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Effects of the installation of offshore pipelines on macrozoobenthic communities (northern and central Adriatic Sea)



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ABSTRACT

Macrozoobenthos living around several pipelines placed at different depths and sediment types in the Western Adriatic Sea was investigated for three years after structures' deployment to detect possible effects due to their installation and presence. Three environmental habitats were considered based on the grain size (silty clay, clayey silt and sand). Samplings were taken within a radius of 100 m from the pipelines and at control sites. Multivariate and univariate analysis showed peculiarities of the three habitats due to the different sediment type, without differences between pipelines and controls inside each group. Silty clay and clayey silt communities appeared quite similar, being mainly represented by opportunistic species typical of the Adriatic coastal area. Benthic populations found at the offshore relict sand were characterized by a higher percentage of sensitive species. Independently of sediment typology, pipelines' installation seems to not affect the benthic populations that appear more influenced by environmental features.

1. Introduction

Nowadays, about 120 offshore platforms have been installed in the northern and central Adriatic Sea (http://unmig.mise.gov.it), producing about 86% (5.239 million of Standard cubic meters) of the natural gas derived by Italy (Ministry of Economic Development, 2013) and representing the highest concentration of fossil fuel extraction platforms in the Mediterranean area.

These platforms are installed in a wide variety of environments, with different depths (from 20 to 80 m) and types of sediment (from coastal mud to relict sand), and are connected to each other and/or with land through about 300 pipelines, extending for a total of around 2300 km and having different length (from a few tens of meters to about 70 km) in relation to the distance among platforms or between the rig and land terminal. These pipelines are laid to or sunken into the sea bottom and their deployment requires different time, depending on their length and deployment modality.

Several studies have been carried out to assess the effects due to the installation of offshore gas and oil platforms on soft-bottom benthic communities (e.g., Wolfson et al., 1979; Montagna and Harper, 1996; Love et al., 1999; Page et al., 1999; Stachowitsch et al., 2002; Currie and Isaacs, 2005; Fabi et al., 2005, 2007; Bigot et al., 2006; Trabucco et al., 2006,

2008; Fontana et al., 2007; Saunders et al., 2007; Terlizzi et al., 2008; Manoukian et al., 2010; Gomiero et al., 2011, 2013a, 2013b; Spagnolo et al., 2014; Punzo et al., 2015, 2017). The importance of benthic organisms in the evaluation of ecosystem stress is well known, being related to the difficulty of these animals to escape natural and anthropogenic disturbances and to the sensibility of several species to environmental changes. In fact, benthic communities are stable under given natural conditions and they have a great responsiveness to environmental or anthropogenic changes. So, these species are commonly regarded as good indicators of environmental impacts (Simboura and Zenetos, 2002), as well as of long-term changes in the ecosystem (Kröncke, 1995).

In the Adriatic Sea, it has been demonstrated that the geographical position of an offshore platform plays an important role in the "timing" of the benthic community restoration (Fabi et al., 2005; Manoukian et al., 2010). In the western side of the basin, the populations' response to the rigs installation appears accelerated in shallow waters (< 40 m; about 2 years after the rig deployment) in respect to offshore environments (about 3 years). This is due to the peculiar physiography of the Adriatic Sea, where currents and the amounts of nutrients, oxygen concentration, salinity, temperature, turbidity and primary and secondary production are strongly affected by the great rivers' inputs (Marini et al., 2008). Due to the variability of these parameters, in

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Fig. 1. Grain-size map and location of the investigated pipelines (1–7). A–L = sampling points. The pipelines length is not in scale. A scheme of the sampling strategy is also reported.

Table 1

Length, distance from the coast, depth, mean grain size and mean Organic Matter content (\pm standard deviation) of the 7 selected pipelines. A-L = sampling points. S-C = silty clay; C-S = clayey silt; S = sand.

Pipeline	P1	P2	Р3	P4	Р5		Р6		P7	
Distance from the coast (km)	34.0	14.0	20.0	60.0	47.0	34.0	53.0	55.0	29.7	25.1
Length (km)	33.0	17.0	5.3	2.9	41	1.0	30	0.0		9.5
Depth (m)	31.0	21.0	24.0	58.0	57.0	52.0	41.0	37.0	33.0	28.0
Sampling point	Α	В	С	D	E	F	G	Н	Ι	L
Sand (%)	2.0 ± 0.0	1.1 ± 0.3	1.9 ± 0.7	$83.2~\pm~0.4$	$84.8~\pm~0.5$	86.4 ± 0.5	73.6 ± 1.2	$18.2~\pm~2.8$	0.7 ± 0.2	0.7 ± 0.3
Silt (%)	47.4 ± 7.7	83.9 ± 9.9	76.7 ± 2.7	$10.1~\pm~0.6$	8.1 ± 0.3	8.1 ± 0.4	$14.4~\pm~0.8$	37.1 ± 2.4	$40.5~\pm~0.6$	43.0 ± 0.2
Clay (%)	50.7 ± 7.8	15.0 ± 10.1	21.4 ± 2.7	6.7 ± 0.1	6.4 ± 0.2	5.5 ± 0.3	11.9 ± 0.5	45.9 ± 2.1	$58.9~\pm~0.6$	56.2 ± 0.1
Sediment type	S-C	C-S	C-S	S	S	S	S	S-C	S-C	S-C
OM (%)	$0.8~\pm~0.1$	$4.9~\pm~1.5$	4.1 ± 1.4	$1.0~\pm~0.2$	$1.1~\pm~0.2$	1.6 ± 0.6	1.6 ± 0.6	$3.7~\pm~1.5$	$4.7~\pm~1.0$	$4.9~\pm~1.0$

inshore areas the benthic fauna is well adapted to changes, therefore its response to environmental and anthropogenic stress is faster than that of the benthic communities living offshore.

