THE EFFECTS OF SEWAGE DISCHARGES ON SHALLOW HARD SUBSTRATE MACROZOOBENTHIC COMMUNITIES IN THE BOSPHORUS

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Evrim Kalkan

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APPROVED BY:

Prof. Miray Bekbölet (Thesis Supervisor)	
Assoc. Prof. Erhan Mutlu (Thesis Co-supervisor)	
Prof. Barış Mater	
Assoc. Prof. Ece Şen	
Assoc. Prof. Melek Türker Saçan	

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The thesis is dedicated to my grandmother, Sabahat Kalkan and to the memory of my mother Hayriye Kalkan.

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THE EFFECTS OF SEWAGE DISCHARGES ON SHALLOW HARD SUBSTRATE MACROZOOBENTHIC COMMUNITIES IN THE BOSPHORUS

In order to determine the pollution effects of sewage discharges on the benthic communities, shallow water hard bottom macrozoobenthic communities were examined along the coasts of the Bosphorus Strait. Samples were collected at 15 stations and environmental parameters measured at 3-month intervals from May 2004 to February 2005. While 9 stations were selected as discharge stations, 6 stations were selected as control stations. A total of 180 samples were collected in May, August and November 2004 and February 2005. Samples were collected from the upper infralittoral zone at dept range 0.5-1m.

The analysis of 180 quadrate samples yielded a total of 167537 individuals belonging to 85 taxa. These are distributed qualitatively among the taxonomic groups as follows: Crustacea 50.59% (43 taxa); Polychaeta 21.18% (18 taxa); Mollusca 14.12% (12 taxa); Nemertea 3.53% (3 taxa); Turbellaria 3.53% (3 taxa) and other groups Cnidaria (2 taxa), Oligochaeta (2 taxa), Pycnogonida (1 taxa) and Echinodermata (1 taxa). Individuals, on the other hand, these are distributed among the taxonomic groups quantitatively as follows: Crustacea 43.99% (73919 ind.); Mollusca 37.25% (62258 ind.); Polychaeta 11.06% (18490 ind.); Oligochaeta 5.79% (9681 ind.) and other groups Turbellaria (1899 ind.), Nemertea (799 ind.), Cnidaria (385 ind.), Pycnogonida (121 ind.) and Echinodermata (5 ind.). With regard to qualitative and quantitative dominance, Crustacea was the most important taxonomic group in the area investigated.

Various univariate, graphical/distributional, multivariate statistical methods and BENTIX index were employed to analyze the data collected from the study area. Analysis of the data revealed clear differences between the sampling sites subjected to sewage discharge and the others. The results suggested that the benthic ecosystem was more or less disturbed in stations subjected to sewage. The typical characteristics of the benthic communities exposed to pollutants such as the prevalence and high dominance of the opportunistic species, low number of species, low diversity and multi-metric benthic index scores and low total faunal abundance were encountered in most of these stations. On the contrary, it could be said that benthic communities was appeared to be healthier in stations non exposed to sewage, characterized by the high number of species, high total faunal abundance, high diversity and multi-metric benthic index scores. It can be construed that the effects of pollution on these communities was quite low.

There is now almost adequate information about the effects of sewage discharges on shallow water hard substratum macrozoobenthic communities, although open questions. The present work has also provided a base for further biomonitoring studies.

İSTANBUL BOĞAZI'NDA YÜZEY ATIKSU DEŞARJLARININ SIĞ SU SERT SUBSTRATUM MAKROZOOBENTİK KOMUNİTELERİ ÜZERİNDEKİ ETKİLERİ

Atıksu deşarjlarının bentik komuniteler üzerindeki kirlilik etkisinin belirlenmesi amacıyla İstanbul Boğazı kıyılarında dağılım gösteren sığ su sert substratum makrozoobentik komuniteleri incelenmiştir. Örneklerin toplanması ve çevresel parametrelerin ölçümü 3 aylık aralıklarla Mayıs 2005 – Şubat 2006 tarihleri arasında gerçekleştirilmiştir. Örnekleme için 15 istasyon seçilmiş, bunlardan dokuzu deşarj istasyonu olarak altısı ise kontrol istasyonu olarak belirlenmiştir. Çalışma süresince toplam 180 adet örnek alınmış, örneklemeler üst infralitoral bölgeden 0.5-1m derinlik aralığından yapılmıştır. 180 kuadrat örneklemesinin analizi sonucu 9 sistemetik gruba ait 85 tür tanımlanmış ve toplam167537 birey sayılmıştır.

