

## Assessment of ecological quality status of Küçükçekmece Bay (Marmara Sea) by applying BENTIX, AMBI, BOPA and BO2A biotic indexes

S. CAĞLAR and S. ALBAYRAK

Istanbul University, Faculty of Science, Department of Biology, 34134 Vezneciler Istanbul, Turkey

Corresponding author: serhatal@istanbul.edu.tr

Received: 26 March 2012; Accepted: 20 June 2012; Published on line: 7 September 2012

### Abstract

This study was carried out to explore the effectiveness of different biotic indexes in the Marmara Sea. The assessment of ecological quality status (EQS) was performed by applying the biotic indexes BENTIX, AMBI, BOPA, BO2A and Shannon-Wiener diversity, in combination with the estimation of total organic carbon (TOC) content of sediments. BOPA and BO2A indexes tended to overestimate the EQS of the stations. BENTIX was the most efficient index as it demonstrated conceivable EQS results with respect to TOC load and successfully determined "acceptable" or "not acceptable" status of the stations. TOC content of sediment, which significantly correlated with several benthic measures (S, N, AMBI, BENTIX), proved to be a valuable proxy measure in evaluating the likelihood of benthic impairment. When overall EQS of northern Marmara Sea was discussed, the region was designated as ecologically disturbed with only 25.7% of the stations in acceptable status.

**Keywords:** Küçükçekmece Bay, Marmara Sea, benthic macroinvertebrates, ecological quality status.

### Introduction

Large amounts of pollutants have been introduced into the Marmara Sea because of increasing settlement, agricultural runoff, industrial activities and maritime transport. In addition to these, Black Sea originated pollutants are also transported to the Marmara Sea via the Bosphorus. Istanbul alone contributes about 40-65% of total anthropogenic discharges (Polat, 1995; Tuğrul & Polat, 1995). Küçükçekmece Bay is located on the northern coast of the Marmara Sea. The Bay, within the west border of Istanbul is inevitably influenced by pollution pressure.

Aquatic environments are subjected to pollution and they are the most severely affected compartments of the world because of the dense human settlement close to coasts (Diaz *et al.*, 2004). Negative impacts of industry, tourism, maritime transport, fisheries, aquaculture etc. on biodiversity are most prominently observed in coastal ecosystems (Simboura & Zenetos, 2002). Sediments are eventual recipients of human-based pollutants, and sediments can accumulate great amounts of organic matter. There is a correlation between amounts of organic carbon and pollutants in coastal sediments. Organic carbon content of sediment can be used as an indicator of pollution. Contamination concentrated at sediment will firstly and

mostly affect benthic organisms that are living dependent on the sea bottom (Shine & Wallace, 2000; Venturini *et al.*, 2004; Hyland *et al.*, 2005). Macrofaunal communities and associated environmental variables, such as organic carbon of sediment, are important tools for detecting the health of coastal marine ecosystems (Magni, 2003).

European Water Framework Directive (WFD 2000/60/EC) established a framework for protecting and enhancing all the water bodies of Europe, including coastal waters. WFD developed the term Ecological Quality Status (EQS), which is assessed as High, Good, Moderate, Poor and Bad, to understand the actual condition of water bodies, and aimed that all water bodies should achieve Good quality status by the year 2015. The EQS would be attributed especially to biological elements as well as hydromorphological and physicochemical elements (Borja *et al.*, 2004; Marin-Guirao *et al.*, 2005).

To determine the EQS, there was a need for developing new methods using different compartments of ecosystem such as phytoplankton, zoobenthos and fish (Borja *et al.*, 2003). AMBI (Borja *et al.*, 2000), BENTIX (Simboura & Zenetos, 2002), BOPA (Dauvin & Ruellet, 2007) and BO2A (Dauvin & Ruellet, 2009) biotic indexes, which are based on sensitivity/tolerance of zoobenthic species or taxa, were proposed for application within the scope of the WFD. Macrozoobenthic communi-

ties are commonly utilised in ecological monitoring studies because they include different species showing tolerance to environmental stress at different levels (Hily, 1984; Dauer, 1993). Benthic invertebrates, utilised as bioindicator, take the first place among biological groups for the purpose of inspecting marine ecosystems because macrobenthos respond to anthropogenic or natural pressure. As most benthic organisms are sessile or sedentary, changes in their populations emerge directly due to the effect of ambient pressure, not due to migration or movement. Assessing the benthic communities gives important clues to determining the extent of environmental stress, whether it is natural or man-mediated (Pearson & Rosenberg, 1978; Pocklington & Wells, 1992; Dauer, 1993; Pancucci-Papadopoulou *et al.*, 1999).

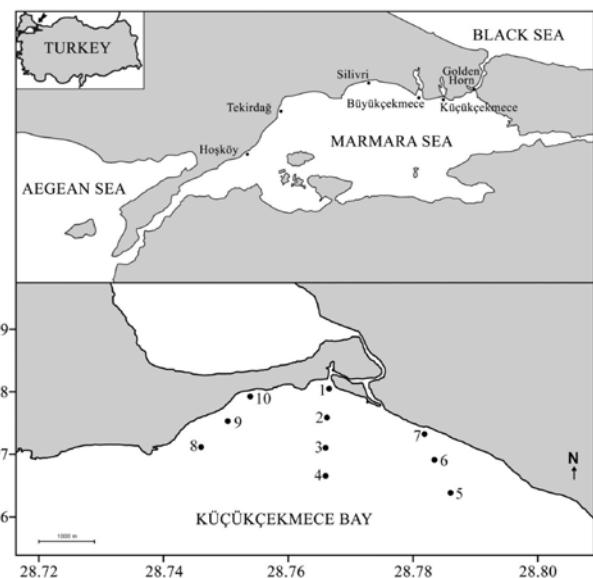
Although many studies were performed throughout European coastal waters with respect to WFD, there are only two research studies carried out in the Marmara Sea using macrozoobenthic communities. Albayrak *et al.* (2006) studied the northern part of this area, between Büyüçekmece Bay and Hoşkoy, whereas Albayrak *et al.* (2010) studied only the Golden Horn estuary. This study utilises macrozoobenthic communities and related biotic indexes as well as total organic carbon (TOC) content of the sediment.

