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Application of the bentix index in assessing ecological quality of hard substrata: a case study from the Bosphorus Strait, Turkey

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Abstract

In this paper, a biotic index (Bentix) has been used for the assessment of ecological quality status of shallow water hard substrate benthic ecosystems affected by coastal sewage discharges in the Bosphorus Strait. A significant difference was observed between the control and the discharge stations with regard to Bentix values (Mann-Whitney U Test, p=0.002) and ecological quality status of the discharge stations was worse than that of controls. The index values revealed that sewage discharges caused serious disturbance in macrozoobenthic communities in the area investigated. Although so far it has been used for soft bottom communities, Bentix (with some species scoring modifications) also appeared to work successfully in hard substrates, at least for the present study.

Keywords: Benthos; Hard substrates; Sewage discharge; Biotic index; Ecological quality; Bosphorus Strait.

Introduction

Marine benthic communities are studied as indicators of changes and disturbance in marine environments. Evaluation of the structure of benthic communities is more advantageous compared to experimental methods. Benthic community parameters are very popular tools for in situ assessment of the ecological status related to pollution in marine environments (GRAY, 1980; WESTON, 1990; DELL VALLS *et al.,* 1998).

It is expected that along a gradient of pollution there is a changing pattern of species abundance owing to the species' different level of response to pollution. Fauna respond to pollution by moving, tolerating it or dying (GRAY *et al.,* 1988). In a benthic community, the most frequently observed response is that some species increase in abundance, many decrease in abundance and others remain unaffected. Thus, species abundance patterns have been documented to reflect the pollution effects integrated over time and hence are widely used in monitoring the pollution effects in subtidal bottoms (GRAY *et al.,* 1988).

A number of publications exist concerning the effects of sewage discharges on macrobenthos living on rocky subtidal substrates (e.g. LITTLER & MURRAY, 1975; AXELRAD *et al.,* 1981; MAY, 1985; FAIRWEATHER, 1990; LÓPEZ GAPPA *et al.,* 1990, 1993; UNDERWOOD & CHAPMAN, 1996; BISHOP *et al.,* 2002; VALLARINO *et al.,* 2002). However, along the coasts of the Mediterranean Sea, although a large amount of sewage is discharged into the sea (EEA, 2006), limited studies have been carried out (e.g. BELLAN-SANTINI, 1968; BELLAN, 1980; TERLIZZI *et al.,* 2002, 2005; PINEDO *et al.,* 2007).

To be able to detect anthropogenic stress and disturbance in macrobenthic communities a number of concepts and numerical techniques (diversity indices, multivariate tools, graphical representations, indicator species and biotic indices) have been developed and summarized in the UNEP/MAP (2004).

Biotic indices approach ecological quality through the use of the indicator organism concept and they take into account changes in taxa. One of these biotic indices has been suggested by United Nations Environmental Programme / Mediterranean Action Plan for use in Mediterranean coastal areas (UNEP/MAP, 2004). This biotic index (Bentix) was designed to fit the Mediterranean benthic ecosystem for the classification of ecological quality status of soft substrate macrozoobenthic communities. Bentix is based on the concept of indicator species and uses the relative percentages of two general ecological groups of species, the 'tolerant' and the 'sensitive' grouped according to their sensitivity or tolerance to disturbance factors (SIMBOURA & ZENETOS, 2002; SIMBOURA *et al.,* 2005).

The purpose of the present study is to test the applicability of the Bentix index in assessing the ecological quality status (ECoQ) of shallow water hard substrate benthic ecosystems affected by coastal sewage discharges in the Bosphorus Strait.

