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Research Article

Golden Horn Estuary: Description of the ecosystem and an attempt to assess its ecological quality status using various classification metrics

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Abstract

In this paper, we describe the pelagic and benthic ecosystem of the Golden Horn estuary opening into the Marmara Sea. To improve the water quality of the estuary, which had long been subject to severe anthropogenic pollution (industrial, chemical, shipping), industrial facilities were moved from the estuary in the 1980s, followed by a rehabilitation plan in the 1990s. Our results, based on chemical parameters and phytoplankton showed some signs of improvement of water conditions in the upper layer. However, macrozoobenthic findings of this study did not reflect such a recovery in bottom life.

An approach to the Ecological Quality Status (EQS) assessment was performed by applying the biotic indices BENTIX, AMBI, BOPA, BO2A. Our final assessment was based on “expert-judgements” and revealed a very disturbed overall ecosystem with “bad” EQS for the station at the head of the estuary, “poor” in the rest of the estuary and “moderate” EQS only in the middle station.

Keywords: Golden Horn; Ecological quality status; Phytoplankton; Zoobenthos.

Introduction

The Golden Horn Estuary is located on the southwest coast of the Bosphorus in the northwestern area of Turkey. It is 7.5 km long and has an average width of 400 m. Its maximum depth is about 40 m at the mouth and decreasing to only a few meters at inner parts, depth is shallower than 10 m for nearly one third of its length (KINACI *et al.,* 2004). It is characterized by a permanently stratified water system similar to the neighbouring Bosphorus and Marmara Sea: the upper layer originating from the Black Sea is approximately 25 m thick with ~20 psu salinity and the lower layer from the

Mediterranean has a salinity of around 38 psu. An additional 2-3 m thick quasi-fresh water zone (~ 10 psu) formed by local industrial and domestic discharges and streams was occasionally present above the two-layered system (ALPAR *et al.,* 1999).

The estuary has long been subjected to severe pollution. Particularly since the 1950s, the increase in settlements and different industrial plants around the estuary has caused dramatic rises in pollution levels, especially by blocking the water circulation because of floating bridges and dry docks. Subsequently, stressful environmental conditions characterized by hypoxia and the formation of hydrogen sulfide prevailed at the inner parts (KIRATLI & BALKIS, 2001; YÜKSEK *et al.,* 2006). To improve the water quality, industrial facilities were moved from the estuary in the 1980s, followed by a rehabilitation plan in the 1990s. Thus, wastewater discharges were gradually taken under control and connected to collector systems. About 4.5 million m^3 of anoxic sediment was dredged from the inner part and pumped into an abandoned stone quarry, increasing the water depth at this part to 4-5 m. After the construction of northern and southern sewerage trunk systems in 1999 and the opening of the floating bridges to ease water circulation in 2000, the pollution in the estuary began to decrease. Recent studies have shown that water quality and ecological conditions were remarkably improved after the rehabilitation activities (ASLAN-YILMAZ *et al.,* 2004; GÖNÜLLÜ *et al.,* 2006; YÜKSEK *et al.,* 2006).

A number of biotic indices have been designed to establish the ecological quality of European coastal waters based on the response of benthic macroinvertebrate communities to natural and man-induced changes in environmental conditions. Species richness and Shannon-Wiener (H′), simple indices characterising species abundance, distribution and diversity, as well as biotic indices like BQI, BENTIX, AMBI, BOPA, BO2A which also incorporate the sensitivity/tolerance of species (BORJA *et al.,* 2000; SIMBOURA & ZENETOS, 2002; ROSENBERG *et al.,* 2004; DAUVIN & RUELLET, 2009) have been used and often intercalibrated (REISS & KRONCKE, 2005; LABRUNE *et al.,* 2006; BIGOT *et al.,* 2008; GREMARE *et al.,* 2009).

However, the Ecological Quality Status (EQS) in transitional waters such as the study area, has been questioned and argued a lot (BLANCHET *et al.,* 2008; BORJA *et al.,* 2008; DAUVIN & RUELLET, 2009).

To date, only a few studies providing data on separate components of the Golden Horn ecosystem have been performed (e.g. ÜNSAL, 1988; TUNCER *et al.,* 2001; ASLAN-YILMAZ *et al.,* 2004; ALTUNKAYNAK *et al.,* 2005). The most comprehensive one, with a holistic approach (YÜKSEK *et al.,* 2006), is assessing environmental changes through macro- and microbiological data and water quality parameters.

The aim of the present work is to present the current status of the ecosystem. Furthermore, an attempt is made to arrive at a single evaluation of the Golden Horn ecosystem ecological quality status by integrating assessments based on biotic indices of different biological elements (phytoplankton, zoobenthos) and integrating multiple physico-chemical parameters.

Materials and Methods

This study was carried out at the Golden Horn Estuary in December 2005 at five stations (Fig. 1). Water samples for physico-chemical analysis were collected by using a 3 liter Ruttner bottle with a thermometer, both from surface and just above

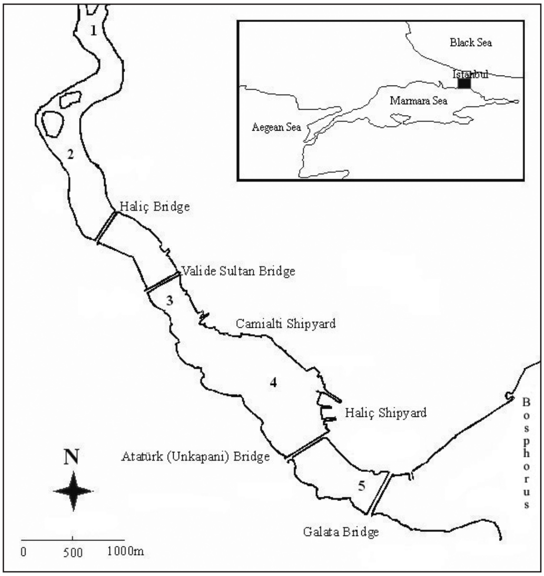


Fig. 1: Map of Golden Horn Estuary showing location of sampling stations.

the sea bed. Temperature, salinity (IVANOFF, 1972) and dissolved oxygen (dO) (WINKLER, 1888) were determined from surface and near sea bed depths. Water visibility was measured with a Secchi disk.