The objective of this study is to explore and compare the effectiveness of different biotic indexes and availability of using the TOC in evaluating the benthic impairment in the northern Marmara Sea by determining the status of the ecological quality of Küçükçekmece Bay and by using historical dataset from previous studies (Albayrak *et al.*, 2006; 2010) in combination with the TOC content of the sediment.

## Materials and Methods

This study was carried out at Küçükçekmece Bay (northern Marmara Sea) in June 2007 (Fig. 1). Sampling of benthic fauna occurred at ten stations, representing the depth transects of the Bay. Benthic material was collected in triplicate with a van Veen grab (surface area: 0.1 m<sup>2</sup>). Samples were sieved through a 1-mm mesh. Subsequently macrozoobenthic organisms were sorted out and preserved in 4% formalin-seawater solution. Specimens were identified to the lowest possible taxon and their individuals were enumerated per unit sample (0.1 m<sup>2</sup>). Mean values for 0.1 m<sup>2</sup> of biotic variables and indexes were calculated for each sampling station. The frequencies of species in the study area were determined using Soyer's (1970) Frequency Index (F) and the results were evaluated as constant ( $F \geq 50\%$ ), common ( $50\% > F \geq 25\%$ ) and rare ( $F < 25\%$ ).

Ecological quality assessment of the Bay was performed by applying Shannon-Wiener (Shannon & Weaver, 1949) community diversity ( $H' \log_2$ ) as well as



**Fig. 1:** The map showing sampling regions from Albayrak *et al.* (2006; 2010) and sampling stations in the Küçükçekmece Bay of this study.

AMBI (Borja *et al.*, 2000) (AMBI version 4.1 with the species list version of February 2010), BENTIX (Simboura & Zenetos, 2002), BOPA (Dauvin & Ruellet, 2007) and BO2A (Dauvin & Ruellet, 2009) [new thresholds for BOPA and BO2A values are due to Ossa-Carretero & Dauvin (2010)] biotic indexes and by considering Weisberg *et al.* (2008). AMBI and BENTIX assess the response of soft-bottom macrozoobenthic communities to environmental stress. Both indexes are based on the relative individual percentages of species classified into groups according to their sensitivity or tolerance to stress. BOPA index uses relative frequencies of polychaetes (all species are accepted as tolerant) and amphipods (all species except *Jassa* are accepted as sensitive). BO2A index is an adaptation of BOPA index for use in the freshwater zones of transitional waters. The formula uses Annelida (including Polychaeta and Clitellata) instead of Polychaeta which is used in BOPA. Shannon-Wiener diversity index ( $H'$ ) is not a biotic index, but it is a measure of species diversity of a particular site and has been widely used by marine biologists. Simboura & Zenetos (2002) indicated a classification scheme for soft-bottom benthic communities based on  $H'$  values in response to five quality status classes of WFD.

Five EQS classes were aggregated into two groups, "acceptable" and "not acceptable", to determine agreement/disagreement between the above-mentioned five indexes. Acceptable status was assigned to "High" or "Good" EQS and scored as 1, whereas not acceptable status was given to "Moderate", "Poor" or "Bad" EQS and scored as 0. The scores of each index were summed for each station (range: 0-5). The sum of scores was used to determine agreement/disagreement between indexes. Full

agreement was measured as 0 or 5, partial agreement as 1 or 4 and disagreement as 2 or 3 (Blanchet *et al.*, 2008).

Mud percentage and TOC content of the sediment and salinity, temperature and dissolved oxygen of seawater at sampling stations were measured. An additional sediment sample was taken by grab and kept under +4°C until analysis. TOC content of the sediment (Loring & Rantala, 1992) and mud percentage (Folk, 1974) were determined. A 3-l Ruttner bottle with thermometer was utilised to measure the salinity, temperature and dissolved oxygen of seawater just above the bottom. Dissolved oxygen content of seawater was analysed using Winkler method (Winkler, 1888). Spearman's rank correlation coefficient (Siegel, 1956) was used to detect the correlation between biotic and abiotic parameters.

## Results

### Physico-chemical variables

The maximum seawater temperature was 23 °C at station 1 (4 m). Temperature was between 18.3 and 21.8 °C at the stations with 10 m depth, between 11.9 and 13.5 °C at the stations with 20 m depth, but it slightly increased to 14.9–15 °C at the stations with 30- and 36-m depth (Table 1). Temperature significantly decreased with increasing depth ( $r_s = -0.628$ ;  $p < 0.05$ ).

Salinity varied between 20.4 psu (st. 1; 4 m) and 36.5 psu (sts. 4, 5 and 8; 30–36 m). Stations with 10 m depth had salinities between 21.2 and 22.7 psu, and stations with 20 m depth had salinities between 26.9 and 29 psu. Salinity significantly increased with increasing depth ( $r_s = 0.966$ ;  $p = 0$ ).

The highest dissolved oxygen value was 12.8 mg·l<sup>-1</sup> at station 1 (4 m). The values gradually decreased with depth. It was between 11.4 and 12.2 mg·l<sup>-1</sup> at 10 m depths, around 8 mg·l<sup>-1</sup> at 20 m depths, between 4.8 and

5.9 mg·l<sup>-1</sup> at 30 m depths and less than one-third of the highest value at 36 m depth (3.8 mg·l<sup>-1</sup>). Dissolved oxygen of the seawater significantly decreased as depth increased ( $r_s = -0.972$ ;  $p = 0$ ).

Mud percentage of the sediment differed from 3.3% (st. 1; 4 m) to 93.3% (st. 3; 20 m), and significantly increased with depth ( $r_s = 0.623$ ;  $p < 0.05$ ).

TOC of the sediment varied between 1.9 mg·g<sup>-1</sup> (st. 1; 4 m) and 24.4 mg·g<sup>-1</sup> (st. 5; 30 m). The lowest value was 5.4 mg·g<sup>-1</sup> at 10-meter stations, 10.4 mg·g<sup>-1</sup> at 20-meter stations, 17.8 mg·g<sup>-1</sup> at 30-meter stations, and 22 mg·g<sup>-1</sup> at 36-meter station. TOC significantly increased with depth ( $r_s = 0.779$ ;  $p < 0.01$ ) and with mud percentage of the sediment ( $r_s = 0.770$ ;  $p < 0.01$ ), but a significant decrease was detected in dissolved oxygen content of the seawater as TOC of the sediment increased ( $r_s = -0.661$ ;  $p < 0.05$ ).