Materials and Methods

Study area

The Bosphorus Strait, one of the two straits in the Turkish Straits System, constitutes a pathway between the Aegean and the Black seas through the Sea of Marmara and the Dardanelles Strait. It is a narrow, elongated, shallow channel of nearly 31 km in length. The Strait has a well-defined two-layer stratification associated with a two-layer pattern of water exchange. The southward flow is driven by the sea level difference between its two ends. The northward flow, on the other hand, is driven by the difference in density, which is predominantly governed by the salinity between the Sea of Marmara and the Black Sea. Consequently, relatively fresh (brackish) Black Sea waters flow towards the Sea of Marmara on top of the more saline and denser waters of the Sea of Marmara flowing in the opposite direction (GUNNERSON & ÖZTURGUT, 1974; OĞUZ *et al.,* 1990).

The salinity of the upper layer varies between 16.5-18.5 psu. On the other hand, the salinity of the lower layer attains a maximum value of 38.5 psu near the Marmara end of the Strait and decreases progressively towards the northern exit (OĞUZ *et al.,* 1990; SUR *et al.,* 1994).

Because of its biological, physiographical and hydrological characteristics, the Bosphorus Strait hosts a unique ecosystem. As a part of the Turkish Straits System, which plays a significant role in the biology of the Mediterranean and the Black Sea basins, the Strait represents a transition zone between the Mediterranean and the Black Sea (ÖZTÜRK & ÖZTÜRK, 1996).

Data set

Quantitative and qualitative data are derived from a study carried out seasonally at 15 stations in May, August, and November 2004 and February 2005 on the rocky shores of the Bosphorus Strait (Fig. 1). Nine stations were chosen as discharge stations, and six stations as control stations. This choice was based mainly on

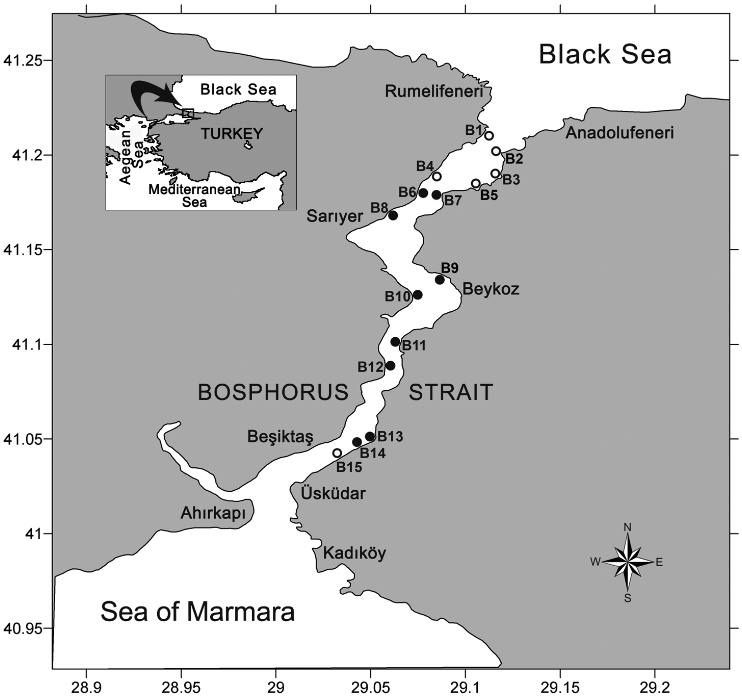

Fig. 1: Map of the study area showing the sampling stations (dots indicate the discharge stations and open circles indicate the control stations).

their distances from the pollution sources. Stations B6, B8, B9, B10, B11, B13 and B14 are directly influenced by sewage produced by the urban Istanbul metropolitan area. Stations B7 and B12 are subjected to indirect effects of sewage discharges. Stations B1, B2, B3, B4, B5 and B15 are located far from any discharge points. In addition, there is no habitation in the area surrounding stations B1, B2, B3 and B5. As far as possible, the stations were standardized with respect to abiotic factors in order to minimize the influence of these nuisance variables on the benthic communities. Location of sampling stations, their biotopes and pressure characterizations are given in Table 1.