Surface water samples, for the determination of nutrients, were collected in 100 ml polyethylene bottles and kept continuously at a temperature of -20° C until their analysis in the laboratory. Nitrate+nitrite (NO_3+NO_2-N) concentrations were analyzed by cadmium reduction method on a Skalar autoanalyser (APHA, 1999). Phosphate (PO_4-P), silicate (SiO4-Si) and chlorophyll *a* (Chl *a*) concentrations were measured by the methods described by PARSONS *et al.* (1984). An additional water sample was taken by Ruttner bottle for qualitative and quantitative composition of surface phytoplankton. The results of countings were summarized as cells per liter (SEMINA, 1978). The small size fraction of phytoplankton (<20μm) was not enumerated. The metrics used in the phytoplankton assessment are composition, abundance, chlorophyll-a concentrations and the Trophic Index (TRIX). The Trophic Status was assessed using the scale proposed by IGNATIADES (2005). The Trophic Index is a linear combination of the logarithms of four state variables [Chl-*a*, N, P and the absolute percentage deviation from oxygen saturation D%O (VOLLENWEIDER *et al.,* 1998)], and characterises the trophic conditions of sea water, identifying four quality classes: highly productive, moderately productive, scarcely productive and open waters. EQS values were calculated for each station according to GIOVANARDI & VOLLENWEIDER (2004) and PENNA *et al.* (2004).

At each site surface sediment samples were taken in order to determine mud percentage and total organic carbon (TOC) content of the sea bed. All samples were kept in a refrigerator (~4° C) immediately after collection. The mud percentages of sediment samples were analysed according to FOLK (1974). The total organic carbon was estimated by the Walkey-Blake method (LORING & RANTALA, 1992).

Benthic samples were collected by van Veen grab with a sampling area of 0.1 m^2 at depths between 4 and 40 m. At each station three replicates were taken for macrozoobenthic fauna analysis. Samples were sieved through the most frequently used screen size of 1 mm. Material was preserved in 4 % formaldehyde prepared in sea water. All macrobenthic organisms were identified to species level when available and individuals of each species were counted. Metrics used for zoobenthos included species number and number of specimens, Shannon-Wiener (SHANNON & WEAVER, 1949) community diversity (H'log2) as well as AMBI (BORJA *et al.,* 2000), BENTIX (SIMBOURA & ZENETOS, 2002), Benthic Opportunistic Polychaetes Amphipods index BOPA (DAUVIN & RUELLET, 2007) and its adaptation Benthic Opportunistic Annelida Amphipod index BO2A (DAUVIN & RUELLET, 2009). For the calculation of AMBI, AMBI version 4.1 with the species list version of December 2007 (available at AZTI's web page http://www.azti.es) was utilized. The BENTIX was calculated according to a species list provided on the web page (http://www.hcmr.gr).

Multivariate analyses were applied to both phytoplankton and macrozoobenthos communities. The Bray-Curtis Similarity Index, was employed in order to determine the similarity between stations and MDS in order to analyse the spatial distribution pattern. Raw data were transformed as log (x+1) transformation (CLARKE & WARWICK, 2001). SIMPER analysis was implemented to identify the dissimilarity level between groups and the percentage contribution of each species to the overall similarity/dissimilarity within each groups that were detected by cluster analysis. In order to determine correlation between biotic and abiotic parameters, additional multivariate analyses were applied such as the Spearman's rank correlation coefficient (SIEGEL, 1956). The significance of the differences in abiotic parameters of the groups detected by cluster and MDS methods were assessed by using one way ANOVA.

Results

Abiotic parameters

The abiotic parameters calculated at sampling stations are presented in Table 1.

Surface water temperature was the same at all stations (12° C) while near the sea bed it was 12° C at the shallower stations and 15° C at the deeper ones. Salinity increased from the innermost part of the estuary towards its mouth to the Bosphorus having a minimum (16.1 psu) at Station 1 and maximum (20.6 psu) at Station 5 for surface waters and between 17.6 and 19.7 psu at the shallower stations for the sea bed and more than 35 psu at the deeper stations. A similar trend was observed also for dissolved oxygen (2.0 $mg \cdot l^{-1}$ at Station 1 and 7.5 $mg \cdot l^{-1}$ at Station 5) for surface waters. Regarding nutrient concentrations, a decreasing trend towards the estuary mouth was noticed for phosphate and silicate [phosphate from 6.79 μg-at $P \cdot l^{-1}$ (st. 1) to 0.87 μg-at $P \cdot l^{-1}$ (st. 5) and silicate from 76.33 μg-at $Si \cdot l^{-1}$ (st.1) to 4.26 μg-at $Si \cdot l^{-1}$ (st. 5)] while nitrate+nitrite levels did not present any pattern (Table 1). The atomic ratio of nitrogen to phosphorus varied between 0.3 (st. 2) and 2.4 (st. 4), being always lower than the Redfield ratio of 16N:1P (REDFIELD *et al.,* 1963). The highest Si:N ratio was 38.3 at Station 2 and the lowest 2.3 at Station 4 while the highest Si:P ratio (11.2) at Station 1 and the lowest (4.9) at Station 5.