### Faunistic composition

A total of 8221 specimens identified to 143 macrozoobenthic species were collected. Polychaeta was the richest group in terms of species number (70 species- 48.9% of total number of species), followed by Crustacea (36 species- 25.2%) and Mollusca (24 species- 16.7%). Mollusca accounted for 48.4% (3897 ind.) of the total individuals in the area, followed by Polychaeta (24.4%-2011 ind.) and Crustacea (22.6%-1861 ind.).

The most dominant species were *Corbula gibba* (Olivier, 1792) (2936 ind.), *Spisula subtruncata* (da Costa, 1778) (714 ind.), *Microdeutopus versicoloratus* (Bate, 1856) (653 ind.), *Apseudes latreillii* (Milne-Edwards, 1828) (529 ind.), *Pomatoceros triquetus* (Linnaeus, 1758) (473 ind.), *Heteromastus filiformis* (Claparede, 1864) (463 ind.), *Sigambra tentaculata* (Treadwell, 1941) (373 ind.) and *Corophium acherusicum* Costa, 1851 (273 ind.).

Six species (*H. filiformis*, *Nephtys hombergii* Savi-

Table 1. Environmental measures at stations.

Station No	Depth (m)	Temperature (°C)	Salinity (psu)	DO (mg·l <sup>-1</sup> )	TOC (mg·g <sup>-1</sup> )	Mud percentage
1	4	23	20.4	12.8	1.9	3.3
2	10	21.8	21.2	12.1	5.7	62.4
3	20	12.8	29	8.3	21.6	93.3
4	36	14.9	36.5	3.8	22	81.1
5	30	15	36.4	5.9	24.4	67.3
6	20	11.9	28.4	8.1	10.4	44.2
7	10	20.7	22.7	11.4	5.4	6.6
8	30	14.9	36.5	4.8	17.8	90.6
9	20	13.5	26.9	8.5	14.4	32.7
10	10	18.3	22.3	12.2	20	68.6

DO: Dissolved oxygen, TOC: Total organic carbon

gny in Lamarck, 1818, *Owenia fusiformis* Delle Chiaje, 1844, *C. gibba*, *Sphaeroma serratum* (Fabricius, 1787), *Jassa marmorata* (Holmes, 1903)) were constant, 18 species (*Capitella capitata* (Fabricius, 1780), *Melinna palmata* Grube, 1870, *Pholoe inornata* Johnston, 1839, *Polydora caeca* (Örsted, 1843), *P. triquetter*, *S. tentaculata*, *Syllis gracilis* Grube, 1840, *Heteronemertea* sp., *Nemertina* sp., *Aspidosiphon muelleri* Diesing, 1851, *Abra alba* (Wood W., 1802), *Mytilus galloprovincialis* Lamarck, 1819, *Pitar rufus* (Poli, 1795), *S. subtruncata*, *Calyptraea chinensis* (Linnaeus, 1758), *Nassarius reticulatus* (Linnaeus, 1758), *Diogenes pugilator* (Roux, 1829) and *Iphinoe elisae* Bacescu, 1950) were common

and other 119 species were rare.

Percentages of individuals of the most dominant species at stations are given in Table 2. Individuals belonging to one species constituted more than half of total individuals of the relevant station at six stations and *S. serratum* formed 100% at station 4 because this station included only one species.

Mean values of univariate indexes (species number, individuals number, H') and biotic indexes (AMBI, BENTIX, BOPA, BO2A) at stations have been summarised in Table 3. BENTIX, BOPA and BO2A were not calculated for stations 4 and 8 because of the operational limits of the indexes.

**Table 2.** Dominant species and their individuals' percentage at stations.

1	2	3	4	5	6	7	8	9	10
<i>S. subtruncata</i> 89	<i>O. fusiformis</i> 45	<i>C. gibba</i> 94	<i>S. serratum</i> 100	<i>C. gibba</i> 82	<i>H. filifor- mis</i> 52	<i>M. versi- culatus</i> 18	<i>S. serratum</i> 31	<i>C. gibba</i> 95	<i>N. hombergii</i> 51
<i>C. gallina</i> 6	<i>N. reticulatus</i> 13			<i>Malacoceros fuliginosus</i> 13	<i>S. tentaculata</i> 17	<i>A. latreillei</i> 15	<i>Corophium insidiosum</i> 19		<i>M. galloprovincialis</i> 8
	<i>D. filum</i> 11				<i>Aonides oxycephala</i> 10	<i>P. triquetter</i> 13	<i>C. gibba</i> 19		

**Table 3.** Mean values (for 0.1 m<sup>2</sup>) of some biotic measures and indexes. S: Species number, N: Individuals number.

	1	2	3	4	5	6	7	8	9	10
<b>S</b>	6	5	3	1	5	18	66	3	13	11
<b>N</b>	253	16	92	4	77	193	1203	5	870	30
<b>H'</b>	0.8	1.9	0.5	0	0.9	2.3	3.9	1.1	0.4	2.5
<b>AMBI</b>	0.1	1.9	4.4	3.0	4.6	3.8	1.9	3.5	4.4	1.9
<b>BENTIX</b>	5.9	4.7	2.3	-	2.2	2.4	3.6	-	2.1	3.0
<b>BOPA</b>	0.00380	0.19629	0.00742	-	0.04595	0.27674	0.09577	-	0.01246	0.23856
<b>BO2A</b>	0.00380	0.19629	0.00742	-	0.04595	0.27674	0.10240	-	0.01246	0.23856