Samples were collected from the upper infralittoral zone at a depth range of 0.5-1 m by scraping the hard substrate on a quadrate of 400 cm^2. At each sampling site and period, three replicates were taken. After collection the samples were fixed in 4% neutral formalin in seawater and they were quickly transferred to the laboratory for further processing. In the laboratory, all macrozoobenthic samples were sieved through a 0.5 mm mesh with tap water, sorted according to major taxonomic groups under a stereomicroscope, preserved in 70% ethanol or 4% neutral formalin and identified to the lowest possible taxonomic level.

Data analysis

The biotic index, Bentix, was calculated for the assessment of the ecological quality (ECoQ) of the study area according to the scores of two ecological groups described by SIMBOURA *et al.* (2005). The formula [6 x %GS + 2 x %GT]/100 assigns the numerical factor '6' to the sensitive taxa group GS and the factor '2' to

Table 1
Coordinates, biotope and pressure characterization of the sampling stations (C: control, D: discharge).

Station	Type	Coordinate	Biotope
B1	C	41°12'45"N, 29°06'40"E	*Cystoseira* spp.community
B2	C	41°12'51"N, 29°07'09"E	*Mytilus galloprovincialis* community
B3	C	41°11'16"N, 29°07'05"E	*Cystoseira* spp. community
B4	C	41°11'12"N, 29°04'42"E	*Mytilus galloprovincialis* community
B5	C	41°10'54"N, 29°06'24"E	*Cystoseira* spp. community
B6	D	41°11'03"N, 29°04'36"E	*Mytilus galloprovincialis* community
B7	D	41°10'38"N, 29°05'15"E	*Mytilus galloprovincialis* community
B8	D	41°10'10"N, 29°03'30"E	*Bryopsis* spp. + *Mytilus galloprovincialis* community
B9	D	41°07'15"N, 29°05'07"E	*Mytilus galloprovincialis* community
B10	D	41°06'22"N, 29°04'18"E	*Bryopsis* spp. community
B11	D	41°06'03"N, 29°03'54"E	*Mytilus galloprovincialis* community
B12	D	41°05'21"N, 29°03'27"E	*Mytilus galloprovincialis* community
B13	D	41°03'00"N, 29°03'12"E	*Bryopsis* spp. community
B14	D	41°02'42"N, 29°02'35"E	*Mytilus galloprovincialis* community
B15	C	41°02'15"N, 29°01'40"E	*Mytilus galloprovincialis* community

the tolerant taxa group GT. The scores of some ambiguous species and some species not found in the score list of Bentix were determined based on whether they were k-strategy or r-strategy species. Stations were classified according to their ECoQ by using the classification scheme given by SIMBOURA & ZENETOS (2002). The Bentix methodology and an extended list of species scores can be downloaded from the internet site: http://www.hcmr.gr/english_site/services/env_aspects/bentix.html.

Besides Bentix, the following indices were applied: Shannon-Wiener diversity index *(H')* (SHANNON & WEAVER, 1963), Pielou's evenness *(J)* (PIELOU, 1969), and Margalef's species richness *(d)* (MARGALEF, 1958) were calculated. The index of dispersion was applied to all data to test randomness.

The numerical abundance data were analyzed using non-metric multi-dimensional scaling (nMDS) techniques, based on Bray Curtis similarity, using the PRIMER package (version 5.0). Clustering aims to find natural groupings of samples such that samples within a group are more similar than samples in different groups. The cluster analysis, based on log (x+1) transformation with the 'Taylor's Power Law' method concepts (TAYLOR, 1961), was performed on the abundance data in order to identify groups of similar stations, among different station groups (control and target) and within the same region and then the similarity data were ordinated by nMDS (CLARK & WARWICK, 2001). The one-way ANOSIM permutation test was used to assess the significant differences between pre-defined groups of sample sites in the cluster analysis. SIMPER analysis was applied in order to identify the percentage contribution of each species to the overall similarity and the dissimilarity between stations. One-way ANOVA (Mann-Whitney U test) using the software package STATISTICA (version 6.0) was applied to test if differences observed in the indices assessment results were statistically significant.