Table 1
Abiotic parameters for surface (S) and bottom (B) water layers from the inner (St. 1) to the mouth (St. 5) of the Golden Horn Estuary (December 2005). dO: Dissolved oxygen, TOC: Total organic carbon.

Parameter/Stations	St. 1	St. 2	St. 3	St. 4	St. 5
Depth (m)	4	5	6	30	40
Temperature (˚C) (S/B)	12/12	12/12	12/12	12/15	12/15
Salinity (psu) (S/B)	16.1/17.6	17.1/19.3	17.5/19.7	18.1/36.2	20.6/35.6
dO (mg/L) (S/B)	2.0/3.6	4.6/5.3	5.4/6.1	5.5/2.2	7.5/2.1
Nitrite+nitrate-N (μg-at/L) (S)	3.24	1.01	2.62	3.72	1.55
Phosphate-P (μg-at/L) (S)	6.79	3.97	2.41	1.53	0.87
Silicate-Si (μg-at/L) (S)	76.33	38.65	24.77	8.53	4.26
N:P	0.5	0.3	1.1	2.4	1.8
Si:N	23.6	38.3	9.5	2.3	2.7
Si:P	11.2	9.7	10.3	5.6	4.9
Mud (%) (B)	98.4	93.6	84.1	99.5	86.5
TOC (mg/g) (B)	51.2	34.1	26.1	45.8	42.7
Visibility (m)	2.2	3.5	3.8	5.1	5.5

Transparency exhibited a decreasing trend from the mouth of the estuary (5.5 m at Station 5) towards the innermost part (2.2 m at Station 1).

Dissolved oxygen content of sea water near the sea bed varied between 2.1 mg·l^{-1} (st.5) and 6.1 mg·l^{-1}(st.3). The lowest values were detected at stations containing saline Mediterranean originated waters.

The substratum at all stations was muddy and characterized by high concentrations of organic carbon. Mud percentages ranged between 84.1 % (st.3) and 99.5 % (st.4) and TOC content between 26.1 mg·g^{-1} (st.3) and 51.2 mg·g^{-1} (st.1).

Biotic parameters

Microphytoplankton composition

From the analysis of the phytoplankton community composition, 64 species belonging to seven different algal groups were identified: 1 cyanophycean (1.6 %), 25 dinoflagellates (39.1%), 2 dictyochophyceans (3.1%), 31 diatoms (48.4%), 2 euglenophyceans (3.1%), 1 chrysophycean (1.6%) and 2 chlorophyceans (3.1%). Four of them (*Phormidium* sp., *Phacus* sp., *Closterium* sp. and *Pediastrum simplex* Meyen) were brackish water species. Regarding species number, *Ceratium* was the most dominant genus with eight species. Diatoms were the most dominant component of the population in respect to number of individuals (57.2%), followed by Dinoflagellates (35.1%). The others (Cyano-, Dictyocho-, Eugleno-, Chryso- and Chlorophyceae) formed only 7.7% of the whole population.

A remarkable increase in total phytoplankton abundance was observed at Station 5 (42.4 x 10^3 cells·l^{-1}) (Table 2). This station was the richest also in terms of species

Table 2
**Metrics for phytoplankton. S: Species number, N: Number of individuals,
H': Community diversity. Percentage of occurence of the dominant phytoplankton species
(DIN: Dinoflagellates, DIA: Diatoms, EUG: Euglenophycean).**

Parameter/Stations	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**
S	16	14	22	20	33
N	19300	14700	5800	29000	42400
H'	2.32	2.76	3.4	3.02	2.5
Chl a	0.3	0.2	0.3	0.9	0.8
Diatoms:Dinoflagellates	113.25	20.66	2.21	0.08	1.94
Dominant species					
Ceratium furca (DIN)					6
Ceratium fusus (DIN)			13	7	8
Heterocapsa triquedra (DIN)				21	
Prorocentrum micans (DIN)				22	7
Prorocentrum scutellum (DIN)				21	11
Cylindrotheca closterium (DIA)		35			
Detonula confervaceae (DIA)	46	24	10		
Leptocylindrus minimus (DIA)	20				
Navicula sp. (DIA)	20	12	24		
Proboscia alata (DIA)		6	17		56
Eutreptiella sp. (EUG)		8	12	12	

diversity. The predominant species of diatoms was *Proboscia alata* (Brightwell) Sundström (23.8 x 10^3 cells·l^{-1}). The most significant dinoflagellates species are shown in Table 2. The lowest total phytoplankton abundance was recorded at Station 3 (5.8 x 10^3 cells·l^{-1}). With regard to numbers of individuals, dinoflagellates were the most common at Station 4 (77.5%) and the least common at Station 1 (0.8%). On the contrary, diatom percent abundance reached the highest level at Station 1 (93.8%), followed by Stations 2 (84.4 %), 5 (65.4%), 3 (58.9 %) and 4 (6.8%). *Detonula confervaceae* (Cleve) Gran (8.8 x 10^3 cells·l^{-1}), *Leptocylindrus minimus* Gran (3.9 x 10^3 cells·l^{-1}) and *Navicula* sp. (3.8 x 10^3 cells·l^{-1}) played a very important role in the increase of diatoms at Station 1.