Reasonably, the benthic populations inhabiting in the surroundings of pipelines should also show a similar recovery pattern, although the pipelines' effects should be weaker due their smaller dimensions in respect to platforms.

Up to date, studies on pipelines have mainly focused on engineering aspects (e.g., Gao et al., 2003; Shabini and Jeng, 2008; Hamouda and Abdel-Salam, 2010; Su et al., 2011; Mattioli et al., 2012; Zhang and Han, 2013, 2014; Changjing et al., 2015), as well as on the assessment of the physical pressure ("obstruction" or "sealing") caused on the seabed (Eastwood et al., 2007; Foden et al., 2011). Literature on the response of benthic biota to pipelines installation consists of short-time studies carried out to evaluate the possible impact of only one pipeline

crossing streams, rivers, estuaries or deployed at sea (Philip and McCart, 1981; Tsui and McCart, 1981; Tillinghast et al., 1987; Young and Mackie, 1991; Pranesh and Kumar, 1993; Rezai et al., 1999; Lewis et al., 2002, 2003). The conclusions of such investigations are quite similar: the recovery of benthic communities depends on the life cycle and motility of the species living in the area. In addition, these Authors affirm that the impact due to natural perturbations is greater than the effects of pipeline construction.

In the other hand, long-term monitoring is essential to assess the effects on benthic communities derived by installation and presence of permanent constructions, even in the case of relatively small-sized structures such as pipelines.

To contribute to fulfill in this gap, the present study aims to evaluate the effects on benthic communities caused by the installation of seven pipelines in various type of sediments in the northern and central

Table 2

A) PERMANOVA analyzing differences among the benthic assemblages of the three groups of pipelines. B) PERMANOVA comparing the benthic assemblage inside each group to evaluate differences between the surroundings of the pipelines (within 100 m) and the control sites. C-S = clayey silt; S-C = silty clay; S = sand. Not significant p > 0.05; significant $0.05 \ge p > 0.01$; highly significant $p \le 0.01$. Results of the Pair-Wise comparison are also reported.

	Source	df	SS	MS	Pseudo-F	P (perm)	Pair-wise test
A	Sediments	2	77,515	38,758	34.908	0.001	$S \neq C-S; S \neq S-C;$ S-C $\neq C-S$
	Res	52	57,734	1110.3			
В	Site (C-S)	1	3404.6	3404.6	1.5507	0.354	
	Res	2	4391.0	2195.5			
	Site (S-C)	1	1347.9	1347.9	0.5976	0.819	
	Res	6	13,534.0	2255.6			
	Site (S)	1	1224.2	1224.2	0.7853	0.615	
	Res	6	9353.3	1558.9			

Adriatic Sea, in the three years after their deployment. It represents the first attempt at large scale to understand if and to what extent such effects can vary in different marine environments.

2. Materials and methods

2.1. Study area and sampling

The Adriatic Sea is an elongated basin with a NW-SE orientation; it

Table 3

is about 800 km long and 200 km wide. The northern area is shallow, rarely exceeding a depth of 46 m, whereas in the central part depth reaches 270 m (Pomo Pit). The continental slope lies ca. 500 km from the northern border of the Adriatic Sea and separates the central part of the basin from the southern one, where depth reaches 1200 m (South Adriatic Pit). The main circulation is dominated by the Eastern Adriatic Current (EAC), flowing counter clockwise from SE to NW along the eastern side, and the Western Adriatic Current (WAC), flowing from NW to SE along the western one.

The western side is characterized by soft bottoms gradually changing from mud inshore to relict sands offshore. Moreover, the inshore area is affected by a great river inflow generating an abundant nutrient input, which enhances primary production near the surface and oxygen demand close to the bottom with periodic bottom hypoxia and anoxia, high organic matter input and high sedimentation rates (Crema et al., 1991; Moodley et al., 1998; Marini et al., 2008; Djakovac et al., 2015). In contrast, the offshore area is characterized by more stable conditions in respect to inshore, being less affected by the Adriatic cyclonic circulation and the terrigenous contribution from rivers.

Seven pipelines (here named with the numbers P1–P7; Fig. 1; Table 1) placed in different types of sediments, varying from silty clay to relict sands, distance from the coast (from 20 to 60 km) and depths (from 21 to 58 m) in the western Adriatic Sea were investigated. The structures have different length and are spread over an area of about 2600 km².