Results and Discussion

The analysis of 180 samples yielded a total of 167,537 specimens belonging to 85 taxa of nine taxonomic groups (Cnidaria, Turbellaria, Nemertea, Polychaeta, Oligochaeta, Pycnogonida, Crustacea, Mollusca and Echinodermata). Crustaceans showed the highest biodiversity (43 species) followed by polychaetes (18 species) and molluscs (12 species). Diversity of other groups was clearly lower (<4 species). Appendix A represents the five most abundant species of benthic fauna in each sampling period and station, providing also their mean dominances (number of ind. of each species / total number of ind.) over the four seasons.

Community descriptive parameters and multivariate analyses

The dispersion index revealed that most species of the benthic community showed aggregated distribution. Therefore, the three replicate quadrates sampled in each sampling site were averaged and interpretations of the data were based on the averaged values.

The means of species richness, diversity and evenness of control and discharge stations are given in Table 2. Comparison of the samplings of the control and discharge stations by Mann-Whitney U test revealed a significant difference in each of the species' richness (p=0.0000) and diversity (p=0.0000). However, there was no significant difference in evenness (p=0.0592) between the control and discharge stations.

The species richness and diversity values of the discharge stations are lower than those of control stations. It is known that these community measures are expected to decrease at high levels of disturbance (HYLAND *et al.,* 2000). In this regard, the decrease of these community measures indicated disturbed community in discharge stations in the present study. In addition, in terms of community diversity, control stations seem better than discharge stations. The Shannon-Wiener index of diversity is one of most commonly used diversity indices in the assessment of pollution effects on marine benthic communities (SIMBOURA & ZENETOS, 2002). However, the use and interpretation of this index (and other diversity indices) are very controversial (CLARKE & WARWICK, 1994; JENNINGS & REYNOLDS, 2000). Although, decrease or increase in community diversity could not be used as a sole indicator of the health or stability of the ecosystem (NYBAKKEN, 1997; SIMBOURA & ZENETOS, 2002), it is known that the pollution perturbed benthic communities have low diversity values (HYLAND *et al.,* 2000). Relatively low diversity values in the discharge stations hence provide evidence for pollution effect on macrozoobenthic communities.

The nMDS configuration that resulted from the entire abundance matrix showed a separation of stations into two different groups corresponding to the control (B1, B2, B3, B4, B5 and B15) and the discharge (B6, B7, B8, B9, B10, B11, B12, B13 and B14) (Fig. 2). The performance of a one-way ANOSIM test gave global R= 0.392 at a significance level of 0.1%, so the separation of the two groups was confirmed.

SIMPER analysis of the transformed entire abundance data was applied to examine the species which contribute to the dissimilarity between control and discharge stations, as the result of the nMDS analysis showed that the control and discharge stations had different faunal compositions. The control stations showed an average similarity of 36.75%. According to SIMPER analysis, five species, *Mytilaster lineatus, Hyale pontica, Mytilus galloprovincialis, Platynereis dumerilii* and *Nereis (Hediste) diversicolor* alone were responsible for 80% of the average similarity. The discharge stations reached an average similarity of 26.54%. Four species, *Mytilus galloprovincialis, Hyale perieri, Echinogammarus olivii* and *Tanais dulongii* alone covered 80% of this value. The control stations were separated from the discharge stations by the presence of relatively high abundance of a few species including *Mytilaster lineatus, Mytilus galloprovincialis, Echinogammarus olivii, Hyale perieri, Jassa marmorata, Nereis (Hediste) diversicolor, Enchytraeus buchholzi, Platynereis dumerilii, Tanais dulongii, Hyale pontica, Erictho-*

Table 2

The mean values of the community descriptive measures at the control and discharge stations.