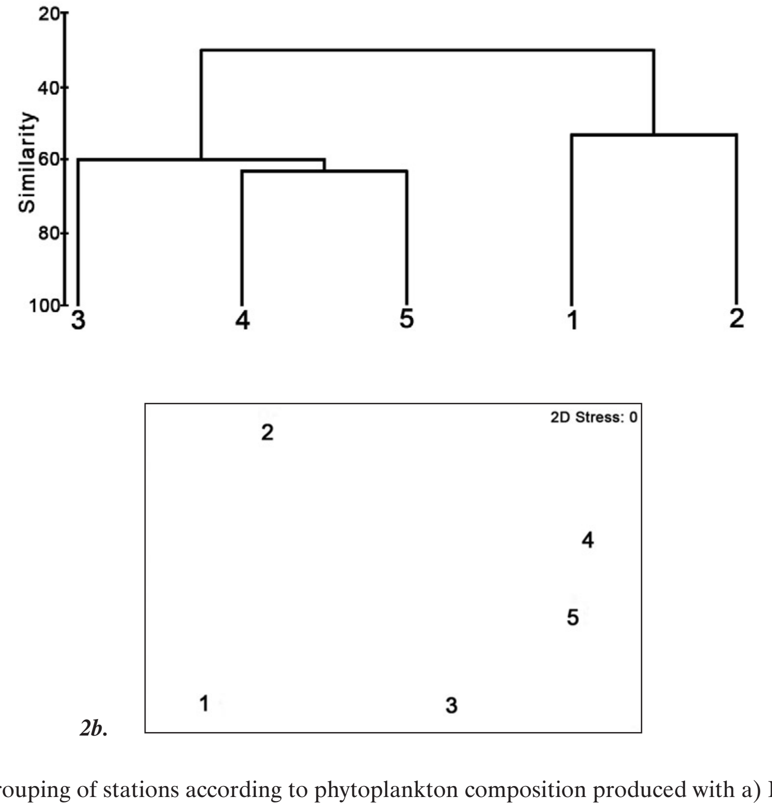
Diatom:dinoflagellate ratio was between 0.08 (St. 4) and 113.25 (St. 1). This ratio decreased due to an increase in dinoflagellates especially at Station 4, while it attained maximum values at the two inner stations due to a decrease in dinoflagellate abundance (Table 2).

The highest species diversity (H′) was found at Station 3 (3.40 bits) and the lowest at Station 1 (2.32 bits). Chlorophyll a concentrations, as an indicator of phytoplankton biomass, ranged between 0.2 μg·l^{-1} (St. 2) and 0.9 μg·l^{-1} (St. 4). Chl *a* values of the outer stations (Sts. 4 and 5) were higher than the inner stations of the estuary.

According to the results derived by the Spearman rank correlation coefficient, salinity and dissolved oxygen values were negatively correlated with phosphorus and silica content ($p<0.01$). Dissolved oxygen was positively correlated with salinity ($p<0.01$) and phosphorus was positively associated with silica ($p<0.01$). Also, dinoflagellate abundance was positively correlated with salinity, dissolved oxygen and chl a ($p<0.05$), while it was negatively correlated with phosphorus and silicate ($p<0.05$).

Cluster analysis and MDS, applied to combine the stations according to their quantitative composition, showed two distinct groups (Fig. 2). The first group included the outer stations St.3, St.4 and St.5 with 61% similarity and the second group stations St.1 and St.2 with 53 % similarity. The average dissimilarity between groups 1 and 2 was 58% according to SIMPER analyses. The species most contributing to dissimilarity were *P. micans* (5.9%), *D. confervaceae* (5.0%), *C. furca* (4.6%), *P. scutellum* (4.0%), *P. compressum* (Bailey) Abe *ex* Dodge (3.8%), *Protoperidinium divergens* (Ehrenberg) Balech (3.5%) and *Scrippsiella trochoidea* (Stein) Loeblich III (3.5%).

Statistically significant differences in phosphorus ($F= 9.93$, $p<0.05$) and Si:N ratio ($F= 17.44$, $p<0.05$) were defined between groups by ANOVA analysis.



2a.

2b.

Fig. 2: Grouping of stations according to phytoplankton composition produced with a) Bray-Curtis group average clustering technique and b) MDS ordination.

Macrozoobenthos composition

A total of 2663 specimens was counted and assigned to 35 macrozoobenthic taxa. Polychaeta was the most dominant group in terms of species (16 species, 45.7%) and abundance (1817 specimens, 68.2%). It was followed by Mollusca (9 species, 25.7%) for species number and by Oligochaeta (693 specimen, 26%) for abundance.

The four most abundant taxa in the study area, making up 87% of all specimens, in decreasing order were *Polydora cornuta* Bosc, 1802, Oligochaeta sp., *Heteromastus filiformis* (Claparede, 1864) and *Malacoceros fuliginosus* (Claparede, 1868).

Mean values of some community parameters and biotic indices are shown in Table 3. Species number varied between $1 \cdot 0.1m^{-2}$ (st.1) and $13 \cdot 0.1m^{-2}$ (st.3) while specimen number between $3 \cdot 0.1m^{-2}$ (st.1) and $309 \cdot 0.1m^{-2}$ (st.5). The dominant species at each station and percentage of dominance within station are also listed in Table 3. Diversity index (H′) values were low at all stations the lowest being $0.35 \cdot 0.1m^{-2}$ (st.1) and the highest $1.46 \cdot 0.1m^{-2}$ (st.3).

The MDS and cluster analyses displayed two distinct groups (Fig. 3). The first group was formed by Stations 2 and 3, the second group by Stations 4 and 5 while Station 1 was clearly separated from both aforementioned groups. Similarity between Stations 2 and 3 was 39.4%, between 4 and 5 was 36.4%. SIMPER analyses detected high dissimilarity between these two groups as 96.6%. The species most contributing to dissimilarity were *P. cornuta* (34 %) and *H. filiformis* (23.9%). Station 1 had a dissimilarity level of 98.2% with first group due to *P. cornuta* (45.7%) and *H. filiformis* (32.9%). Dissimilarity between Station 1 and the second group was 99.3%. Oligochaeta sp. (67.5%) and *M. fuliginosus* (19.1%) were dominantly responsible for this dissimilarity.