**Table 4.** Spearman's rank-correlation coefficient (r<sub>s</sub>) between biotic and abiotic parameters.

		S	N	H'	AMBI	BENTIX	BOPA	BO2A
<b>Depth</b>	<b>r<sub>s</sub></b> <b>p</b>	<b>-0.571</b> 0.042	-0.524 0.060	-0.486 0.077	<b>0.703</b> 0.012	<b>-0.831</b> 0.011	0.075 0.430	0.075 0.430
<b>Mud percentage</b>	<b>r<sub>s</sub></b> <b>p</b>	<b>-0.762</b> 0.005	<b>-0.733</b> 0.008	-0.248 0.244	0.406 0.122	-0.521 0.185	0.214 0.305	0.214 0.305
<b>DO</b>	<b>r<sub>s</sub></b> <b>p</b>	0.518 0.062	0.479 0.081	0.382 0.138	<b>-0.640</b> 0.023	<b>0.807</b> 0.015	-0.143 0.368	-0.143 0.368
<b>TOC</b>	<b>r<sub>s</sub></b> <b>p</b>	<b>-0.598</b> 0.034	<b>-0.552</b> 0.049	-0.406 0.122	<b>0.665</b> 0.018	<b>-0.786</b> 0.021	0.048 0.455	0.048 0.455

S: Species number, N: Individual number, DO: Dissolved oxygen. Statistically significant correlations are in bold.

According to Spearman's rank correlation coefficient (Table 4), species and individual numbers negatively correlated with TOC ( $p<0.05$ ) and mud percentage ( $p<0.01$ ). AMBI positively correlated with TOC and depth, negatively with dissolved oxygen, whereas, BENTIX negatively with TOC and depth, positively with dissolved oxygen ( $p<0.05$ ). Another negative correlation was between depth and species number ( $p<0.05$ ). A statistically significant correlation could not be determined between abiotic parameters and  $H'$ , BOPA and BO2A ( $p>0.05$ ).

#### Assessment of ecological quality status

Station 1 was assessed as "High", station 7 as "Good", stations 2 and 6 as "Moderate", stations 9 and 10 as "Poor" and stations 3, 4, 5 and 8 as "Bad".

All biotic indexes (AMBI, BENTIX, BOPA and BO2A) agreed with final assessment at stations 1 and 7. However, some disagreements were observed. Especially, BOPA and BO2A tended to overestimate the EQS of the stations. Both indexes indicated "High" EQS at stations 3 and 9, "Good" EQS at station 5, whereas final assessment was "Bad" for stations 3 and 5 and "Poor" for station 9. Individuals of tolerant bivalve *C. gibba* formed from 82% to 95% of total abundance at those stations, and BOPA and BO2A overestimated such stations because they accept only polychaetes and amphipod genus *Jassa* as tolerant. AMBI and BENTIX showed more conceivable results with final assessment. However, they had different scores at some stations due to placing some species in contrary ecological groups. For example, *N. hombergii*, the most dominant species (51%) at station 10, was placed in group II by AMBI, but it was accepted as tolerant by BENTIX, so AMBI classified as "Good" and BENTIX as "Moderate" the station 10 where the TOC of sediment was high as  $20 \text{ mg}\cdot\text{g}^{-1}$  and of which final assessment was "Poor".

Level of agreement/disagreement between indexes was calculated according to the sum of scores (Table 5). All indexes indicated "not acceptable" status for station 6, and full agreement was observed only at this station.

Partial agreement was seen for the stations 1, 7 and 10, and disagreement for the stations 2, 3, 5 and 9. Stations 4 and 8 were not taken into account because BENTIX, BOPA and BO2A were not calculated for these two stations. There was a significant agreement between only AMBI and BENTIX ( $p<0.001$ ).

Shannon-Wiener diversity index ( $H'$ ) classified all stations as "not acceptable", 60% being in "Bad" EQS. AMBI classified half of the stations as "acceptable" and half as "not acceptable", BENTIX classified 37.5% as "acceptable" and 62.5% as "not acceptable". However, AMBI evaluated only 10% of the stations in "High" EQS, whereas BENTIX did the same for just 25%. Both BOPA and BO2A indexes classified 62.5% of the stations as "acceptable" (37.5% in "High" and 25% in "Good" EQS) and 37.5% as "not acceptable".

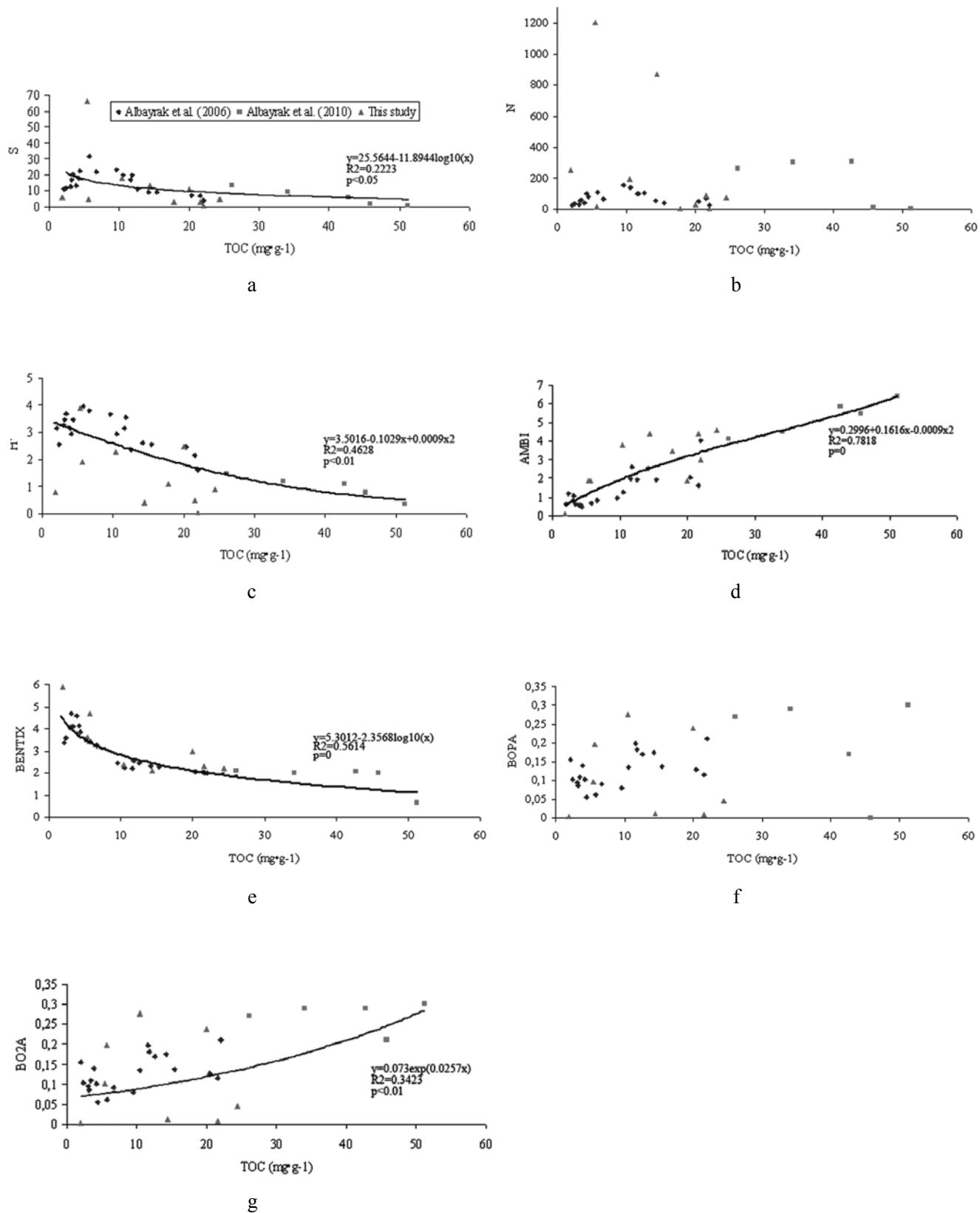
#### TOC and benthic variables/indexes relations