Community descriptive measures	Control Stations	Discharge Stations
Shannon diversity *(H')*	3.03 ± 0.32	2.53 ± 0.52
Evennes *(J)*	0.61 ± 0.06	0.57 ± 0.59
Margalef's species richness *(d)*	5.78 ± 0.28	5.90 ± 1.16

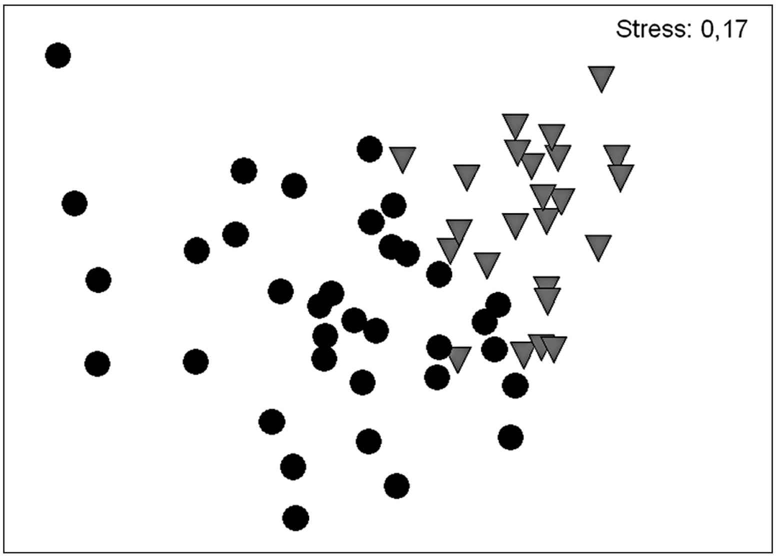

Fig. 2: Non-metric multidimensional scaling ordination (nMDS) plot derived from log-transformed abundance data. Circles indicate discharge stations and triangles indicate control stations.

nius brasiliensis, Opheliidae (sp.) and *Stenothoe tergestina* (Table 3). It is quite clear that only a few species are important in characterizing and differentiating the groups.

Bentix

As mentioned in materials and methods, the species are classified into one of the two ecological groups. These groups are: GS (includes species sensitive to disturbance in general and also the indifferent to disturbance)and GT(includes species tolerant to disturbance) (SIMBOURA *et al.,* 2005).

The percentages of sensitive species (GS) were higher than those of tolerant species (GT) at the majority of samplings at stations B1, B2, B3, B4 and B5. The high

Table 3

Results of SIMPER analysis showing species contributing most to dissimilarity between control and discharge stations. A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	**Contribution (%)**	
	Control	**Discharge**
Mytilaster lineatus	39.59	9.67
Mytilus galloprovincialis	17.34	43.66
Nereis (Hediste) diversicolor	7.73	1.28
Platynereis dumerilii	7.26	3.30
Hyale perieri	6.61	12.49
Tanais dulongii	0.53	7.12
Echinogammarus olivii	6.15	12.34

percentages of tolerant species (GT), on the other hand, were found at all sampling periods of B6, B7, B8, B9, B10, B11, B12, B13, B14 and B15. As Figure 3 shows, while discharge stations were characterized by the high ratio of tolerant species, relatively high abundance of sensitive species (GS) was found mostly at control stations, except B15.

The bivalve *Mytilus galloprovincialis,* the amphipods *Hyale perieri, Echinogammarus olivii* and *Jassa marmorata,* the tanaid *Tanais dulongii* and the oligochaete *Enchytraeus buchholzi* were found most abundant at discharge stations (see Appendix A). On the other hand, in the majority of control stations *Mytilaster lineatus* (Bivalvia), *Hyale pontica* (Amphipoda), Opheliidae (sp.) (Polychaeta), *Nereis (Hediste) diversicolor* (Polychaeta) and *Mytilus galloprovincialis* (Bivalvia) were the most dominant species. As is evident, those species having high dominance in the majority of control and discharge stations also contribute to the dissimilarity between control and discharge stations in SIMPER analysis (Table 3).