Sistematik gruplar içerdikleri tür sayısına (kalitatif baskınlık) göre karşılaştırıldığında toplam tür sayısının %50.59'ine (43 tür) sahip olan Crustacea'nın en başta olduğu, bu grubu sırasıyla %21.18 (18 tür) ile Polychaeta, % 14.12 (12 tür) ile Mollusca, % 3.53 (3 tür) ile Nemertea, % 3.53 (3 tür) ile Turbellaria ve diğer gruplar Cnidaria, Oligochaeta, Pycnogonida ve Echinodermata'nın (toplam tür sayısının % 7.06; 6 tür) takip ettiği görülmüştür. Sistematik gruplar içerdikleri birey sayıları açısından (kantitatif baskınlık) gore karşılaştırıldıklarında ise toplam birey sayısının % 43.99'una (73919 birey) sahip olan Crustacea'nın en başta olduğu, bu grubu sırasıyla %37.25 (62258 birey) ile Mollusca, %11.06 (18490 birey) Polychaeta, % 5.79 (9681 birey) Oligochaeta ve diğer gruplar Turbellaria, Nemertea, Cnidaria, Pycnogonida ve Echinodermata (toplam birey sayısının % 2; 3209 birey) takip ettiği görülmüştür. Tür sayısı ve birey sayısı baskınlıkları açısından değerlendirildiğinde Crustacea'nın çalışılan bölgenin en önemli grubu olduğu görülmektedir.

Çalışma alanından elde edilen verilerin analizi tek değişkenli, grafiksel/ dağılımsal, çok değişkenli istatistiksel metotlar ve BENTIX indisi kullanılarak yapılmıştır. Analizler atıksu deşarjı etkisindeki istasyonlar ile diğerleri arasında açık bir farklılık olduğunu ortaya koymuştur. Sonuçlar atıksu deşarjı etkisi altındaki istasyonlarda bentik ekosistemin az veya çok zarara uğradığını göstermiştir. Bu istasyonlarda firsatçı türlerin hâkimiyeti ve baskınlığı, düşük tür sayısı, düşük çeşitlilik ve multi-metrik bentik indis değerleri ve düşük toplam faunal bolluk değerleri gibi kirletici etkisine maruz kalmış bentik komunitelerin tipik özellikleri ile karşılaşılmıştır. Buna karşın atık su etkisine maruz kalmayan ve yüksek tür sayısı, yüksek toplam faunal bolluk, yüksek çeşitlilik ve yine yüksek multi-metrik bentik indis değerleri ile karakterize olan istasyonlarda bentik komunitelerin daha sağlıklı göründüğü söylenebilir. Bu istasyonlarda kirlilik etkisinin düşük olduğu yorumu yapılabilir.

Böylelikle, sorulara açık olmasına rağmen, şu an için atıksu deşarjlarının sığ su makrozoobentik komuniteleri üzerindeki etkisiyle ilgili yeterli sayılabilecek düzeyde bilgiye ulaşılmıştır. Bu çalışma aynı zamanda ileride yapılacak olan izleme çalışmaları için de bir zemin oluşturmuştur.

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1. INTRODUCTION

Human impact on living resources has escalated over the last century and threatened the balance of many parts of the ecosystem (Rosenberg et al., 2004). The seas have been considered the ultimate dumping grounds for the wastes of human societies. People have felt until recently that the immerse volume of the world's oceans had an infinitive capacity for absorbing all of their waste. They have also learned that some of their wastes in very small amounts have significant effects on communities and species. The discharge of human sewage and garbage into coastal waters is practiced throughout the world. The sewage may or may not have had some treatment before discharge. Sewage adds a large volume of small particles to the water and also large amounts of nutrients. In small volumes and with adequate diffusing pipes, it is difficult to detect any long term effect on the communities of the open coasts. In large volumes and in semi enclosed embayments, the effect can be devastating (Nybakken, 1996).

Marine coastal zones have been widely recognized as the most vulnerable, suffering the impact of most anthropogenic activities. Inventory-making and classification of marine biodiversity in coastal ecosystems is fundamental in order to comply with the urgency of the present times which calls for a sustainable environment. Monitoring, conservation and restoration, if necessary, are possible, only when there is knowledge. And very little is known (Zenetos *et al.*, 2000). The estimation of marine pollution has become a priority subject in recent years (Thomas, 1993). Human impacts such as shipping (oil spills, bioinvasion), industry (chemical effluents), dredging and dumping, fishing and mariculture, biological invasions, tourism, etc are typically monitored over periods of several years (Pearson and Rosenberg, 1978; Chapman et al., 1995; Simboura and Zenetos, 2002).

Benthos is one of three major ecological groups into which marine organisms are divided, the other two being the nekton and the plankton. The benthos are organisms and communities found on or near the seabed. This includes those animals (zoobenthos) and plants (phytobenthos) living on (epifauna) or in (endofauna) marine substrata as well as those that swim in close proximity to the bottom without ever really leaving it (Fairbridge, 1960). In terms of size, this is generally divided into three categories: meiobenthos, the organisms that pass through a 0.5 mm sieve; macrobenthos, those that are caught by grabs or dredges but retained on the 0.5 m sieve; and epibenthos, those organisms than live on rather than in the seabed. Those in the latter category are usually larger (Fairbridge, 1960; Holme and McIntyre, 1984).