Pipelines 1–4, being deployed on a granulometrically homogenous seabed, were sampled at about half of their length (sampling points named A, B, C and D; Fig. 1 and Table 1). Pipeline 5 was installed on a

Summary of SIMPER analysis applied to data of the three groups. Average abundances (Avg. abund.), % contribution (Contrib%), % cumulative contribution (Cum.
%) to the average similarity are given for each species. Only the first ten species for each group are reported. Average dissimilarity (%) between groups is also
ndicated. $C-S = clayey silt; S-C = silty clay; S = sand.$

C-S & S-C	C-S Avg Abund.	S-C Avg Abund.	Contrib. %	Cum. %	Avg Dissimilarity %
Corbula gibba (Olivi, 1792)	5.9	2.0	2.6	2.6	65.1
Nucula nucleus (Linnaeus, 1758)	3.0	0.0	1.8	4.4	
Antalis inaequicostata (Dautzenberg, 1891)	2.3	0.0	1.4	5.8	
PARAONIDAE Cerruti, 1909	0.0	2.2	1.4	7.2	
Glycera rouxi Audouin & Milne Edwards, 1833	0.0	2.1	1.4	8.6	
Capitella capitata (Fabricius, 1780)	2.6	0.3	1.4	10.0	
Upogebia tipica (Nardo, 1869)	2.2	0.0	1.4	11.4	
Nassarius incrassatus (Strøm, 1768)	2.1	0.0	1.3	12.7	
Glycera unicornis Lamarck, 1818	2.0	0.0	1.3	14.0	
Amphiura chiajei Forbes, 1843	0.8	2.1	1.1	15.1	
S & S-C	S Avg Abund.	S-C Avg Abund.	Contrib. %	Cum. %	Avg Dissimilarity %
Ditrupa arietina (O. F. Müller, 1776)	6.0	0.0	1.3	1.3	77.3
SABELLIDAE Latreille, 1825	4.9	0.2	1.0	2.3	
Prionospio sp. Malmgren, 1867	4.3	0.0	1.0	3.3	
Myrtea spinifera (Montagu, 1803)	4.5	0.2	0.9	4.2	
Hilbigneris gracilis (Ehlers, 1868)	4.3	0.2	0.9	5.1	
SIPUNCULA	4.9	0.8	0.9	7.0	
Aspidosiphon muelleri Diesing, 1851	4.8	0.8	0.8	7.8	
Prionospio cirrifera (Wirén, 1883)	5.7	2.0	0.8	8.6	
PARAONIDAE Cerruti, 1909	5.8	2.2	0.8	9.4	
Ophelina cylindricaudata (Hansen, 1879)	4.1	0.4	0.8	10.2	
S & C-S	S Avg Abund.	C-S Avg Abund.	Contrib. %	Cum. %	Avg Dissimilarity %
PARONIDAE Cerruti 1909	5.8	0.0	11	1.1	80.2

PARONIDAE Cerruti, 1909	5.8	0.0	1.1	1.1	80.2
Ditrupa arietina (O. F. Müller, 1776)	6.0	0.0	1.1	2.2	
SABELLIDAE Latreille, 1825	4.9	0.0	0.9	3.2	
Prionospio cirrifera (Wirén, 1883)	5.7	1.5	0.9	4.1	
Myrtea spinifera (Montagu, 1803)	4.5	0.0	0.9	5.0	
Aphelochaeta filiformis (Keferstein, 1862)	4.4	0.0	0.8	5.9	
Prionospio sp. Malmgren, 1867	4.3	0.0	0.8	6.7	
Glycera rouxi Audouin & Milne Edwards, 1833	4.3	0.0	0.8	7.5	
Ampharete acutifrons (Grube, 1860)	4.3	0.0	0.8	8.3	
Aspidosiphon muelleri Diesing, 1851	4.8	0.6	0.8	9.1	

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Table 4

Summary of SIMPER analysis applied to data of each group. Average abundances (Avg. abund.), % contribution (Contrib%), and % cumulative contribution (Cum.%) to the average similarity are given for each species. Only the first ten species for each group are reported. Average similarity (%) inside each group is also indicated.