The polychaete, *Nereis (Hediste) diversicolor* has been reported as a characteristic species of polluted areas (ANGER, 1975). This species was found also on the edge of a grossly polluted area by GHIRARDELLI & PIGNATTI (1968) and in a polluted harbor area by TULKKI (1968). According to SMYTH *et al.* (1974) it was highly abundant and widely distributed on polluted shores. The other polychaete, *Platynereis dumerilii* was accepted as a dominant secondary colonizer (SANDERS *et al.,* 1972; GRASSLE & GRASSLE, 1974). In addition, these two species were classified as first order opportunistic species by SIMBOURA & ZENETOS (2002) and classified into the tolerant group of species scored as 2 in the extended list of

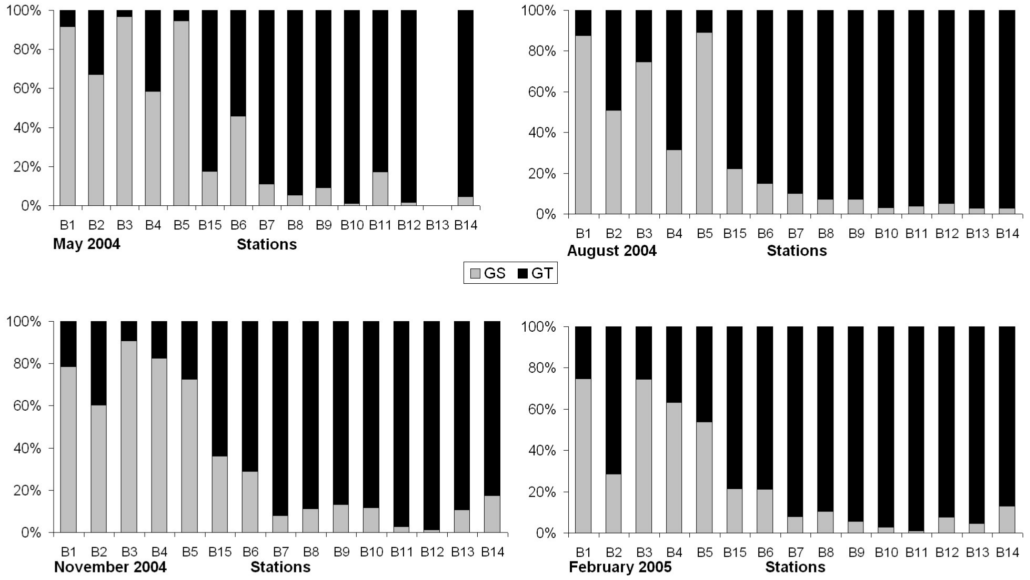

Fig. 3: Evaluation of the percentage of the Ecological Groups (GS and GT) for each sampling period.

species scores of Bentix methodology. However, these species contributed significantly to observed differences between control and discharge stations by their relatively high abundance in controls (Table 3). Therefore, these two polychaetes, characteristic for control stations, could not be accepted as tolerant to disturbance (GT) for the present study.

In fact *Nereis (Hediste) diversicolor* is a euryhaline species common to estuaries (SIMBOURA & NICOLAIDOU, 2001) which is naturally adapted to the salinity variations and the brackish water environment of the upper water layer of the Bosphorus Strait. It is widely known that in highly stratified salinity environments, as is the case in the study area, the conditions above and below the halocline can have significant impact on the benthic fauna composition and that the salinity variations above the halocline may create a more stressful environment than below the halocline (ROSENBERG *et al.,* 2004). In addition, the coastal zone, where the samples of the present study were collected, can be accepted as a naturally stressed environment for the benthic community due to its shallow depth, strong currents and wave-induced hydraulic disturbance. In these type environments some species tolerant to natural or anthropogenic instability may dominate and underestimate the ecological quality evaluation.