The benthic environment is a fundamental compartment of any aquatic ecosystem. The bottom environment of the seas adjacent to urban areas is grossly polluted by the discharge of untreated effluent from cities and wastewater from the factories. Such pollution results in serious environmental disturbances of the marine bottom ecosystem (Leppäkoski, 1971; Rosenberg, 1973; Pearson and Rosenberg, 1978; Tsutsumi and Kikuchi, 1983; Furota, 1987; Tsutsumi et al., 1991). The sea bottom is generally recognized as a sink for many pollutants entering the marine environment and effects may be apparent in benthic communities. In these environments we need an extensive knowledge of the natural ecosystem to enable the conservation of nature and the regulation of human development (Sandulli et al., 1989).

Differences in physical and biological conditions are the main causes of spatiotemporal variation in marine communities (Dayton, 1984; Sousa, 1984; Terlizzi et al., 2002). Natural process can include disturbances such as wave action, temperature, irradiance or salinity whilst biological process may include settlement, recruitment, predation and competition. Anthropogenic disturbance has the potential to alter the patterns of natural variability. Thus, it is crucial to understand the patterns of natural variability to distinguish them from the variability induced by human disturbance on marine habitats (Underwood, 1994).

Marine benthic communities are routinely studied as indicators of change and disturbance in marine environment (Gray, 1981). Assessing pattern in the structure of benthic communities has several advantages over experimental methods for the detection of anthropogenic disturbance. Measures of benthic community structure in marine ecosystems have been subject to a number of investigations to assessment of in situ

alterations of residential community structure related to pollution-induced changes for the stated reasons that (Gray, 1980; Weston, 1990; Dell Valls et al., 1998):

- The organisms are sedentary, thus reflecting local conditions
- Many species reside at the same sediment-water interface where many pollutants concentrate
- These communities are taxonomically diverse consisting of species that exhibit different tolerances to stress
- The lifespan of many species allows community structure to integrate and reflect sources of stress over time
- They are commercially important and or are important food sources for economically or recreationally important species
- They have important role in cycling nutrients and other chemicals between the sediments and the water column.

The benthos can integrate conditions over a period of time rather than reflecting conditions just at the time of sampling. They have advantages over pelagic organisms in that they are immobile and are therefore more useful in assessing local effects (Warwick et. al., 1990; Kröncke, 1995). The extent and impact of sewage pollution in the marine environment may be assessed using changes in numbers, diversity and community structure of macrobenthic invertebrates (Ismail, 1992) because the species which comprise the communities of bottom-living invertebrates in the sea vary in their tolerance to pollution.

Along a gradient of pollution it is generally believed that there will be a changing pattern of species abundances as each species will have a different level of response to the pollutant. Species living in sediments must in response to pollutant move, tolerate it or die. In a given community the most frequent response is that some species increase in abundance and others remain unaffected. The patterns of species abundance found will reflect effects of the pollutant integrated over time, and are therefore widely used to monitor effects of pollutants in subtidal sediments.

Species picked for their sensitivity or tolerance to various parameters is named as indicator species. Based on the literature, zoobenthic species can be grouped into two major groups: the sensitive ones and the tolerant ones. The sensitive ones characterizing a habitat type by their dominance (% abundance) or their exclusive presence in the specific habitats. The tolerant; these are the so called resistant species and the first grade or second grade opportunistic ones. So in the general scheme presented, the sensitive species are used to define the habitat type. Moreover, the sensitive species are well adapted to their specific environment. The tolerant species are generally opportunistic with low ecological requirements but sometimes they to various parameters, historically pollution (Simboura and Zenetos, 2002). Hence, some sensitive species decrease in relative importance, some tolerant species remain unaffected, and some may benefit from the changed condition increase (Warwick, 1988; Elias, 1992).

Only few papers described the effects of sewage on macrobenthic assemblages living on hard substrata and most of them focused on the intertidal, e.g. Fairweather, 1988. Along the coasts of the Mediterranean Sea, despite a large amount of domestic and industrial sewage is discharged to the sea (UNEP, 1989), only one published account exists concerning the effects of sewage effluents on macrobenthos living on rocky subtidal substrates (Terlizzi et al., 2002).