The Spearman rank correlation coefficient (r_s), revealed statistically significant negative correlations ($p<0.05$) between organic carbon (TOC) and species number and between TOC and community diversity.

ANOVA defined statistically significant differences in respect to some ecological factors between groups such as dissolved oxygen ($F= 42$ $p<0.05$), salinity ($F= 1348$, $p<0.01$), transparency ($F= 61$, $p<0.05$), temperature ($F=0$, $p=0$) and depth ($F= 21$, $p<0.05$). Mud percentage and TOC content of the sediment did not show any significant difference between groups ($p>0.05$).

Assessment of Ecological Quality Status

Following the rationale that the benthic communities represent a long-term record of local water quality conditions, we decided to compare ecological quality results with water quality using the TRIX index. The TRIX index calculated in the Golden Horn ranged from 2.6 (St. 2) to 3.3 (St. 4) but was overall lower than 4 which shows low trophic level but high quality state in the estuary. TRIX was positively correlated with nitrogen ($p<0.01$), but the index did not demonstrate any significant relation with other environmental parameters.

Altogether 35 macrozoobenthic species of different sensitivity status were identified in our samples. Often when various estuarine benthic indices show discrepancies in their assessments of benthic condition, they are reflecting different aspects of benthic condition. The application of the macrozoobenthic metrics H′, AMBI, BENTIX, BOPA, BO2A did not lead to a clear assessment (Table 4). In only one station (St.1) all indices H′, AMBI, BENTIX, BOPA, BO2A concurred in the ecological status assessment.

Table 3
Metrics for macrozoobenthos. Mean values for S: Species number, N: Number of individuals, H': Community diversity. Percentage of occurence of the dominant macrozoobenthos species (POL: Polychaeta, MOL: Mollusca).

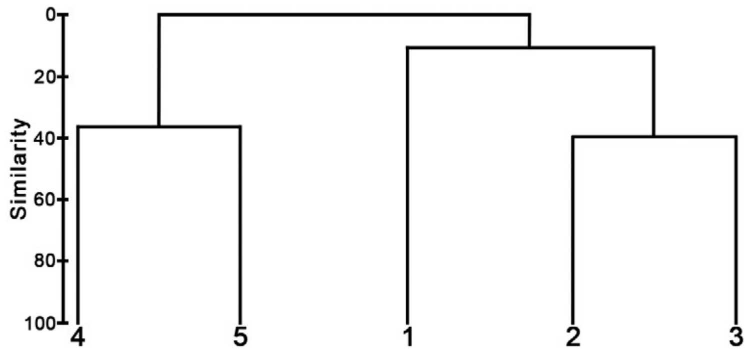
Parameter/Stations	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**
S/0.1m^2	1	9	13	2	6
N/0.1m^2	3	303	262	12	309
H'/0.1m^2	0.35	1.19	1.46	0.77	1.09
Dominant species					
Malacoceros fuliginosus (POL)	75	10.7			24.9
Polydora cornuta (POL)		78.6	11.9		
Neanthes succinea (POL)		3.9	6.2		
Heteromastus filiformis (POL)			65.2		
Mytilus galloprovincialis (MOL)			6.1		
Corbula gibba (MOL)				8.5	
Oligochaeta sp.				82.8	67.4

Table 4
Assessments derived by various biotic and abiotic indices and final assessment for the study stations.

	St. 1	**St. 2**	**St. 3**	**St. 4**	**St. 5**
H' (Macrozoobenthos)	0.35 Bad	1.19 Bad	1.46 Bad	0.77 Bad	1.09 Bad
BENTIX	0.67 Bad	2.01 Poor	2.12 Poor	2.00 Poor	2.07 Poor
AMBI	6.42 Bad	4.51 Poor	4.11 Moderate	5.47 Poor	5.86 Bad
BOPA	0.30 Bad	0.29 Bad	0.27 Bad	0 High	0.17 Moderate
BO2A	0.30 Bad	0.29 Bad	0.27 Bad	0.21 Poor	0.29 Bad
TOC (mg/g)	51.2 High risk	34.1 High risk	26.1 Intermediate risk	45.8 High risk	42.7 High risk
dO (mg/L) bottom	3.6 Moderate	5.3 Good	6.1 High	2.2 Poor	2.1 Poor
Final assessment	**BAD**	**POOR**	**MODERATE**	**POOR/BAD**	**POOR**

The EQS derived by the Community diversity index (H′) was low at all stations, ranging from bad to bad/poor. Low Shannon (H′) index is not necessarily a sign of degradation, being probably related to the natural condition of this transitional system. Thus, H' index is not representative in the Golden Horn estuary, a transitional ecosystem, as the classification scale for H′ is based on coastal waters (ZENETOS & SIMBOURA, 2001).

The BENTIX values were around the lowest limits, showing 'poor' EQS conditions, at four stations and only the innermost station, classified in the 'bad' EQS. This is to be expected as two of the benthic samples were azoic.



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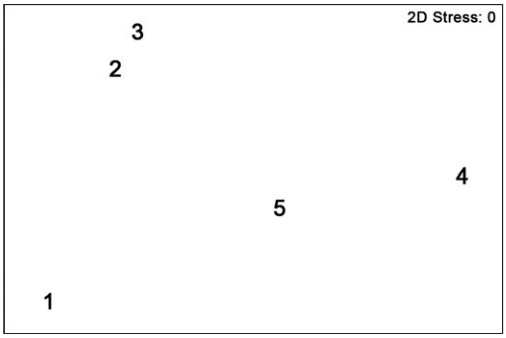

3b.

