*-vs-*Cs* (Table 3). More specifically, the macroalgae *Padina pavonica* (L.) Thivy 1960, *Caulerpa cylindracea* Sonder 1845, *Flabellia petiolata* (Turra) Nizamuddin 1987, and articulated corallines of the genus *Jania* characterized control locations, while showing low to very low abundance at *I* (Table 2). In contrast, *Halimeda tuna* (J. Ellis & Solander) J.V. Lamouroux 1816, the morphological group of Filamentous Algae, and *Caulerpa prolifera* (Forsskål) J.V. Lamouroux 1809, mostly characterized assemblages at *I*, with the latter found exclusively at the impact location (Table 2).

No significant effects of the effluent on the mean total cover and the mean number of taxa were detected (Table 4, Fig. 4).

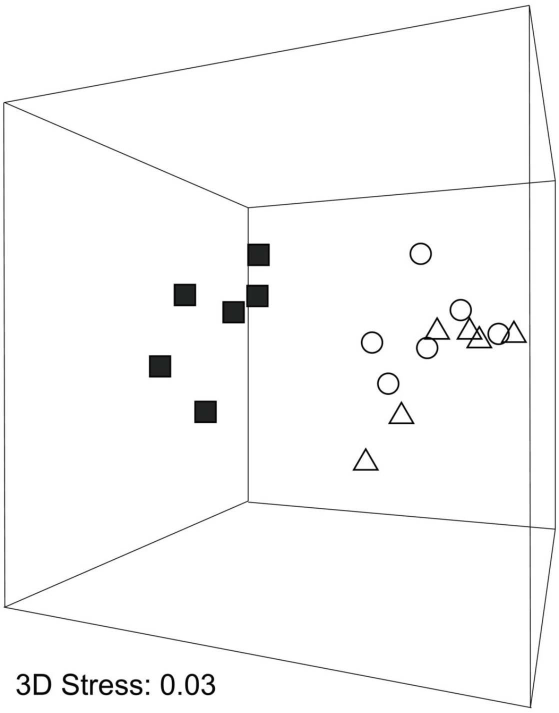

Fig. 3. 3-D non-metric multidimensional scaling ordination (nMDS) based on Bray–Curtis dissimilarities of site centroids for *I* (black squares), C1 (blank circles) and C2 (blank triangles)

Table 3. Summary of results of SIMPER analysis based on Bray-Curtis dissimilarities (data untransformed). Only taxa whose contribution to total dissimilarity between *I*-vs-*Cs* was equal or exceeded 1% were reported

	Cs	**I**		
Taxon	**Average abundance**	**Average abundance**	**% contribution to dissimilarities**	**Cumulative % contribution**
Padina pavonica	19.9	11.4	16.1	16.1
Filamentous Algae	7.1	21.0	14.1	30.2
Caulerpa cylindracea	16.7	3.6	12.8	43.0
Flabellia petiolata	9.9	1.1	8.9	51.9
Halimeda tuna	2.7	9.3	7.5	59.4
Peyssonnelia spp.	10.1	6.9	6.8	66.2
Caulerpa prolifera	0.0	7.1	6.6	72.8
Jania spp.	6.4	0.9	5.5	78.3
Dictyota spp.	3.7	5.8	5.5	83.8
ECR	1.6	4.3	4.2	88.0
Cliona celata	0.2	1.3	1.4	89.4
Sarcotragus spinosulus	0.5	0.9	1.2	90.6

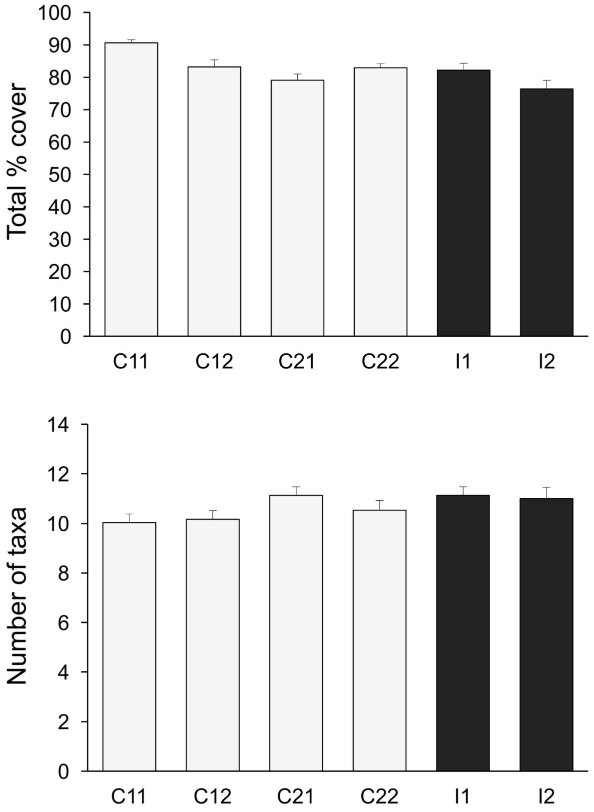

Fig. 4. Mean (± SE, $n = 30$) total % cover and number of taxa for each of the two sites (indicated with 1 and 2) at *I* (black bars), C1 and C2 (white bars)

Discussion

We detected a significant change between *I*-vs-*Cs* (at $\alpha = 0.10$), indicating that the discharge of heated seawater from the effluent has the potential to alter the structure of nearby sessile assemblages with respect to unaffected areas. However, our data were limited to a single time of sampling preventing further considerations on temporal patterns of such changes. The effects of thermal discharge might be quite variable among seasons (Langford, 1990; Kim et al., 2013; Teixeira et al. 2012), and strongly dependent on the ambient temperature of seawater, which could contribute especially in the warm season to exacerbate the effects of heated waters on aquatic organisms, since sublethal and lethal limits can be more easily approximated (Langford, 1990). Further investigations are therefore needed to appreciate temporal variations in the effect of thermal discharge on macroinvertebrates and macroalgae inhabiting subtidal reefs.