There has been more research done on the effects of sewage pollution on soft bottom benthic communities (Pearson and Rosenberg, 1978; Dauer and Conner, 1980; Austen et al., 1989) compared the assemblages that live on hard substrata. Soft bottom benthic communities are considered to be useful for monitoring purposes because they are relatively immobile, their taxonomy is reasonably well known (above the species level) and extensive literature discusses the effects of sewage on these assemblages (Warwick, 1993). On the other hand, the advantages of using hard bottom assemblages for monitoring the effects of sewage pollution are that they are sessile, reflect local conditions and can be assessed without destructive sampling methods (Warwick, 1993; Roberts, 1996).

1.1. Concept of Mathematical Ecology

The need of the interpretation of the macrobenthic data and its use in detecting anthropogenic stress, disturbance and change has led to the development of an extensive number of concepts and numerical techniques: diversity indices, multivariate tools, graphical representations, indicator species, biotic indices (Eliot, 1993, 1994).

Among them, diversity indices are basically an approach to biological quality through the structure of the community. The Shannon-Wiener index of diversity is, without doubt, one of most commonly used diversity indices in the assessment of pollution in marine benthic communities. Ranges of variation of community diversity index H' corresponding to five ecological quality classes should be defined for various habitat types accordingly (Simboura and Zenetos, 2002) (Table 1.1). However, the use and interpretation of this and other indicators (i.e. Hill numbers, Simpson, number of species) has been subjected too much debate (Clarke and Warwick, 1994; Jennings and Reynolds, 2000). The values of all these indices are influenced by sampling methodology, sample size and identification procedures. Consequently, species richness and community diversity values can only be compared if the sampling methodology has been followed, including same level of taxonomic expertise (Simboura and Zenetos, 2002).

Pollution Cla	ssification	H'	Ecological Quality
Normal/Pristin	ne	H' > 5.0	High
Slightly	polluted,	4 < H' ≤5	Good
Moderately po	olluted	$3 < H' \le 4$	Moderate
Heavily polluted		$1,5 < H' \le 3$	Poor
Azoic to very	polluted	$0 < H' \le 1.5$	Bad

Table 1.1. Ecological quality assessment using the community diversity index (H').

Graphical representation methods of the community structure are widely used in ecological assessment of benthic ecosystems. Relative abundances or biomasses of different species are plotted as a curve, which retains more information about the distribution than a single index (Warwick and Clark, 1991). The log-normal distribution method (Gray, 1981) compares species abundance patterns with theoretical models. Another graphical representation method, the abundance biomass comparison (ABC) plots is commonly used in coastal waters and a lesser degree in estuarine waters (Warwick, 1986). ABC as a conceptual method was evaluated for the determination of stress level of faunistic community at each station. The stations are under stable unpolluted conditions, where the benthic community is approaching equilibrium, the biomass become increasingly dominated by one or a few large species, each represented by rather few individuals and also they are in equilibrium with available resources. The expected curves for these unpolluted communities are the biomass curve above abundance curve through its entire length. In other words, indicating higher "numbers diversity" than "biomass diversity". If abundance and biomass curves cross each other one or more times those areas recognized as moderately polluted. Under these circumstances the large competitive dominant individuals are eliminated and equality between numerical and biomass dominants is reduced. If the abundance curve above biomass curve through its entire length those areas are called as grossly polluted areas. As pollution become more severe, benthic communities would become increasingly dominated numerically by one or a few very small species (usually annelids), and few larger species are present although these would contribute proportionally more to the total community biomass in relation to their abundance than would the small numerical dominants (Warwick et al., 1987).

The rarefaction curves is the another graphical representation methods of the community structure. Rarefaction curves (Sanders, 1968) were among the earliest to be used in marine studies. They are plots of the number of individuals on the x-axis against the number of species on the y-axis. The more diverse the community is the steeper and more evaluated is the rarefaction curves. This method may also provide clues about the pollution status of the communities. Steeper and elevated curves indicate more diverse communities which are relatively less affected by pollution.

Multivariate techniques unlike diversity measures take into account changes in taxa and base their comparisons on the extent to which different data sets share particular species, at comparable levels of abundance and biomass.

For each of these classes of methods there are appropriate statistical tests to determine the significance of differences between replicated community samples in either time or space. For univariete indices, classical ANOVA is appropriate, and for graphical and multivariate methods there are multivariate equivalents, including the simulation/ permutation test ANOSIM which does not make the assumption of multivariate normality in the data.

An ecological quality assessment tool proposed recently is the relative abundance of indicator species (fragile and/or opportunistic) in respect to the total fauna, which however requires the definition of reference levels for each habitat type and the definition of the ranges of variation for each quality class to use for classification purposes (De Boer et al., 2001; Zenetos and Simboura, 2001).

Biotic indices approach ecological quality through the use of the indicator organism concept and like multivariate methods they take into account changes in taxa. Although taxonomy may vary widely, the methodology behind establishing biotic indices may be universal. The most popular one in biotic indices is BENTIX (Simboura and Zenetos, 2002).