Fig. 3: Grouping of stations based on macrozoobenthos composition produced with a) Bray-Curtis group average clustering technique and b) MDS ordination.

The AMBI values were between 4.11·0.1m^{-2} (St. 3, indicating 'moderate' EQS conditions) and 6.42·0.1m^{-2} (St. 1, indicating 'bad' EQS).

The assessment derived by the Opportunistic Polychaetes to Amphipod ratio index (BOPA) was consistent with BENTIX and AMBI for St. 1, but differed in the other stations. Its adaptation for freshwater and transitional waters, the BO2A index which incorporates the oligochaetes among the opportunistic taxa, led to assessments closer to those produced by other indices. Yet, it classified St. 4 towards the mouth of the estuary in the 'poor' EQS, although the species diversity is very low, dominated by Oligochaeta (>82% in abundance).

Following TOC thresholds identified by MAGNI *et al.* (2009) it is indicated that risks of reduced benthic diversity should be relatively high at TOC values > about 28 mg·g^{-1} (st 1, 2, 4 and 5), and intermediate at values in between 10 and 28 as is the case of station 3.

Considering the interpretations assessment presented in Table 4, and abiotic measurements shown in Table 1, we conclude the following classification of the ecological status, which in most cases coincides with the worst of the values in the biological elements.

Station 1, located at the innermost part of the estuary, was classified as 'bad'. Also, all biotic indices were classified this station as of 'bad'EQS (Table 4). Two replicates of this station were azoic. Only eight specimens belonging to only three species were encountered from the third replicate. All three species [*Neanthes succinea* (Frey&Leuckart, 1847), *Streblospio gynobranchiata* Rice&Levin, 1998, *M. fuliginosus*] are pollution tolerant. The TOC level measured at this station was 51.2 $mg \cdot g^{-1}$.

Station 2 is considered as of 'poor' EQS. Species variety and abundance, 9 species distributed among 303 specimens, are not very low. However, the most abundant species are highly tolerant of pollution. *P. cornuta*, an alien species, contributed 78.6% and *M. fuliginosus* 10.7% to the total specimens at this station. BENTIX and AMBI classified this station as poor, BOPA and BO2A as bad. TOC content of the sediment was also quite high.

The highest ecological status in the Golden Horn Estuary of 'moderate' was assigned to Station 3. Here, the mean species number was 13 and specimen number 262 per 0.1 m^2. The pollution tolerant species *H. filiformis* (65.2%) and *P. cornuta* (11.9%) dominated this station. *Athanas nitescens* (Leach, 1814), a sensitive crustacean, is present at this station representing 0.4% of all individuals. Moreover, the crustacean *Melita palmata* (Montagu, 1804), the mollusc *Modiolula phaseolina* (Philippi, 1844) and *Parvicardium exiguum* (Gmelin, 1791), which formed 1.3% of all specimens, are sensitive to organic enrichment. Station 3 had the lowest TOC value (26.1 $mg \cdot g^{-1}$) in the estuary, despite still being high.

A total of four species and 35 specimens was obtained from Station 4 while means were 2 species/0.1m^2 and 12 individuals/0.1m^2, respectively. All of the species are tolerant to pollution. This station was classified as 'poor to bad' due to its high TOC content (45.8 $mg \cdot g^{-1}$), low oxygen concentration near bottom (2.2 $mg \cdot l^{-1}$) which results in low diversity values.

Station 5 was rated as 'poor'. Mean species number is low (6 species/0.1m^2) but specimen number relatively high (309 individuals/0.1m^2). The deterioration in oxygen conditions in the near bottom layer (2.1 $mg \cdot l^{-1}$) as opposed to the highly oxygenated surface waters (7.5 $mg \cdot l^{-1}$) and high organic content (42.7 $mg \cdot g^{-1}$) mirror a very disturbed ecosystem. Indeed, the most abundant species such as Oligochaeta sp. 67.4% and *M. fuliginosus* 24.9% are highly tolerant of pollution.

Discussion

When marine ecosystem assessments began for the European Water Framework Directive (WFD) in the early 2000, benthos was the biological element most often assessed. Today, the European Marine Strategy requires integrated assessments of marine quality based on ecological evaluation of several biological elements (phytoplankton, benthos, algae, phanerogams and fishes) together with assessments by different disciplines (chemists, physicists, engineers, managers, etc.) to reach agreement on the final assignment of ecological status. Integration of information to this high level has rarely, if ever, been attempted. An integrative assessment using multiple ecosystem components, in assessing ecological status was made by BORJA *et al.* (2009).

Based on the availability of ecological quality thresholds of different physico-chemical parameters (nutrients, dO, TOC) and biological elements (phytoplankton, zoobenthos), different station assessments were combined into a final one. Comparison with historical data illustrates how the assessed integrated quality has changed over time.

YÜKSEK *et al.* (2005; 2006) studied the historical changes in biodiversity of the Golden Horn Estuary, and claimed a remarkable recovery in marine life and improving water quality after rehabilitation studies in 1990s. Upper water layer chemical conditions and phytoplankton community composition findings in this study support this claim.