Group/taxa	Avg Abund.	Contrib. %	Cum. %	Avg Similarity %
Silty clay (A, H, I, L)				61.4
Sternaspis scutata (Ranzani, 1817)	4.5	7.2	7.2	
Hyala vitrea (Montagu, 1803)	3.1	4.9	12.1	
Glycera rouxi Audouin & Milne Edwards, 1833	2.1	3.4	15.5	
NEMERTEA	1.8	3.3	18.8	
Nepthys hystricis McIntosh, 1900	2.1	3.1	21.9	
Amphiura chiajei Forbes, 1843	2.1	2.9	24.8	
Ampelisca intermedia Bellan-Santini & Diviacco, 1990	1.9	2.9	27.7	
Prionospio cirrifera (Wirén, 1883)	2.0	2.8	30.5	
Labioleanira yhleni (Malmgren, 1867)	1.8	2.7	33.2	
Kurtiella bidentata (Montagu, 1803)	1.7	2.5	35.7	
Clayey silt (B, C)				46.4
Corbula gibba (Olivi, 1792)	5.9	7.7	7.7	
Sternaspis scutata (Ranzani, 1817)	4.8	4.8	12.5	
Hyala vitrea (Montagu, 1803)	3.2	3.7	16.2	
Ampelisca intermedia Bellan-Santini & Diviacco, 1990	2.6	3.5	19.7	
Nucula nitidosa Winckworth, 1930	2.4	2.9	22.6	
Capitella capitata (Fabricius, 1780)	2.6	2.8	25.4	
Kurtiella bidentata (Montagu, 1803)	2.9	2.3	27.7	
Antalis inaequicostata (Dautzenberg, 1891)	2.3	2.3	30.0	
Nucula nucleus (Linnaeus, 1758)	3.0	2.3	32.3	
Upogebia tipica (Nardo, 1869)	2.2	2.2	34.5	
Sand (D, E, F, G)				73.7
Prionospio cirrifera (Wirén, 1883)	5.7	1.5	1.5	
PARONIDAE Cerruti, 1909	5.8	1.5	3.0	
Ditrupa arietina (O. F. Müller, 1776)	6.0	1.2	4.2	
Hilbigneris gracilis (Ehlers, 1868)	4.3	1.1	5.3	
Myrtea spinifera (Montagu, 1803)	4.5	1.1	6.4	
Glycera rouxi Audouin & Milne Edwards, 1833	4.3	1.1	7.5	
Aspidosiphon muelleri Diesing, 1851	4.8	1.1	8.6	
SIPUNCULA	4.9	1.1	9.7	
Prionospio sp. Malmgren, 1867	4.3	1.1	10.8	
SABELLIDAE Latreille, 1825	4.9	1.1	11.9	



Fig. 2. PCO ordination with projection of individual taxa onto the ordination axes. Pipelines A, H, I, L = silty clay; pipelines B, C = clayey silt; pipelines D, E, F, G = sand.

sandy bottom but, due to its length (> 40 km) and depth range (from 47 to 60 m), it was sampled at two different points (points E and F). Similarly, investigations on P6 and P7 were carried out at two different points along their route, because they were installed on non-homogenous bottoms (P6: points G and H; P7: points I and L; Fig. 1 and Table 1).

Each pipeline was sampled for 3 years starting just from its installation (first sampling: within 3 months from installation; 2 surveys/ year, in winter and summer). At each survey six sampling stations were randomly selected within a radius of 100 m from each point. This distance was established as the maximum distance of influence of the structures on benthic community during specific monitoring programs (unpublished data). In addition, six reference sites 1000 m away from the structure (K) were sampled for each sampling point on the pipelines (A-L). At each site six replicates of sediment were collected using a Van-Veen grab (capacity = 13 L; surface = 0.1 m^2). The samples were sieved on board through a 0.5 mm mesh and the benthic organisms preserved in 5% buffered formalin. In the laboratory, macrofauna was sorted using a stereomicroscope and a binocular microscope, identified to the species level when possible, counted and weighed (fresh weight). The taxonomic nomenclature followed the World Register of Marine Species (WoRMS; Horton et al., 2018). Sediment samples were taken through a box corer and analysed for their grain particle size and organic matter content (OM). Particle size was analysed according to the Udden-Wentworth Phi classification (Wentworth, 1922). Each sample was washed in 16% hydrogen peroxide for 24 h and then wet sieved on a 63-µm mesh to sort out the fine fraction. The sand fraction was sieved through a stack of geological test-sieves ranging from 0 Phi to +4 Phi. The fine fraction was analysed by sedigraph. Organic matter (OM) was estimated according to Schumacher (2002).

2.2. Data analysis

To gather the pipelines, a hierarchical Cluster Analysis was applied to granulomeric data. Prior to analysis, the sediment grain size were normalized and a matrix based on Euclidean distance was calculated. The Cluster analysis grouped P and K into three groups: silty clay (S-C), clayey silt (C-S) and sand (S; Table 1).

Table 5

Number of taxa (total and mean richness ± standard deviation) recorded at each group (pipelines and reference sites).

Phylum/Subphylum	Silty clay (A, I	H, I, L)	Clayey silt (B	, C)	Sand (D, E, F	7, G)
	Total	Average	Total	Average	Total	Average
Porifera					3	2.0 ± 0.7
Cnidaria	5	2.5 ± 1.0	8	5.0 ± 3.0	8	5.8 ± 1.0
Platyhelminthes			1	1.0 ± 0.0	1	1.0 ± 0.0
Nematoda	1	0.3 ± 0.3				
Nemertea	1	1.0 ± 0.0	1	1.0 ± 0.0	1	1.0 ± 0.0
Mollusca	23	13.8 ± 1.5	49	33.5 ± 13.5	110	72.8 ± 5.0
Polychaeta	57	31.8 ± 3.3	44	31.5 ± 9.5	118	93.3 ± 3.8
Echiura	1	1.0 ± 0.0				
Sipuncula	2	1.8 ± 0.3	2	1.5 ± 0.5	5	3.8 ± 0.3
Pycnogonida					1	1.0 ± 0.0
Crustacea	30	14.8 ± 2.3	27	17.0 ± 8.0	122	82.3 ± 10.1
Bryozoa					5	3.0 ± 1.1
Echinodermata	8	6.5 ± 0.6	12	7.5 ± 3.5	18	13.0 ± 1.4
Tunicata					4	2.0 ± 0.4
Total	128	$73.3~\pm~5.5$	144	98.0 ± 38.0	395	$280.8~\pm~21.7$