Figure 2 demonstrates relationships between benthic measures/biotic indexes and TOC content of sediment. The distribution of species number, Shannon-Wiener community diversity, AMBI, BENTIX and BO2A significantly ( $p<0.05$ ) indicated deterioration of benthic life with increasing organic matter load. Individuals' number exhibited an insignificant ( $p>0.05$ ) bimodal curve with first peak around  $8 \text{ mg}\cdot\text{g}^{-1}$  TOC and second peak around  $43 \text{ mg}\cdot\text{g}^{-1}$  TOC while BOPA exhibited an insignificant ( $p>0.05$ ) sigmoid curve.

Hyland *et al.* (2005), Albayrak *et al.* (2006) and Magni *et al.* (2009) indicated TOC thresholds for assessing the risk of benthic impairment in relation to organic matter load. TOC and benthic variables/indexes data from Albayrak *et al.* (2006) and (2010), both from different parts of Northern Marmara Sea including 20 and 5 stations respectively, were merged with data of this study including 10 stations. And mean values from totally 35 stations were summarised in Table 6 in accordance with those thresholds. At all three thresholds, BENTIX showed the most compatible EQS assessment for TOC load levels. At Albayrak *et al.* (2006) thresholds, BENTIX indicated

**Table 5.** Levels used for measurement of agreement/disagreement between indexes (after Blanchet *et al.*, 2008).

Sum of scores	Interpretation
0	Full agreement of the five indexes on "Moderate" or worse EcoQ status (not acceptable)
1	Partial agreement (four agreements out of five indexes) of the five indexes on "Moderate" or worse EcoQ status (not acceptable)
2	Disagreement between the five indexes on the EcoQ status of the station
3	Disagreement between the five indexes on the EcoQ status of the station
4	Partial agreement (four agreements out of five indexes) of the five indexes on "Good" or higher EcoQ status (acceptable)
5	Full agreement of the five indexes on "Good" or better EcoQ status (acceptable)



**Fig. 2:** Distribution of benthic measures and biotic indexes, from data of Albayrak *et al.* (2006; 2010) and this study, along a TOC gradient. a) Species number (S), b) Individuals' number (N), c) Shannon-Wiener community diversity (H'), d) AMBI, e) BEN-TIX, f) BOPA, g) BO2A. (Trend lines for b and f were not indicated because they were not significant,  $p > 0.05$ )

**Table 6.** Mean values of biotic variables and indexes, from merged dataset of Albayrak *et al.* (2006; 2010) and this study, within TOC thresholds in accordance with Albayrak *et al.* (2006), Hyland *et al.* (2005) and Magni *et al.* (2009) thresholds.

	S	N	H'	AMBI	BENTIX	BOPA	BO2A
<b>Albayrak <i>et al.</i> (2006) thresholds</b>							
Low (1-5.9 mg·g <sup>-1</sup> )	19	185.7	2.9	0.9	4.2	0.10018	0.10084
Moderate (6-11.9 mg·g <sup>-1</sup> )	21.1	114.2	3.3	1.5	2.9	0.13782	0.13782
High (12-21.9 mg·g <sup>-1</sup> )	9.2	150.1	1.9	2.7	2.3	0.13319	0.13319
Very high (>22 mg·g <sup>-1</sup> )	5.3	118.4	1	4.4	1.8	0.17521	0.21646
<b>Hyland <i>et al.</i> (2005) thresholds</b>							
Low (<10 mg·g <sup>-1</sup> )	20.2	169.4	3	0.9	4	0.09618	0.09674
Intermediate (10-35 mg·g <sup>-1</sup> )	10.8	135.8	2	2.7	2.4	0.15608	0.15608
High (>35 mg·g <sup>-1</sup> )	3	108	0.7	5.9	1.5	0.15666	0.26666
<b>Magni <i>et al.</i> (2009) thresholds</b>							
Low (<10 mg·g <sup>-1</sup> )	20.2	169.4	3	0.9	4	0.09618	0.09674
Intermediate (10-28 mg·g <sup>-1</sup> )	10.9	127	2.1	2.6	2.4	0.14821	0.14821
High (>28 mg·g <sup>-1</sup> )	4.5	156.7	0.8	5.5	1.6	0.19000	0.27250

S: Species number, N: Individuals number.