In BENTIX, species are categorized according to three ecological groups. Also an attempt was made to compile a list of indicator species assigning a score ranging from 1-3 corresponding to each one of the three ecological groups. The information to classify the species into the ecological groups was derived from works providing ecological characterization of species (Borja et al., 2000; Corbera and Cardell, 1995; Simboura and Nicolaidou, 2001) and reviewing the literature cited in Simboura and Zenetos, 2002.

Group 1 (GI) includes species which are sensitive to disturbance in general. These species corresponds to the k-strategy species, with relatively long life, slow growth, and high biomass (Gray, 1979). Also species indifferent to disturbance, always present in low densities with non-significant variations with time are included in this group, as they cannot be considered as tolerant by any degree. Species belonging to this group were assigned with the score 1. Group 2 (GII) includes species tolerant to disturbance or stress whose populations may respond to enrichment or other source of pollution by an increase of densities (slight unbalanced situations). Also this group includes second order opportunistic species, or late successional colonizers with r-strategy; species with short life span, fast growth, early sexual maturation, and larvae throughout the year. Species belonging to this group were assigned with the score 2. Group 3 (GIII) includes the first

order opportunistic species (pronounced unbalanced situations), pioneers, colonizers, or species tolerant to hypoxia. Species belonging to this group were assigned with the score 2 (Simboura and Zenetos, 2002).

Based on the above descriptions, it appears that species belong to the tolerant species, the second order and first order opportunistic species. The use of Bentix can produce a series of continuous values from 2 to 6. According to these values, sampling sites are classified (Table 1.2).

Pollution Classification	BENTIX	Ecological Quality Status (ECoQ)
Normal/Pristine	$4.5 \le \text{BENTIX} < 6.0$	High
Slightly polluted, transitional	$3.5 \le \text{BENTIX} < 4.5$	Good
Moderately polluted	$2.5 \leq \text{BENTIX} < 3.5$	Moderate
Heavily polluted	$2.0 \le \text{BENTIX} < 2.5$	Poor
Azoic	0	Bad

Table 1.2. Classification of ecological quality according to the range of the Benthic index.

1.2. The Study Area

The Bosphorus Strait, which is 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 and shallow channel of nearly 31 km length. The Strait has a well defined two-layer stratification and associated a two-layer water of 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) water of the Black Sea flows towards the Sea of Marmara on top of the oppositely flowing more saline and denser waters of the Marmara Sea (Gunnerson and 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 possesses a unique ecosystem. As being a part of the Turkish Straits System, which plays significant roles in the biology of the Mediterranean and the Black Sea basins, Strait represents a transition zone between the Mediterranean and the Black Sea (Öztürk and Öztürk, 1996).

The north-western Black Sea coastal waters, transported towards the Bosphorus region by alongshore currents (Sur et al., 1994), are drastically polluted by large inputs of nutrients and organic matter via riverine and wastewater discharges (Bologa, 1985; Mee, 1992). The polluted Black Sea surface water, before spreading into the Marmara upper layer, is further contaminated by the waste discharged into the Bosphorus from the city of Istanbul by the numerous industries and approximately the 15 million population (Orhon et al., 1994). In addition, vertical mixing provides nutrient input from the Marmara lower layer, especially in the Marmara-Bosphorus junction region (Baştürk et al., 1990; Polat, 1995).

First studies related to benthos of the Bosphorus Strait were performed by Ostroumoff (1896), Marion (1898) and Sowinsky (1898). In first two studies benthic species essamblages were examined comparatively in different sites of the Turkish Strait System. In addition, Sowinsky (1898) examined the Amphipoda and Isopoda faunas of the Bosphorus. After these studies, Ninni (1923) and Devedjian (1926) reported some benthic organisms marketed in "Istanbul Fisheries Market".

The most detailed study related to macrozoobenthic fauna of the Bosphorus Strait and surrounding area was performed by Demir (1952-54) in the Strait and Prince Islands (Sea of Marmara). Demir (1952-1954) reported a total of 418 species belonging to various macrozoobenthic groups in this study. The study of Tortonese (1959) included some detailed observations related to the benthos of the Bosphorus and its surrounding area in

the Sea of Marmara. Another like Tortonese (1959) was performed by Caspers (1968) in the Strait and its Black Sea and Marmara junctions. In this study, the Bosphorus Strait was considered as a transition zone between the Sea of Marmara and the Black Sea and macrozoobenthic communities of the area were examined with regard to species richness and abundance.