In 1972 the surface water of the inner part was anoxic (0 $mg{\cdot}l^{-1}$) but the outer part had a high oxygen level (8.01 $mg{\cdot}l^{-1}$) while the bottom sea water at 25 m depth had low dissolved oxygen values at the middle and outer parts of the estuary as 2.35 $mg{\cdot}l^{-1}$ and 2.89 $mg{\cdot}l^{-1}$, respectively (YÜCE, 1972). An increase in oxygen concentrations was observed in the Golden Horn following recovery studies. Minimum value of dO at the surface of outer estuary was ~3 $mg{\cdot}l^{-1}$ in 1998 but reached ~5 $mg{\cdot}l^{-1}$ in 2000 (YÜKSEK *et al.,* 2006) and was measured to be 7.5 $mg{\cdot}l^{-1}$ in the present study (December 2005). Such a recovery was also declared by GÖNÜLLÜ *et al.* (2006), both for the surface and bottom at the inner part of the Golden Horn estuary. BEST *et al.* (2007) proposed thresholds of dO for five EQ classes fitting the needs of the European Water Framework Directive. In our study, EQS assignments following BEST *et al.* (scheme) produced uneven results among surface and bottom layers (see Table 1). Worth noting is that the oxygen values were determined in winter, it is possible to find lower values in summer. In the stratified water body structure in the Golden Horn, the upper layer is formed by Black Sea brackish waters with a thickness of 25 m and the lower layer by saline Mediterranean originated waters entering via the Marmara Sea. Considering that the renewal rate for the Mediterranean waters in the Marmara Sea is between six and seven years, it is clear that much of the oxygen is depleted during this period and that dO drops to 2 $mg{\cdot}l^{-1}$ by the time the Mediterranean originated waters reach the estuary (KIRATLI *et al.,* 2000; ALPAR *et al.,* 2003). This explains the low dO values of bottom waters near the stations 4 and 5. On the other hand, the upper water layer formed by Black Sea brackish waters, with the exception of Station 1 which is located at head of the estuary and is closest to two streams loading pollutants in high quantities, had relatively high dO content.

The highest surface inorganic phosphate value was observed at the mouth of the estuary in 1998 (10.36 $\mu g\text{-at } P{\cdot}l^{-1}$) and decreased to 1.12 $\mu g\text{-at } P{\cdot}l^{-1}$ in 2001 (YÜKSEK *et al.,* 2006) and further to 0.87 $\mu g\text{-at } P{\cdot}l^{-1}$ in the present study. Moreover, a remarkable decrease of silicate and nitrite+nitrate concentrations was measured at the outer estuary (YÜKSEK *et al.,* 2005). In our study, nutrient levels (phosphorus and silicate) at the outermost station of the Golden Horn were lower than the innermost. High levels of silicate was detected at stations 1 (76.33 $\mu g\text{-at } Si{\cdot}l^{-1}$) and 2 (38.65 $\mu g\text{-at } Si{\cdot}l^{-1}$), the phosphorus content reached its highest level at Station 1 (6.79 $\mu g\text{-at } P{\cdot}l^{-1}$). The inflow of dense domestic and industrial wastewater by the Alibeyköy and Kağıthane rivers towards to Station 1 is obviously responsible for these high nutrient levels at the inner parts (GÖNÜLLÜ *et al.,* 2005). Nutrient levels at the outer part are reduced due to the Black Sea originated Bosphorus waters entering the estuary.

In the Golden Horn, the atomic ratio of N:P was estimated to be much lower than the normal Redfield ratio (16:1) for phytoplankton growth (REDFIELD *et al.,* 1963) which indicates that the estuary is oligotrophic in nature and that Nitrogen is the limiting nutrient. Indeed, the nitrogen limitation of phytoplankton growth is common in coastal systems (NIXON, 1986). Diatom growth in marine waters is likely to be limited by dissolved silica when Si:N ratios are less than 1 according to Redfield ratios (REDFIELD *et al.,* 1963; PIEHLER *et al.,* 2004). In the present study, the Si:N ratios were higher than 1, so silicate is not likely to be a potentially limiting factor for the growth especially of diatoms in the sampling period in the Golden Horn. As a result, this situation caused diatom dominance in terms of diversity and abundance, particularly so at the head of the estuary.

YÜKSEK *et al.* (2006) detected Chl *a* concentrations as generally below 10 $\mu g \cdot l^{-1}$ at the lower estuary and around 50 $\mu g \cdot l^{-1}$ at the upper parts in the year 2000. According to Chl *a* based assesments (IGNATIADES, 2005), Chl *a* values in this study showed that the area is generally oligotrophic in nature (0.2-0.8 $\mu g \cdot l^{-1}$) while mesotrophic waters were observed only at Station 4 (0.9 $\mu g \cdot l^{-1}$). Similarily, the TRIX index revealed a low trophic level but high quality state in the estuary. All chl *a*, TRIX and nutrient values reflected a low trophic level in the estuary and this context shows a recruitment in water column conditions.

Previous phytoplankton studies carried out in the Golden Horn demonstrated that species diversity of the pelagic ecosystem was very limited (SAYDAM *et al.,* 1986; UYSAL, 1987). In 1995, 24 phytoplankton species were identified in the upper layer whereas in the period after rehabilitation species number increased year by year: 43 phytoplankton species were recorded in 1998, 57 in 1999, 81 in 2000, 92 in 2001 and 60 in 2002 (TAŞ, 2003; TAŞ & OKUŞ, 2003). In the present study, 64 species are reported during one sampling period only. Given the limited sampling effort, the finding of such rich species diversity may be regarded as a sign of improvement of water conditions in the upper layer.

However, the macrozoobenthic findings of this study did not reflect such a recovery in bottom life. ÜNSAL (1988) studied the benthic fauna of the Golden Horn between the years 1985-1986 and stated that three pollution indicator species were dominant constituting 89 % in abundance. In this study, carried out in 2005, four of the most abundant pollution tolerant taxa made up 87 % of all specimens. Unfortunately, two studies with a twenty year interval revealed similar results. However, as opposed to two azoic stations in the area reported by ÜNSAL (1988) only two azoic replicates were found in this study (St. 1) and yet the station was not azoic. This alone may be accepted as an improvement sign.