“Good” EQS at Low TOC, “Moderate” EQS at Moderate TOC, “Poor” EQS at High TOC and “Bad” EQS at Very High TOC. At both Hyland *et al.* (2005) and Magni *et al.* (2009) thresholds, it indicated “Good” EQS at Low TOC, “Poor” EQS at Intermediate TOC and “Bad” EQS at High TOC. However, all other biotic indexes also indicated deteriorating EQS with increasing TOC load, despite not being gradually as BENTIX showed.

## Discussion

Reaching a final assessment for EQS of a particular site should not be subjective or arbitrary. Biotic indexes provide numerical data to minimise the subjective judgment; however, they also involve subjectivity at least when selecting species as sensitive or tolerant (Marin-Guirao *et al.*, 2005; Weisberg *et al.*, 2008). One of the main problems about applying these biotic indexes is classifying some species into different ecological groups. Many species in this study such as polychaetes *Amphicteis gunneri* (M. Sars, 1835), *Eumida sanguinea* (Orsted, 1843), bivalves *Anodontia fragilis* (Philippi, 1836), *Myrtea spinifera* (Montagu, 1803), crustaceans *Ampelisca diadema* (Costa, 1853) and *Sphaeroma serratum* (Fabricius, 1787) were placed in contrary ecological groups by BENTIX and AMBI. The case of *Sphaeroma serratum* is remarkable at Station 4, with 22 mg·g<sup>-1</sup> TOC in its sediment. BENTIX accepted this species as sensitive (EGI), but AMBI placed it in Group III, which included species tolerant to excess organic matter enrichment. In our opinion, *S. serratum*, surviving alone under such high TOC content, should also be accepted as tolerant by BENTIX. Marin-Guirao *et al.* (2005) and Prato

*et al.* (2009) also presented some examples on this problem and recommended a consensus for placing the species to a particular ecological group. The same problem is in question for BOPA and BO2A methods. Both of these indexes accept all polychaetes as tolerant and all amphipods except the genus *Jassa* as sensitive. However, many polychaeta species in this study such as *Haplosyllis spongicola* (Grube, 1855) and *Magelona alleni* Wilson, 1958 were accepted as sensitive, and many amphipods such as *Corophium acutum* Chevreux, 1908 and *Gammarella fucicola* (Leach, 1814) were accepted as tolerant by BENTIX and AMBI. Ossa-Carretero *et al.* (2009) also denoted that Polychaeta contains both sensitive and tolerant species. Such different classification of species or higher taxa caused biotic indexes to give discrete results. Moreover, some taxonomical problems are also present and must be solved. For example, *Pholoe synophtalmica* Claparede, 1868 is a synonym of *Pholoe inornata* Johnston, 1839, but AMBI accepts them as separate species and places the former species into Group II and the latter species into Group IV.

H' showed 50% agreement with final assessment, four of these five stations included only a few species and H' indicated bad quality status because it is extremely sensitive to species number. BOPA and BO2A showed 50%, AMBI showed 40%, BENTIX showed 37.5% agreement with final assessment. Success of AMBI and BENTIX methods at identifying the effect of organic pollution has been exhibited by many studies. However, neither these two biotic indexes nor the BOPA and BO2A indexes could successively detect the ecological quality where less number of species was encountered, similar to the case of six stations in this study. When the remain-

ning four stations (6, 7, 9 and 10) were considered, including at least eleven species/0.1 m<sup>2</sup>, it was seen that AMBI showed 75% while BENTIX, BOPA and BO2A showed 50% agreement with final assessment. Especially at station 7, including 66 species/0.1 m<sup>2</sup>, all four biotic indexes successively detected the ecological quality. Although BOPA and BO2A have the advantage of reducing taxonomical effort, they have some disadvantages as well. One of them is that they accept all polychaetes as tolerant and all amphipods (except the genus *Jassa*) as sensitive. Another one is that only polychaetes (or annelids) and amphipods (except *Jassa*) directly affect the calculation, and other species are not sufficiently taken into account. These indexes must be carefully used when "other species" have important relative frequency. Dauvin & Ruellet (2007) also denoted that it could be difficult, if not impossible, to interpret the results in a poor ecosystem where these two categories of organisms are rare or absent.

All biotic indexes tended to overestimate the EQS of the stations in this study. Especially BOPA and BO2A indexes classified more stations as "High" quality status. Biotic indexes are not very efficient in transitional ecosystems because these ecosystems naturally host many tolerant and opportunistic species adapted to the natural stress of the environment. AMBI works better only for slightly and moderately polluted lagoons, but BENTIX is more effective than AMBI for heavily polluted lagoons with poor quality (Simboula & Reizopoulou, 2008). Pranovi *et al.* (2007) cited that biotic indexes seem to generally overestimate the EQS of not strictly marine ecosystems, partially such as our study area. These indexes mostly showed "High" or "Good" conditions in Venice lagoon. Especially BOPA showed low discrimination by classifying nearly all the samples in the "High" category. However, BENTIX was more sensitive than AMBI to the organic matter content increase in the sediment and related changes in macrobenthic assemblage, and BENTIX was better than AMBI in discriminating the stations. BOPA classified a station in the extremely polluted Golden Horn estuary (Albayrak *et al.*, 2010) as "High" because the station did not include any polychaete species, but more than 80% of the individuals belonged to tolerant Oligochaeta. On the other hand, BO2A classified the same station as "Poor". This was why BO2A was developed especially as an adaptation of BOPA for oligohaline environments. Ossa-Carretero *et al.* (2009) denoted that BOPA overestimated the status in their study area and indicated a need to calibrate the thresholds between EQS classes. Prato *et al.* (2009) have indicated that BENTIX tends to reveal extreme values in EQS because species are ascribed only to two ecological groups rather than five ecological groups of AMBI. Moreover, AMBI sets a wider "Good" class (1.2-3.3) compared to "High" (0-1.2) and "Moderate" (3.3-4.3) classes, while BENTIX sets a wider "High" class (4.5-6) compared to its "Good" (3.5-4.5) and "Moderate" (2.5-3.5) classes. However, Simboula & Argyrou (2010) indicated that a large number of sites were classified as "Good" by AMBI, as "Moderate" by BENTIX in Greece (Eastern Mediterranean), and AMBI overestimates the EQS in Eastern Mediterranean areas. Boundary among "Good" and "Moderate" classes is critical for the evaluation of an index, and ability of an index is to indicate "Moderate" status. Pinedo & Jordana (2008) denoted that the tolerant groups in the pollution succession model are more closely related to the opportunistic taxa in the Mediterranean. The succession model has a closer association and shows a greater variety of combinations between the tolerant and opportunistic groups in the "Moderate" class in the Eastern Mediterranean. Simboula & Argyrou (2010) demonstrated that the tolerant group is not only more associated with the opportunistic taxa, both groups are equally important in the "Moderate" class in the Eastern Mediterranean. BENTIX gives equal weight to the tolerant group GIII and to opportunistic groups GIV and GV, while AMBI gives less weight to the tolerant group than opportunistic groups. Accordingly, BENTIX gathered tolerant and opportunistic groups in the "Moderate" class. BENTIX captured this close association between tolerant and opportunistic groups in the Mediterranean ecosystems by its structural features, assigning cases, where tolerants and opportunists combine at a cumulative percentage of more than 60%, to the "Moderate" class. By correlating the tolerant and opportunistic groups with giving equal weight, BENTIX seems to be more successful in detecting EQS in the Eastern Mediterranean, where tolerant and opportunistic groups have an equally important role in the response of benthic communities to stressors.