Various groups of macrozoobenthos were separately examined within many faunistic studies in the Bosphorus Strait. Hydroid polyp fauna was examined by Albayrak and Balkıs (2000), Polychaete fauna was examined by La Greca (1949), Rullier (1963) and Gillet and Ünsal (2000) in the Bosphorus. Vermes fauna was examined by Balkıs and Albayrak (2001). Hitherto recorded species of Decapoda from the Turkish Straits System including the Bosphorus Strait were reviewed by Müller (1986). Amphipod and isopod faunas of the Strait were examined in detail by Sowinsky (1898) as mentioned above. After nearly a century, amphipod fauna of the same area was studied by Balkıs and Albayrak (1994). Mollusca fauna of the Strait and the Sea of Marmara and Albayrak and Balkıs (1996a, 1996b) in the Strait again. Echinoderm fauna of the Bosphorus Strait and the Sea of Marmara was examined by Tortonese and Demir (1960). After 46 years, echinoderms of the Strait studied by Albayrak (1996). In addition, review of on the biology of Turkish Strait System by Öztürk and Öztürk (1996) includes some notes related to the benthos of the study area.

As is understood from above, past benthic studies in the Bosphorus Strait were mostly focused on separate systematic groups and their representative species. However, our knowledge of pollution impact on macrozoobenthic communities in the Bosphorus Strait is very poor. Although the large amount of domestic sewage discharged to the sea along the coasts of the Bosphorus Strait, only three studies (Unsal, 1988; Topaloğlu and Kihara, 1993; Uysal et al., 2002) related to the effects of pollution on macrobenthos were performed.

Unsal (1988) studied the pollution effects on benthic fauna distribution, especially on polychaetes in the Golden Horn (an estuary located in the southern part of the European coast of the Bosphorus Strait). Topaloğlu and Kihara (1993) examined the influence of

pollution on the ecological stability and the structure of the hard bottom macrozoobenthic community of the Beykoz inlet in the northern part of the Anatolian coast of the Bosphorus Strait. Uysal et al. (2002) examined the spatial and temporal distribution of soft bottom macrozoobenthic communities which are under the influence of Strait's lower-layer sewage discharge in the Bosphorus Strait and its surrounding area.

The main objectives of the present study are;

- Contribution to the knowledge of pollution effects on benthic life
- The identification of human-induced stresses on hard substrate macrozoobenthic communities
- Analysis of ecosystem responses to stress
- Assessment of ecosystem health and ecological quality status of the hard substrate biocoenoses
- Setting up the initial base for biomonitoring in the Bosphorus Strait.

To be able to determine the degree of disturbance and stress on the benthic communities in the sampling sites by sewage discharges, numerical indices, univariate, multivariate and distributional methods and BENTIX (a biological quality index) are used

2. MATERIALS AND METHODS

2.1. Sampling and Treatment of Macrozoobenthos

The study was carried out seasonally at 15 stations from May 2004 to February 2005 in the rocky shores of the Bosphorus Strait, since the surface effluents in the study area are discharged to the rocky shores (Figure 2.1). While 9 stations were selected as target (discharge) stations, 6 stations were selected as control stations. This choice was based on mainly their distances from the main sources of pollution. Stations B6, B8, B9, B10, B11, B13 and B14 located on the coasts of the Bosphorus Strait were directly influenced by sewage produced by urban area of the Istanbul metropolitan. Stations B7 and B12 were subjected to indirect effects of sewage discharges. Stations B1, B2, B3, B4, B5 and B15 were less affected by human activities and there were not any discharge points close to these stations. In addition, there is not any inhabitance at the surrounding area of stations B1, B2, B3 and B5. As far as possible, the stations were standardized with respect to water depth and sediment type in order to minimize the influence of these nuisance variables (which may have unwanted effect on the findings of the research) on the benthic communities. The depth of sampling sites 0.5 m and they are under the influence of the hydrographic conditions of the upper water layer of the Bosphorus Strait. Therefore, water column overlaying the sampling sites possesses the characteristics of brackish waters of Black Sea origin. Consequently, the sampling sites can be accepted as upper infralittoral rocky bottom brackish water habitats. Location of sampling sites and the information about their biotopes are given in Table 2.1 to be able to describe the study area.

A total of 180 samples were collected in May, August and November 2004 and February 2005. Samples were collected from the upper infralittoral zone at a dept ranging from 0.5 to 1m. Macrozoobenthic samplings were carried out using a metal frame (quadrate). The area covered by the quadrate is 400 cm² (20x20cm), which is the minimum necessary quadrate area for the investigation of hard substratum assemblage (Bellan-Santini, 1969; Stirn, 1981). For each sampling site and period, three replicates were taken in order to achieve the minimum necessary area for a statistical investigation of hard

substrate (Stirn, 1981). All organisms within the quadrate were quickly and thoroughly removed from the rocky substratum using a spatula and fixed in 4% neutral formalin solution. Thereafter, they were transferred to the laboratory for further process.



Figure 2.1. Location of sampling sites.