Multivariate analyses were performed to analyse the composition of macrofauna communities. Prior to any analysis, the species abundance data were fourth-root transformed to reduce the contribution of prevalent taxa and therefore increase the importance of less abundance species. Afterwards, the Gower exc 0e0 similarity matrix was calculated. Gower coefficient is well-suited for quantitative abundance data excluding double-zeros from comparison (Legendre and Legendre, 1998).

A permutational analysis of variance (PERMANOVA; Clarke, 1993; Clarke and Warwick, 2001) based on the abundance data of the species was used to verify if: a) there were differences in terms of benthic composition among the three groups identified on the base of their grain size (1-way PERMANOVA with Sediment as three levels fixed factor), b) there was a homogeneity between P and K sites inside each group (1-way PERMANOVA with site as two levels fixed factor), and c) there was a homogeneity between the two sampling seasons inside each group (1-way PERMANOVA with season as two levels fixed factor). As no differences were found between winter and summer, the seasonal data were treated together.

Significant terms derived by PERMANOVA were investigated using Pair-Wise comparison.

Moreover, multivariate analyses were performed to identify how the benthic assemblage changed in relation to the type of sediments. Firstly, the unconstrained Principal Coordinates (PCO) plot on averaged data was applied and a projection plot was drawn onto PCO axes (Anderson et al., 2008). Successively, similarity percentage breakdown procedure (SIMPER; Clarke and Warwick, 2001) was used to determine the contribution of individual taxa to dissimilarities among the three groups and to similarities inside each group.

To verify possible spatial and temporal differences between the P and K sites, univariate measures such as species abundance (N; number of individuals m⁻²), species richness (R), and Shannon–Weiner diversity (H'; Pielou, 1974) were calculated in each sampling year and the mean values (\pm standard deviation) were computed within each group.

Considering that no transformation of data achieved assumptions of normality and homoscedasticity, statistical differences between P and K sites over time were evaluated through a (non-parametric) rank-based ANOVA (Hettmansperger and McKean, 2011; Kloke and McKean, 2014), testing the main effects and interactions of sites and years. When the rank-based ANOVA indicated significant main effects (p < 0.05), differences between sites and/or years were tested through the Tukey test (Zar, 1984).

BENTIX index (Zenetos et al., 2004) (Bentix Add-In v. 1.0) was performed for each group comparing sites (P and K) over time to determine the environmental status. This index is based on the relative individual percentages of species classified into two groups according to their sensitivity or tolerance to stress (Simboura and Argyrou, 2010). The thresholds utilized are those reported in Simboura and Argyrou (2010). BENTIX is generally applied to assess the Environmental Quality Status (EQS) of estuarine and coastal waters but it has been also tested and recently adopted to evaluate the stress level induced by offshore activities (Gomiero et al., 2013a, 2013b; Spagnolo et al., 2014; Punzo et al., 2017).

Finally, to verify the existence of a significant relationship between data on benthic communities and environmental data, the Distance based Linear Models (DistLM) forward selection procedure was utilized (Anderson et al., 2008). DistLM performs a multivariate multiple regression on the basis of any distance measure and a forward selection of the predictor variables, individually, with tests by permutation. The results are a marginal test, fitting each variable individually and ignoring other variables and a conditional test, fitting each variable one at a time, (Anderson et al., 2008). The distance-based redundancy analysis (dbRDA, Legendre and Anderson, 1999) was performed to explicitly investigate the relationship between the community assemblages and the environmental variables pointed out by the DISTLM forward procedure (Anderson et al., 2008).

Both univariate and multivariate analyses were conducted with PRIMER[™] ecological software package vers. 6 & PERMANOVA + (Clarke, 1993; Clarke and Warwick, 2001), while the rank-based ANOVA was performed using the Rfit package (Kloke and Mckean, 2012) from the software R.

3. Results

Multivariate patterns - PERMANOVA applied to biotic data confirmed the differences in the macrozoobenthic communities inhabiting S, C-S and S-C groups identified on the basis of the sediment composition (Table 2). Instead, no differences were highlighted between P and K sites inside each group; for this reason SIMPER analysis was performed grouping P and K sites inside each group.

This analysis (Table 3) showed the highest dissimilarity (80.2%) between S and C-S, with the polychaetes Paraonidae nd Cerruti, 1909, *Ditrupa arietina* (O. F. Müller, 1776) and Sabellidae nd Latreille, 1825 as main contributors, all collected at S but not at C-S. The lowest dissimilarity was obtained between C-S and S-C (65.1%). In this case, the main contributors were the molluscs *Corbula gibba* (Olivi, 1792), *Nucula nucleus* (Linnaeus, 1758) and *Antalis inaequicostata* (Dautzenberg, 1891), more abundant or only observed at C-S.