BENTIX agreed on the "acceptable" or "not acceptable" status in 87.5% of the stations, Shannon-Wiener diversity index in 80%, AMBI in 70%, BOPA and BO2A in 62.5%. Although these biotic indexes generally failed to detect the final assessment at five classes of EQS, they, especially BENTIX, were more successful in determining the "acceptable" or "not acceptable" status.

Although organic matter in surface sediments is an important food source for benthic fauna, excessive amounts of organic enrichment, often accompanied by other chemical stressors, may cause oxygen depletion and an increase in toxic by-products resulting in reduction in species richness, abundance and biomass of benthic fauna which are in close association with bottom sediments (Hyland *et al.*, 2005). Data from Figure 2 and Table 6 clearly exhibited degrading EQS and decreasing species richness as TOC increased. Therefore, TOC content of sediment was considered as a valuable proxy measure by evaluating the benthic impairment and by significantly correlating to several benthic measures.

A successful biotic index should assign worsening benthic life with organic matter load. Mean values of all biotic indexes (AMBI, BENTIX, BOPA and BO2A) demonstrated deteriorating EQS as TOC level increased

(Table 6). However, BENTIX was the most efficient index showing conceivable EQS results with increasing TOC load. Pranovi *et al.* (2007) also indicated that BENTIX was more sensitive than AMBI to increases in the organic matter content in the bottom sediments and to related changes in the macrobenthic assemblages of Venice lagoon. Albayrak *et al.* (2006) studied the EQS of northern Marmara Sea and indicated that AMBI failed to detect the spatial differentiation of EQS, but BENTIX succeeded in 70% of the cases in producing an ecologically relevant classification reflecting the environmental pressures. Albayrak *et al.* (2010) studied the ecological quality status of the extremely polluted Golden Horn Estuary in İstanbul and stated both AMBI and BENTIX showed 80% agreement with final assessment whereas BOPA showed 20% and BO2A showed 40% agreement. In this study, AMBI and especially BENTIX were more successful in identifying the ecological quality.

Although the whole northern Marmara Sea was designated as ecologically disturbed with only 25.7% of the stations in “acceptable” status, a decreasing pattern of TOC and healing EQS were determined from east towards west. Only 2 stations out of 25 stations (8%) at Golden Horn, Küçükçekmece, Büyükçekmece and Silivri were in “acceptable” status, whereas 7 stations out of 10 stations (70%) at Tekirdağ and Hoşköy were in “acceptable” status (Albayrak *et al.*, 2006; 2010 and this study).

## Acknowledgements

Authors are grateful to Prof.Dr. Melih Ertan Çınar from Aegean University for his helps in identifying some polychaete species, to Assoc.Prof.Dr. Murat Sezgin from Sinop University for some amphipod species and to Assist.Prof.Dr. Mehmet Baki Yokeş from Haliç University for some chiton species. This work was supported by the Research Fund of the İstanbul University, project number: T-770.

## References

Albayrak, S., Balkis, H., Zenetos, A., Kurun, A. & Kubanç, C., 2006. Ecological quality status of coastal benthic ecosystems in the Sea of Marmara. *Marine Pollution Bulletin*, 52 (7): 790-799.

Albayrak, S., Balkis, N., Balkis, H., Zenetos, A., Kurun, A., Karhan, S.Ü., Çağlar, S. & Balci, M., 2010. Golden Horn Estuary: Description of the ecosystem and an attempt to assess its ecological quality status using various classification metrics. *Mediterranean Marine Science*, 11 (2): 295-313.

Blanchet, H., Lavesque, N., Ruellet, T., Dauvin, J.C., Sauriau, P.G. *et al.*, 2008. Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats - Implications for the implementation of the European Water Framework Directive. *Ecological Indicators*, 8 (4): 360-372.

Borja, A., Franco, J. & Perez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin*, 40 (12): 1100-1114.

Borja, A., Franco, J., Valencia, V., Bald, J., Muxika, I. *et al.*, 2004. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. *Marine Pollution Bulletin*, 48 (3-4): 209-218.

Borja, A., Muxika, I. & Franco, J., 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. *Marine Pollution Bulletin*, 46: 835-845.

Dauer, D.M., 1993. Biological criteria, environmental health and estuarine macrobenthic community structure. *Marine Pollution Bulletin*, 26 (5): 249-257.

Dauvin, J.C. & Ruellet, T., 2007. Polychaete/amphipod ratio revisited. *Marine Pollution Bulletin*, 55 (1-6): 215-224.

Dauvin, J.C. & Ruellet, T., 2009. The estuarine quality paradox: Is it possible to define an ecological quality status for specific modified and naturally stressed estuarine ecosystems? *Marine Pollution Bulletin*, 59 (1-3): 38-47.

de la Ossa-Carretero, J.A., del Pilar-Ruso, Y., Gimenez-Casalduero, F. & Sanchez-Lizaso, J.L., 2009. Testing BOPA index in sewage affected soft-bottom communities in the north-western Mediterranean. *Marine Pollution Bulletin*, 58 (3): 332-340.