In the laboratory, all macrozoobenthic samples were sieved through a 500 µm mesh with fresh water in order to remove formalin, mud and material smaller than 500 µm. Retained material was put into a plastic tub. Firstly, the extracted fauna were separated into taxonomic groups. Thereafter, all organisms were identified to the lowest possible taxonomic level using a Nikon SMZ-U stereomicroscope and an Olympus CX21 compound microscope. For statistical analyses, all individuals of each species were counted and wet weights were measured for each species in each replicate by a digital scale to the nearest 0.0001 g. All organisms are stored in 70% ethanol or 4% neutral formalin solution. The specimens were stored in the personal collection.

All macrozoobenthic taxa were identified using the following sources; for Cnidaria Manuel (1987), for Turbellaria Prudhoe (1982), for Nemertea Bürger (1895), for Polychaeta Fauvel (1923, 1927), Day (1967), Fauchald (1977), Bianchi (1981), Campoy (1982), Ergen and *et al.* (2000), for Oligochaeta Nielsen et al. (1959), for Pycnogonida Bouvier (1923), Krapp (1973), for Crustacea Bacescu (1951), Zariquiey Alvarez (1968), Bellan-Santini and et al. (1982, 1989, 1993, 1998), Jacobs (1987), Taiti et al. (1996), Kırkım (1998), for Mollusca Parenzan (1970, 1974, 1976), Nordsieck (1972), Poppe and Goto (1993) and for Echinodermata Tortonese (1965). Taxonomic nomenclature and classification of the identified species follows Fauchald (1977), Martin and Davis (2001) and electronic databases; MarBEF-Marine Biodiversity and Ecosystem Functioning (2004) and CLEMAM-Check List of European Marine Mollusca (2004).

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

Table 2.1. Coordinates and biotope characterization of sampling sites.

2.2. Measurements of Abiotic Parameters

During the present study, main environmental parameters, such as temperature, salinity, and dissolved oxygen were measured at each sampling. In addition, nitrite and nitrate, total phosphate and silicate, which increase by sewage discharges and cause changes on environmental conditions, were measured. Moreover, the fecal coliform was measured at each sampling as an indicator of the existence of sewage.

Water temperature was measured by mercury thermometer on site. Salinity and dissolved oxygen were measured by using a WTW LF 320 conductivity meter and a WTW Oxi 330 oximeter respectively. Salinity and dissolved oxygen samples were collected using 50 mL dark glass bottles. Salinity and dissolved oxygen were also measured on site.

Water samples of each analysis were collected from a depth as close as possible to the bottom to be able to detect clearly water properties of the bottom. Before water samples for nitrite and nitrate, total phosphate (TP) and silicate (Si) analyses were taken, polyethylene bottles were rinsed out by sea water. For nutrient analyses High Density PE bottles, cleaned with HCL 5%, were used. All samples were pre-filtered though 5µm syringe filters (Sartorius MiniSart 17594-Q) and then sorted in polyethylene bottles. These samples were immediately transferred to the laboratory and frozen to -20°C. All further analyses of frozen samples were performed in the laboratory in the following days after the sampling.

For microbiological analyses, samples were collected by 250mL sterile, dark glass bottles. Like other samples they were immediately transferred to the laboratory for analyses. In 6 hours they were analyzed according to APHA (1999) in the laboratory. Samples were aseptically filtered from 0.45 μ m membrane filter (Sartorius 13906-50-AJN) with sterile metal filtering set. Filters were then transferred on media and incubated in required temperature. MFC medium was chosen for fecal coliform analysis and samples were incubated for 24 hour at 44.5 ± 0.1°C. Blue colonies were assumed as fecal coliform at the end of the incubation period. The results were given as colony forming unit (CFU)/100mL.

Samples for nitrite and nitrate, total phosphate (TP) and silicate (Si) were preserved at -20°C. All parameters were measured on a Bran+Luebbe AA3 Autoanalyser according to Grasshoff et al. (1983).

Nitrate is reduced to nitrite by a copper-cadmium column. The nitrite then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylene diamine dihydrochloride to form a purple azo dye.

Persulfate digestion method was performed by adding $5mL K_2S_2O_8$ to 50mL samples and autoclaving at $110^{\circ}C$ for 30 min. Digested samples were then analyzed at Bran+Luebbe AA3 Autoanalyser for total phosphate.

The determination of soluble silicate is based on the reduction of silico-molybdate in acidic solution to molybdenum blue by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to minimize interference from phosphates.