Inside each group the highest similarity (73.7%) was obtained for S and the lowest (46.3%) for C-S (Table 4).

PCO plot (78.8% of total variation) well showed this pattern,

significant $p > 0.05$; s	Source of variability	DF	$R_{\rm m}$				$N_{\rm m}$				$\mathrm{H'_m}$			
			RD	Mean RD	F	<i>p</i> -Value	RD	Mean RD	F	<i>p</i> -Value	RD	Mean RD	F	<i>p</i> -Value
S-C group (A, H, I, L)	Site (P vs K)	1	52.0561	52.0561	14.9676	0.0014 P > K	0.6246	0.6246	0.1268	0.7265	0.4128	0.4128	4.3013	0.0546
	Year	2	23.6105	11.805	3.3944	0.0591	7.8225	3.9113	0.7938	0.4691	0.8918	0.4459	4.6460	$0.0256 \ 3 > 1$
	Site \times year	2	3.6283	1.8142	0.5216	0.6033	3.0620	1.5310	0.3107	0.7372	0.0312	0.0156	0.1629	0.8511
C-S group (B, C)	site (P vs K)	1	101.3078	101.3078	8.9795	0.0241 P > K	0.0000	0.0000	0.0000	1.0000	0.3255	0.3255	1.2604	0.3045
	year	2	2.7136	1.3568	0.1203	0.8888	41.9101	20.9550	0.4060	0.6833	0.1736	0.0868	0.3361	0.7272
	Site \times year	2	2.4121	1.2061	0.1069	0.9003	24.1209	12.0604	0.2337	0.7985	0.0211	0.0101	0.0409	0.9602
S group (D, E, F, G)	Site (P vs K)	1	187.5462	187.5462	17.6677	0.0005 P > K	266.7232	266.7232	2.8247	0.1101	0.0000	0.0000	0.0000	1.0000
	Year	2	176.6355	88.3177	8.3199	0.0028.3 > 1	145.9675	72.9838	0.7729	0.4764	0.0314	0.0157	0.2246	0.8010
	Site \times year	2	15.4814	7.7407	0.7292	0.4960	62.5154	31.257	0.3310	0.7225	0.0310	0.0155	0.2215	0.8030

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highlighting the similarities inside each group and the uniqueness of the groups (Fig. 2).

Univariate patterns - Overall, 510 taxa were identified. They were mainly polychaetes (144), crustaceans (138) represented above all by amphipods (65) and decapods (44), and molluscs (135), especially bivalves (80) and gastropods (52). The highest number of taxa (395) were observed at S (R_m: 280.8 \pm 21.7), 144 at C-S (R_m: 98.0 \pm 38.0) and 128 at S-C (R_m : 73.3 ± 5.5; Table 5).

Porifera, Pycnogonida, Bryozoa and Tunicata were found only at S, Nemertea and Echiura only at S-C. Platyhelminthes were absent at S-C.

Analyzing the variation of R_m inside each group over time, a statistical difference among years was only found for S, due to a progressive increment of the index both at P and K sites (Table 6, Fig. 3). Instead, significant differences were highlighted within each group between P and K, with the highest values at the former.

The lowest density (N) was obtained for S-C, where it varied between 284.2 \pm 21.1 and 392.1 \pm 68.9 ind m $^{-2}$ (P) and between 257.9 ± 1.5 and 381.6 ± 8.2 ind m⁻² (K). Polychaetes, e.g. Sternaspis scutata (Ranzani, 1817), Paraonidae nd Cerruti, 1909, and molluscs such as Hyala vitrea (Montagu, 1803) and C. gibba, were the most abundant taxa contributing from 51% to 57% and from 21% to 24% to the total density at P and K sites respectively.

The highest density was observed for S, varying between 3792.1 ± 707.6 and 5244.7 ± 95.7 ind m⁻² (P) and between 2415.8 \pm 675.6 and 3571.1 \pm 1142.1 ind m $^{-2}$ (K). In this case, the polychaetes D. arietina, Paraonidae nd, Prionospio cirrifera Wirén, 1883 and Sabellidae nd Latreille, 1825 were the most abundant taxa. Intermediate N values were recorded for C-S (K: 478.9 \pm $26.3 \le N \le 2142.1 \pm 1700.0 \text{ ind } \text{m}^{-2}$; P: $868.4 \pm 79.0 \le N \le 1000 \text{ km}^{-2}$ 1915.8 \pm 926.3 ind m⁻²). Within this group *C. gibba*, *H. vitrea*, *N.* nucleus and S. scutata dominated the community. In particular C. gibba represented 24% of the total density both at P and K sites. No statistical differences were found inside each group comparing both site and time (Table 6, Fig. 3).

The highest values of H' were also obtained for S; a homogeneity was observed inside each group except for S-C, characterized by an increasing diversification from the first monitoring year to the third one (Table 6, Fig. 3).

Environmental status - The BENTIX index (Fig. 4) classified the surroundings of S-C and C-S pipelines and respective reference sites as "moderate", and S sites (P and K) as "good". It was due to a high dominance of opportunistic taxa in the first two typologies of sediments (e.g., C. gibba, S. scutata, H. vitrea), ranging from 67.2% to 81.7% (S-C) and from 67.2% and 86.3% (C-S). The lowest value within these two groups was recorded at C-S K in the third year.