Diaz, R.J., Solan, M. & Valente, R.M., 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management*, 73 (3): 165-181.

Folk, L.R., 1974. *Petrology of Sedimentary Rocks*. Hemphill Publishing, Austin, Texas, 182 pp.

Hily, C., 1984. *Variabilite de la macrofaune benthique dans les milieux hypertrophiques de la Rade de Brest*. Vol. 1-2. Thèse de Doctorat d'Etat, Université de Bretagne Occidentale, 337 pp.

Hyland, J., Balthis, L., Karakassis, I., Magni, P., Petrov, A. *et al.*, 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. *Marine Ecology Progress Series*, 295: 91-103.

Loring, D.H. & Rantala, R.T.T., 1992. Manual for the geochemical analyses of marine sediments and suspended particulate matter. *Earth-Science Reviews*, 32 (4): 235-283.

Magni, P., 2003. Biological benthic tools as indicators of coastal marine ecosystems health. *Chemistry & Ecology*, 19 (5): 363-372.

Magni, P., Tagliapietra, D., Lardicci, C., Balthis, L., Castelli, A. *et al.*, 2009. Animal-sediment relationships: Evaluating the ‘Pearson–Rosenberg paradigm’ in Mediterranean coastal lagoons. *Marine Pollution Bulletin*, 58 (4): 478-486.

Marin-Guirao, L., Cesar, A., Marin, A., Lloret, J. & Vita, R., 2005. Establishing the ecological quality status of soft-bottom mining-impacted coastal water bodies in the scope of the Water Framework Directive. *Marine Pollution Bulletin*, 50 (4): 374-387.

Ossa-Carretero, J.A. & Dauvin, J.C., 2010. A comparison of two biotic indices, AMBI and BOPA/BO2A, for assessing the ecological quality status (EcoQS) of benthic macro-invertebrates. *Transitional Waters Bulletin*, 4 (1): 12-24.

Pancucci-Papadopoulou, M.A., Simboura, N., Zenetos, A., Thessalou-Legaki, M. & Nicolaïdou, A., 1999. Benthic

invertebrate communities of NW Rodos (Rhodes) Island (SE Aegean Sea) as related to hydrological regime and geographical location. *Israel Journal of Zoology*, 45 (3): 371-393.

Pearson, T.H. & Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography & Marine Biology: An Annual Review*, 16: 229-311.

Pinedo, S. & Jordana, E., 2008. Spain (Catalonia and Balearic Islands). p. 62-70. In: *Water Framework Directive Intercalibration Technical Report. Part 3. Coastal and Transitional Waters*. Carletti, A., Heiskanen, A.S. (Eds). JRC Scientific and Technical Reports. JRC & ies.

Pocklington, P. & Wells, P.G., 1992. Polychaetes: key taxa for marine environmental quality monitoring. *Marine Pollution Bulletin*, 24: 593-598.

Polat, S.Ç., 1995. *Nutrient and organic carbon budgets in the Sea of Marmara: A progressive effort of the biogeochemical cycles of carbon, nitrogen and phosphorus*. PhD Thesis. Middle East Technical University, Turkey, 215 pp.

Pranovi, F., Ponte, F. & Torricelli, P., 2007. Application of biotic indices and relationship with structural and functional features of macrobenthic community in the lagoon of Venice: an example over a long time series of data. *Marine Pollution Bulletin*, 54 (10): 1607-1618.

Prato, S., Morgana, J.G., la Valle, P., Finoia, M.G., Lattanzi, L. et al., 2009. Application of biotic and taxonomic distinctness indices in assessing the Ecological Quality Status of two coastal lakes: Caprolace and Fogliano lakes (Central Italy). *Ecological Indicators*, 9 (3): 568-583.

Shannon, C.E. & Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, 117 pp.

Shine, J. & Wallace, G., 2000. Chemical aspects of organic carbon and ecological stress in benthic ecosystems. p. 40-44. In: *Ad hoc Benthic Indicator Group-Results of Initial Planning Meeting. IOC Technical Series* 57, 6-9 Decem-ber 1999. UNESCO, Paris.

Siegel, S., 1956. *Non-parametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York, 312 pp.

Simboula, N. & Argyrou, M., 2010. An insight into the performance of benthic classification indices tested in Eastern Mediterranean coastal waters. *Marine Pollution Bulletin*, 60 (5): 701-709.

Simboula, N. & Reizopoulou, S., 2008. An intercalibration of classification metrics of benthic macroinvertebrates in coastal and transitional ecosystems of the Eastern Mediterranean ecoregion (Greece). *Marine Pollution Bulletin*, 56 (1): 116-126.

Simboula, N. & Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. *Mediterranean Marine Science*, 3 (2): 77-111.

Soyer, J., 1970. Bionomie benthique du plateau continental de la côte catalana Francaise. III: Les peuplements de Copépodes Harpacticoides (Crustacea). *Vie Millieu*, 21 (2): 377-511.

Tuğrul, S. & Polat, Ç., 1995. Quantitative comparison of the influxes of nutrients and organic carbon into the Sea of Marmara both from anthropogenic inputs and from the Black Sea. *Water Science & Technology*, 32 (2): 115-121.

Venturini, N., Tommasi, L.R., Bicego, M.C. & Martins, C.C., 2004. Characterization of the benthic environment of a coastal area adjacent to an oil refinery, Todos os Santos Bay (NE-Brazil). *Brazilian Journal of Oceanography*, 52 (2): 123-134.

Weisberg, S.B., Thompson, B., Ranasinghe, J.A., Montagne, D.E., Cadieu, D.B. et al., 2008. The level of agreement among experts applying best professional judgment to assess the condition of benthic infaunal communities. *Ecological Indicators*, 8 (4): 389-394.

Winkler, L.W., 1888. The determination of dissolved oxygen in water. *Deutschen Chemischen Gesellschaft*, Berlin, 21: 2843-2855.