In contrast to other studies (e.g., Teixeira et al., 2012), no effects of thermal discharge on total abundance and taxon richness were detected. This could depend on the fact that the stretch of coast where the effluent was positioned is particularly exposed to dominant winds and currents and subjected to an intense hydrodynamic regime, which contributed to enhance seawater mixing and helped dissipating the plume of heated waters. It is worth noting also that, the water body northward the effluent received

Table 4. Summary of results of asymmetrical ANOVA on mean total cover and number of taxa

		Total % cover			**No. of taxa**		
Source of variation	**d.f.**	**MS**	***F***	***P***	**MS**	***F***	***P***
Lo	2	960.1	1.856	0.270	15.3	7.719	0.068
I-vs-*Cs*	1	872.7	1.687	0.285	14.4	0.894	0.518
Cs	1	1047.3	1.989	0.294	16.1	5.694	0.139
Si(Lo)	3	517.4	2.661	0.098	2.0	0.399	0.753
Si(*I*)	1	498.8	2.230	0.209	0.3	0.031	0.868
Si(*Cs*)	2	526.5	2.928	0.118	2.8	0.883	0.449
St(Si(Lo))	12	194.2	1.803	0.052	5.0	1.196	0.293
St(Si(*I*))	4	223.6	1.275	0.322	8.5	1.863	0.129
St(Si(*Cs*))	8	179.8	1.915	0.063	3.2	0.802	0.604
Res	162	108.1			4.0		
Res *I*	54	175.4			4.5		
Res *Cs*	108	93.9			4.0		

the heated water only when winds blew from S-SE sectors (one of the main wind direction in the region, along with N-NE winds, Gaeta et al. 2020), so that rocky reef assemblages at *I* were not permanently under the influence of thermal discharge.

The effect of heated seawater discharge, however, seemed to be strong enough to cause changes in composition of sessile assemblages and shifts in the relative abundance of dominant taxa between *I* and control locations. This was mostly due to changes in the structure of macroalgal stands characterizing subtidal reefs close to the effluent, which were dominated by filamentous algae, *H. tuna* and *C. prolifera*, whereas *P. pavonica, F. petiolata*, articulated corallines, and *C. cylindracea* characterized control locations. Such changes could be related to the intrinsic thermal tolerance of the different species. *C. prolifera* and *H. tuna*, for instance, have been found to perform better in warmer waters, with higher optimal temperature or higher survival temperature limits with respect to other species, such as *P. pavonica* (Savva et al., 2018). Interestingly, *C. cylindracea* was more abundant at control locations, despite its relative insensitiveness in terms of growth rates and photosynthetic yield to increasing ambient temperature (Sampeiro-Ramos et al., 2015). Nevertheless, increased seawater temperature could have detrimental effects on the physiology of early thalli of this invasive species, causing a reduction of growth rates and photosynthetic efficiency if occurring during winter (Flagella et al., 2008), possibly explaining the reduced abundance at *I*.

The increase of seawater temperature is not the only stress associated to the effluent discharge, and other environmental alterations could have participated in determining the observed patterns. Other studies in the region highlighted that the increased hydrodynamism caused by the outflow, with the ensuing increase in resuspension of sediments, could affect macrobenthic assemblages nearby the effluent (Marano et al., 2000). The proliferation of filamentous algae at *I*, and found in other areas subjected to thermal discharge (e.g., Choi, 2008), can be interpreted as a symptom of generalized environmental stress caused by the increase in seawater temperature (Johnson et al., 2017; Dijkstra et al., 2019), turbidity and sedimentation which favored this group of tolerant and opportunistic algae (Airoldi, 1998).

The Mediterranean Sea is undergoing to increasing levels of human pressures threatening its unique marine biodiversity (Lejeusne et al., 2010; Micheli et al. 2013;), with non-native species introduction and climate change being the most alarming threats to this basin due to their potential to cause regime shifts to marine ecosystems (Rilov & Galil, 2009; Mannino et al., 2017; Bevilacqua et al., 2019). Subtidal rocky reefs are of crucial importance for the functioning of coastal ecosystems and they are particularly sensitive to the ongoing environmental changes (Sala et al., 2011; Strain et al. 2014), which place this marine habitat among the most endangered ones at a basin scale (Bevilacqua et al., 2020). Our findings demonstrated that thermal pollution from coastal power plants could play a role in modifying the structure of sessile assemblages on subtidal reefs. Current rates of warming of the Mediterranean Sea could combine increasing the magnitude and the spatial extent of these effects, and potentially participating to drive undesirable ecological changes, stressing the need for further long-term assessments of the impact of point sources of thermal pollution on these important habitats.

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