On sand, the lowest BENTIX value (3.51) was recorded at P sites during the first year as a consequence of a great abundance of D. arietina, classified as an opportunistic species by the index. The percentages of sensitive taxa was always higher than those recorded at C-S and S-C, varying between 38.2% and 52.2% at P and between 43.1% and 48.8% at K sites.

Relationship between biotic and abiotic variables - All the environmental variables except silt were statistically significant or highly significant on the basis of the marginal tests (Table 7a). In the subsequent sequential test depth became unnecessary, and OM not statistically significant for the combined model (Table 7b). Sand was the variable with the greatest power, explaining 56.13% of the variation in the biotic data, followed by clay (20.80%). The first two dbRDA axes (Fig. 5) explained 100% of the fitted variation, accounting for 76.9% of the total variation in the resemblance matrix. Also in this case there was a clear separation among the three groups linked with the grain size and the distribution pattern of the pipelines was similar to that obtained by the PCO plot. This similarity indicates that the constrained representation explains the most salient pattern of variation across the data cloud as a whole.



Fig. 3. Temporal variation of univariate indices of macrofaunal communities (\pm standard deviation) on each type of substrate. Rm = mean species richness; N = density; H' = Diversity index; K = control site; P = pipeline.

4. Discussion

The sustainable exploitation of the oceans and their natural resources requires for a deep knowledge of the impacts caused by human activities on the different components of the marine ecosystems, in order to allow the development of measures and policies aimed to mitigate impacts and avoid detriment to the marine environment and its resources. Usually, the installation of pipelines is not considered as impacting the surrounding environment (Philip and McCart, 1981; Tsui and McCart, 1981; Tillinghast et al., 1987; Young and Mackie, 1991; Pranesh and Kumar, 1993; Rezai et al., 1999; Lewis et al., 2002, 2003); nevertheless, there is still a scarce scientific evidence around the world supporting such assumption, especially with regards to the possible effects induced by laying and presence of these structures on the biotic components.

Based on such statement, in this study a number of pipelines installed at different distance from the Italian coast of the Adriatic Sea (from 20 to 60 km), depth (from 21 to 58 m) and sediment typology were investigated, to get a reliable picture of the possible effects of these structures in the three years after deployment on the local benthic communities.

As a matter of fact, we found significant differences between benthic communities living in the surroundings of pipelines installed on S-C and C-S (from 20 to around 35 m depth) and those occurring on S (40–60 m depth).

S-C and C-S communities appeared rather similar between themselves and very different from S community. The similarity between the S-C and C-S was due to the occurrence of a few common species, such as *H. vitrea, S. scutata, Ampelisca intermedia* Bellan-Santini & Diviacco, 1990 and *Kurtiella bidentata* (Montagu, 1803), most of which belonging to the Biocoenosis of coastal terrigenous mud (Pérès and Picard, 1964), which dominated both in terms of species richness (21% of the total population at C-S, 30% at S-C) and density (25% of the total population at C-S, 70% at S-C).

The differences between these two types of substrate were due to

few species, above all the bivalve *C. gibba*; it belongs to the Biocoenosis of unstable soft bottoms and is known as a pioneer species in recolonization of defaunated bottoms (Bonvicini Pagliai et al., 1985; Curini Galletti, 1987; Crema, 1989) thanks to its high reproduction potential. It is widespread on soft bottoms along the northern Adriatic coast (Aleffi and Bettoso, 2000; Hrs-Brenko, 2006; Fabi et al., 2008) characterized by environmental instability. According with Aleffi and Bettoso (2000), the greater abundance of *C. gibba* at C-S sediments could be linked to the lower depth where B and C pipelines are installed in respect to A, H, I and L. These findings also agree with the *Corbula* abundance range reported for the same area by Crema et al. (1991), Simonini et al. (2004) and Moodley et al. (1998).

More broadly, most of the above mentioned species are considered as opportunistic by BENTIX index that classified both S-C and C-S environments as moderate in each study year, without differences between P and K sites. This classification is congruent with the features of the northern Adriatic area and suggests that the installation and presence of pipelines in such environments do not affect the local benthic communities. It is worthy to note that, in this basin, the zoobenthic assemblages do not appear particularly stressed even by other widespread human activities, such as mariculture and harbour sediment dumping (Danovaro et al., 2004; Simonini et al., 2005a, 2008; Fabi et al., 2009; Punzo et al., 2012). Surprisingly, even potentially impacting fishing activities (e.g., hydraulic dredge for clams, rapido-trawl for soles) have low, short-time effects on benthic communities in the northern Adriatic Sea (Pranovi and Giovanardi, 1994; Pranovi et al., 1998, 2000; Morello et al., 2005), due to the occurrence of either opportunistic species or other K-strategy organisms which, however, can assume an opportunistic behaviour in particular situations of direct disturbance (Pranovi et al., 1998).