Sampling efforts revealed a total of 35 macrozoobenthic taxa belonging to different taxonomic groups but none of them belonged to Echinodermata. The absence of echinoderms is another indicator of pollution. Moreover, five alien species [polychaetes *Polydora cornuta, Streblospio gynobranchiata, Desdemona ornata* Banse, 1957, *Ficopomatus enigmaticus* (Fauvel, 1923), bivalve *Tapes philippinarum* (Adams & Reeve, 1850)] existed in the study area with an abundance of 31.2% within total individuals. Alien species seem to be favoured in estuarine and polluted environments since they are robust to unsuitable conditions so they can readily colonize such areas (ZIBROWIUS, 1992; ÇINAR *et al.,* 2006). The presence of aliens with high abundance shows stressed conditions in the estuary.

Low species diversity is a sign of organic pollution whereas low species diversity together with low abundace an indication of acidic or toxic contamination (MASON *et al.,* 1985). Mean species numbers of stations were not very high in all stations but extremely low at Stations 1 ($1 \cdot 0.1m^{-2}$) and 4 ($2 \cdot 0.1m^{-2}$). Moreover, also mean specimen numbers were also very low at $2 \cdot 0.1 m^{-2}$ at Station 1 and $12 \cdot 0.1 m^{-2}$ at Station 4 whereas at least $262 \cdot 0.1 m^{-2}$ at the other three stations. Station 1 is located at the uppermost part while Station 4 between two shipyards. Sediment accumulation rate in the Golden Horn is very high at 3.5 cm per year (TEKSÖZ *et al.,* 1991). The estuary receives 50.000.000 $m^3 \cdot year^{-1}$ water and 59.000 $m^3 \cdot year^{-1}$ sedimentary material from two main polluted streams, namely the Alibeyköy and Kağıthane, flowing into the estuary (DALAN, 1988). Moreover, 1.900.000 tonnes of liquid waste and 49.000 tonnes of solid waste are discharged here by many industrial plants (TEZCAN & DURGUNOĞLU, 1977). Metal pollution in the estuary has been known since the beginning of the 17th century (KIRATLI *et al.,* 2000). A study carried out by TUNCER *et al.* (2001) near the Atatürk Bridge showed that Cu values were approximately four times higher than shale average (KRAUSKOPF, 1979) while Zn were ten times, Cd twenty times, Pb twenty-five times and Ag just 130 times higher. CUMALI & GÜVEN (2008) indicated high oil pollution in the Golden Horn especially near the Haliç Bridge (174.5 $\mu g \cdot l^{-1}$) and the Atatürk Bridge (104.9 $\mu g \cdot l^{-1}$) and claimed that the Alibeyköy and Kağıthane streams were the sources of this oil pollution. Moreover, the estuary is affected by oil pollution coming from ship accidents occurring in the Bosphorus or Marmara Sea (GÜVEN *et al.,* 2004). The above mentioned heavy metal and oil pollution quantities show the presence of toxic pollution in the estuary and it is assumed that low species and specimen diversity in the Stations 1 and 4 are caused by this pollution.

Results from indices H′, BENTIX, AMBI, BOPA and BO2A did not lead to a clear assessment (Table 4). In only one station (St.1) all indices H′, BENTIX, AMBI, BOPA, BO2A concurred in the ecological status assessment.

Shannon-Wiener diversity index (H′) values of macrozoobenthic fauna were low at all stations, the highest being $1.46 \cdot 0.1 m^{-2}$ bits, indicating ‘bad’ EQS. However, low Shannon (H’) index and species number (S) are not necessarily a sign of degradation, being probably related to natural condition of transitional systems (DAUVIN & RUELLET, 2007) such as our study area.

BENTIX showed ‘bad’ EQS for Station 1 of which two replicates are azoic and ‘poor’ for the remaining four stations while AMBI was ‘bad’ for Stations 1 and 5, ‘poor’ for Stations 2 and 4, ‘moderate’ for Station 3. The Benthic Opportunistic Polychaetes Amphipods index (BOPA) which ignores the Oligochaeta led to meaningless classification for the stations towards the mouth of the estuary almost exclusively dominated by Oligochaeta. It is clear that BENTIX tends to underestimate the EQS in slightly or moderately disturbed lagoons as opposed to AMBI and BOPA that in our results appear to overestimate the situation. BENTIX’s tendency to underestimate the EQS in transitional ecosystems is due to the fact that in estuaries there is a natural dominance of tolerant species which is the so-called Estuarine Quality Paradox (SIMBOURA & REIZOPOULOU, 2008). The BO2A index, which has been designed for use in the freshwater zones of transitional waters (i.e., up to the upper limit of tidal range) is more sensitive and produced results more consistent when combining abiotic and biotic assessments. The supreme performance of BO2A index versus the aforementioned bioindicators is probably due to the fact that it is developed specifically for such variable anthropogenized systems comprising a variety of conditions (DAUVIN & RUELLET, 2009).

Our final assessment based on "expert-judgements" was 'bad' for Station 1, 'poor to bad' for Station 4, 'poor' for Stations 2 and 5 and 'moderate' for Station 3.

Whereas this analysis was based on our personal, yet extensive, knowledge of the study area and the location of sampling stations, it helps to illustrate that the identified thresholds for BO2A index appear to be realistic and predictive of disturbed condition where clear evidence of disturbance exists. The results made biological and ecological sense and physico-chemical improvements were often correlated with improvements in the quality of benthos.

In conclusion, the findings of this study revealed some improvements in water column properties but benthic life is still unhealthy. It is suggested that recovery studies must be continuously carried out until all compartments of the estuary become rehabilitated, especially focusing on the bottom environment.

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