Two peracarids, *Tanais dulongii* and *Echinogammarus olivii,* were other important species encountered in the study area in terms of their relative abundance, frequency of occurrence and indicator potentials. There are few available studies concerning the pollution tolerance of these two species. *T. dulongii* appears to be tolerant of organic pollution and physical disturbance (ADAMI *et al.,* 2004; SALAS *et al.,* 2005). It has furthermore been found abundant at intertidal sites with high metal concentrations in the sediments (REISH *et al.,* 1997). On the other hand, both *T. dulongii* and *E. olivii* were classified as sensitive or indifferent to disturbance (GS) in the extended list of species scores of Bentix. However, SIMPER analysis showed that both species contributed significantly to observed differences between control and discharge stations and one-fifth of the average similarity of discharge stations was contributed by these species (Table 3). *E. olivii* was one of the most abundant species in the area investigated. This species provided approximately 21% of the cumulative dominance in discharge stations but 3% in controls. Likewise, *T. dulongii* provided approximately 7% of the cumulative dominance in discharge stations whereas only 0.25% in controls. Therefore, these two peracarids, characteristic for discharge station, can be accepted as tolerant to disturbance (GT) in the present study.

The other important species to contribute to the difference of control and discharge stations in the present study were *Jassa marmorata, Hyale perieri* and *H. pontica.* BELLAN-SANTINI (1981) proposed that the ratio of the abundance of certain peracarid genera might represent a reliable indicator of pollution. Specifically, the author suggested that the ratio of the mean dominance of the genera *Jassa* and *Hyale* reflect the degree of pollution (the value is higher under increased pollution). In the present study, *Jassa* species, especially *J. marmorata,* seem to be tolerant of disturbance, always present in high densities, with significant variations with time. Likewise, one of the *Hyale* species, *H. perieri,* a characteristic species of the discharge stations and whose populations respond to pollution by an increase of density, appears to be tolerant of disturbance or stress. However, the other *Hyale* species, *H. pontica* appears to be sensitive to disturbance and was found as a characteristic for the control stations.

The Bentix index in the control stations reached an average value of 4.53 ± 1.01 classifying the community into the good class which indicates minor environmental disturbance (EC, 2003). On the other hand, in the discharge stations the average value decreased to 2.38 ± 0.35 (poor class) indicating heavily a polluted environment. The difference observed between the control and the discharge stations was proved statistically significant by using the Mann-Whitney U Test (p=0.001).

According to Bentix, B1, B3 and B5 were classified as normal/pristine and presented high ecological quality status (ECoQ) in their all samplings. Stations B2 and B4 were classified as slightly polluted, transitional and evaluated as good ECoQ in the majority of their samplings. Stations B6 and B15 were classified as moderately polluted and assigned to moderate ECoQ in most of their samplings. Stations B7, B8, B9, B10, B11, B12, B13 and B14 were classified as heavily polluted and presented poor ECoQ in all of their samplings (Fig. 4). Consequently, all stations classified as heavily polluted and with poor ECoQ, were directly affected by sewage discharges (discharge stations). Although station B6 was also directly affected by sewage discharges, it was classified as moderately polluted and presented moderate ECoQ. All other stations, except B15, classified as normal/pristine, slightly polluted-transitional, were not directly affected by sewage discharge (control stations).

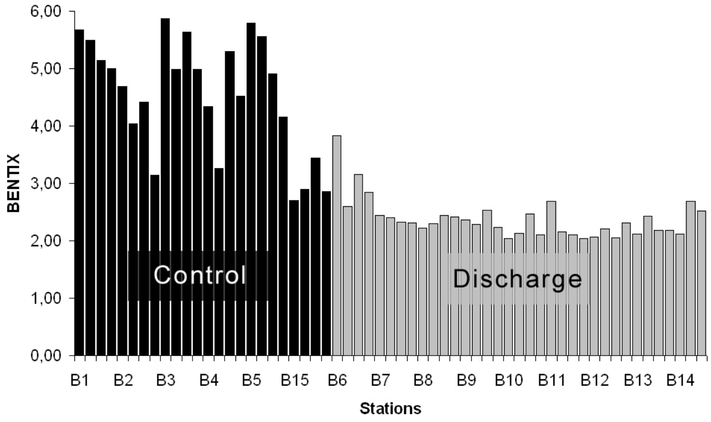

Fig. 4: Bentix index trend in the study area.