Differently from the C-S and S-C pipelines, a very diversified population was found in the surroundings of S P and K sites. The presence of a such offshore community, consisting of many taxa each represented by a few individuals, had been already described by Vatova (1947). Species typical of the Biocoenosis of well-sorted sand, such as the



Fig. 4. Values of BENTIX and environmental status classification obtained for each group of pipelines. P = pipeline; K = control site; 1-3 = monitoring years.

polychaetes Owenia fusiformis Delle Chiaje, 1844 Hilbigneris gracilis (Ehlers, 1868) and Syllis parapari San Martín & López, 2000 and the gastropod C. cylindracea, dominated as richness (18% of the total population). Instead, organisms belonging to the Biocoenosis of unstable soft bottoms, especially D. arietina (Pérès and Picard, 1957), and indicators of organic matter such as P. cirrifera and Myrtea spinifera (Montagu, 1803) (Bianchi et al., 1993), appeared more important in terms of density. As C. gibba, D. arietina is a pioneer species whose abundance tends to increase during the development of transitional communities after environmental changes (Pérès and Picard, 1964). Indeed, it was more abundant at S-P sites in comparison to K sites (about 3 times) in the first year after the pipeline installation, gradually decreasing in the subsequent years until reaching comparable densities to those recorded at K sites. This suggests an initial and lightly unstable situation just after pipelines deployment in such environment, likely due to the higher stability which characterizes the offshore Adriatic basin.

The occurrence at the offshore sandy habitat of organic matter indicators, in spite of the low organic matter content, it is not surprising

Table 7				
Summary of DistLM analysis. Not sig percentage of variance explained.	mificant p > 0.05; significant	t 0.05 $> p > 0.01$; highly signifi	cant $p < 0.01$. Prop.: proportion; Cum.:	cumulative
(a) Marginal tests	F	d	Prop.	
Sand	10.234	0.001	0.561	
Silt	0.786	0.727	0.089	
Clay	6.863	0.003	0.462	
OM	3.742	0.034	0.319	
Depth	7.538	0.002	0.485	
(b) Sequential tests	F	р	Prop.	Cum. %
Sand	10.235	0.003	0.561	56.13
Clay	6.290	0.030	0.208	76.89
OM	2.057	0.128	0.059	82.79



Fig. 5. Distance-based RDA ordination relating environmental variables to benthic fauna data. Pipelines A, H, I, L = silty clay; pipelines B, C = clayey sit; pipelines D, E, F, G = sand.

having been also found in other studies carried out in the northern and central Adriatic Sea since 1940 (Vatova, 1947; Simonini et al., 2004, 2005b). Moreover, it is noteworthy that the densities of these taxa at S-P and K sites were very similar, suggesting a spatial distribution depending on natural features rather than human activities, exclusively represented in this area by fishery and gas extraction. In spite of the initial disturbance, BENTIX classification indicated a good environmental status everywhere since the first year after the pipelines installation, with comparable percentages of sensitive and tolerant taxa, proving the absence of or a very limited impact.

In the overall, the results of this study show that, as expected, the installation of pipelines in the northern and central Adriatic Sea does not cause any defaunation nor evident variation in the local benthic assemblages, as instead observed in the case of offshore platforms placed in the same areas just after installation (Fabi et al., 2005, 2007; Manoukian et al., 2010; Gomiero et al., 2013a). According with Semprucci et al. (2010), the qualitative and quantitative differences among the benthic populations inhabiting the investigated areas are dependent of environmental features. These findings agree with those reported by Lewis et al. (2002), who did not observe any relevant change in the benthic communities inhabiting the Clonakilty Bay (western Ireland) one month before and six months after the installation of a pipeline. Also, Rezai et al. (1999) evidenced a minimum disturbance and a very rapid recovery of the coral community around one month after the construction of a submarine pipeline at Redang Island (Malaysia).

Nevertheless, in the central and northern Adriatic sea the presence of pipelines seems to increase the quantity of species in respect to areas without structures independently of the type of the substrate, as demonstrated by the higher values of species richness recorded in the surroundings of the structures in comparison to the reference sites. This may be related to abundant detritus, preys, and predators close to the pipelines, which may create micro-currents that facilitate sediment resuspension and detritus deposition on the seabed, attracting suspension and deposit feeders. Obviously the effect is lower than that observed around gas platforms, where the shells of *Mytilus galloprovincialis* Lamarck, 1819 specimens falling down from the submerged parts of the structures provide natural hard substrates which form new habitat for marine epifaunal organisms rarely occurring in soft bottom communities (Wolfson et al., 1979; Page et al., 1999; Stachowitsch et al., 2002; Fabi et al., 2005, 2007; Currie and Isaacs, 2005; Trabucco et al., 2006, 2008; Manoukian et al., 2010; Gomiero et al., 2013a).

Besides to increase the available scientific knowledge on the impacts induced by the pipeline construction, the findings of this study may be also relevant for Descriptor 6 'Sea floor integrity' of the Marine Strategy Framework Directive (MSFD; EU, 2012), including offshore installations among the main pressures on the seafloor, as they provide useful data to classify the northern and central Adriatic Sea as an area moving towards or failing the achievement of the Good Environmental Status.

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