Ecological quality status of the discharge stations was worse than that of controls with regard to Bentix values. As a very descriptive and effective tool, the Bentix index precisely classifies the benthic communities into ecological quality classes. According to the authors of the index, its robustness lies in the fact that it is independent of habitat type and sample size. It has therefore a potential for global application. Its effectiveness in discriminating between ecological classes is because of its ability to reflect the faunal composition in relation with the resistance of species to disturbance factors (SIMBOURA & ZENETOS, 2002). The present work is the first case of applying the index to hard substrate benthic data. Although so far it has been used for soft bottom communities (e.g. DAUVIN *et al.*,

2007; SIMBOURA & REIZOPOULOU, 2007; SIMBOURA *et al.,* 2005, 2007; ALBAYRAK *et al.,* 2006; MARÍN-GUIRAO *et al.,* 2005; ZENETOS *et al.,* 2004), Bentix (with some species scoring modifications) appeared to work successfully also in hard substrate communities, at least for the present study. In this sense, it can be construed that the macrozoobenthic communities of the discharge stations, with low Bentix values and worse ecological quality status, were affected by pollution.

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Appendix A:

The most abundant species and their mean dominances (number of ind. of each species / total number of ind.) at each sampling period and station.

Eco. Gr.	Tax. Group	Species	Control Stations						Discharge Stations								
			B1	B2	B3	B4	B5	B15	B6	B7	B8	B9	B10	B11	B12	B13	B14
GS	Mol	*Mytilaster lineatus*	34.86	10.91	40.81	28.65	27.55	7.57	13.85		5.58						
GS	Pol	*Nereis (Hediste) diversicolor*	17.10		11.76		13.95										
GS	Pol	*Platynereis dumerilii*		11.01	5.86							5.67					
GS	Pol	Opheliidae (sp.)	6.05			6.55	9.76									6.37	
GS	Cru	*Ampithoe helleri*					6.23										
GS	Cru	*Hyale pontica*	7.60	10.19	10.44		5.59	4.38									
GS	Cru	*Dynamene bidentatus*	6.09														
GS	Cru	*Sphaeroma serratum*												7.96			
GS	Cru	*Idotea pelagica*				10.19				4.14							
GT	Mol	*Mytilus galloprovincialis*		22.44		10.04		30.84	8.14	47.43	26.30	18.53	11.61	38.65	49.05	23.95	27.83
GT	Tur	Macrostomida (sp.)														7.37	
GT	Pol	Fabriciinae (sp.)														5.23	
GT	Pol	*Capitella capitata*								37.86	6.90						
GT	Oli	*Enchytraeus buchholzi*				17.71					21.08	14.86	2.97				18.18
GT	Cru	*Ampithoe ramondi*													1.84		
GT	Cru	*Echinogammarus olivii*						22.63	20.37		25.91		5.90	34.94			13.32
GT	Cru	*Hyale perieri*		11.96					10.51	6.13		4.43	32.31	8.17	13.71		
GT	Cru	*Ericthonius brasiliensis*			7.55												
GT	Cru	*Jassa marmorata*								20.66				3.37	26.06		16.12
GT	Cru	*Jassa ocia*								4.89							
GT	Cru	*Stenothoe tergestina*						6.14							4.05		
GT	Cru	*Tanais dulongii*							18.35		5.55	23.01	35.11				

Eco. Gr: Ecolocical groups of species: GS: sensitive taxa group, GT: tolerant taxa group
Taxonomic groups - Tur: Turbellaria, Nem: Nemertea, Pol: Polychaeta, Oli: Oligochaeta, Cru: Crustacea, Mol: Mollusca.