2.3. Statistical Analyses

Statistical methods were used in order to interpret the data and simplify their complex structure, for better understanding similarities and dissimilarities between stations and common environmental factors which causes groups. A variety of univariate methods (Shannon-Weaner diversity index, species richness as Margalef's and evenness as Pielou's), multivariate methods (clustering techniques, multi-dimensional scaling (MDS), analyses of similarity (ANOSIM), Kruskal-Wallis test and the similarities per centages procedure (SIMPER) and a distributional method (abundance-biomass comparison and rarefaction curves) were employed in the analyses of the biological data set. The index of dispersion was applied to all data to test the randomness. As the result of this analysis, random distribution could not be determined and most species of the benthic community showed aggregated distribution. Therefore, average abundance and biomass data from three replicates of each sampling were used in the statistical analyses.

In order to highlight the structure of communities in the studied habitats, the following ecological indices were used. The numerical dominance by abundance (*NDA*) and by biomass (*NDB*) on a scale of 1 m², and Soyer's frequency index (*F*) were estimated. Shannon-Weaner diversity index (*H'*) (Shannon & Weaver, 1963), Pielou's (Pielou, 1969) evenness (*J*) of distribution of individuals among species and Margalef's species richness (d) were calculated on a log₂ basis. The Shannon-Wiener index of diversity is, without doubt, one of most commonly used diversity indices in the assessment of pollution in marine benthic communities. The increase in values of Shannon-Weaner and Pielou's indices usually indicates an improvement in the ecological health (Albayrak et al., 2006). In addition, the BENTIX index (Simboura and Zenetos, 2002) was calculated for the assessment of ecological quality status classification of the study area.

Soyer's Frequency Index (F):

F = m x 100 / Mm= total number of samples in which a given species was recorded M= total number of samples

Numerical dominance by abundance (NDA):

NDA= n x 100 / N n= total number of individuals of a given species for all samples N= total number of individuals found in all samples

Numerical dominance by biomass (NDB):

NDA= n x 100 / N n= total biomass of individuals of a given species for all samples N= total biomass of individuals found in all samples

Diversity Index (H'):

$$H' = -\sum_{i=1}^{k} p_i \log_2 p_i$$

k= species number,

 p_i = proportion of the frequency of the species to the total frequency $(p_i = \frac{f_i}{n})$

Eveness Index (J):

J= H' / ln S H': Shannon – Weaver diversity index S: species number

Margalef's richness (d):

 $d=(S-1)/\log_2 N$

S: species number N: total number of individuals found in all samples

BENTIX:

 $BENTIX = \{6 X \%GI + 2 X (\% GII + \% GIII)\}/100$

GI: the per centage of species which are sensitive to disturbance in general GII: the per centage of second-order opportunistic species GIII: the per centage of the first order opportunistic species

The numerical abundance data were analyzed using cluster and multidimensional scaling (MDS) 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 method "Taylor's Power Law" concepts (Taylor, 1961), was performed to the abundance data in order to identify groups of similar stations and among different station groups (control and target) and within the same region and then the similarity data were ordinated by MDS (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. Two-way crossed ANOSIM was used to assess the significant differences between sampling periods (dates) and pre-defined groups of sample sites in the cluster analysis. SIMPER analysis was applied in order to identify the per centage contribution of each species to the overall similarity and the dissimilarity between stations. Firstly, the groups obtained from the cluster analysis were defined before ANOSIM analysis. With ANOSIM test, the differences between control and target stations were checked. Secondly, by applying Simper to the data we found that which species cause differences between groups. The above analyses were applied to examine the similarity degree of sampled in both space and time.

As an indicator of pollution-induced stress, ABC (Abundance-Biomass Comparison) curves (Warwick, 1986; Warwick et al., 1987), which use k-dominance curves, were plotted for each station. The advantage of distribution plots is that the distribution of species abundances among individuals and the distribution of species biomasses among individuals can be compared on the same terms. Since the two have different units of measurement, this is not possible with diversity indices. The relation between species abundance and species biomass curves can indicate pollution-induced stress. Where the biomass curve lies above the abundance curve the assemblage will not be affected by pollution, where the 2 curves cross one another the first stage of pollution-induced change occurred, and where the abundance curve lies above the biomass curve the assemblage will form a grossly polluted habitat. In the present study, cumulative dominance curves could not be used because most dominant species in terms of abundance and biomass caused false impression of disturbance. Therefore, partial dominance (%) curves were used in the present study to be able to obtain exact results.

ANOVA was employed to examine the statistical differences between sampling sites. In the present study, the variances are not homogeneous, a non-parametric ANOVA (Kruskal-Wallis) was used. The non-parametric Mann-Whitney U test and Kruskal-Wallis (ANOVA) test were employed for analyzing the differences of the number of species (S), numerical abundance (N), numerical biomass (B), species richness (D), Shannon-Weaner diversity index (H') and Pielou's evenness index (J) between control and target stations and seasons using the software package STATISTICA (version 6.0)

3. RESULTS AND DISCUSSION

3.1. Biotic Environment

The samples, 180 in total, were collected in May, August, and November 2004 and February 2005 at 15 stations along the coast of the Bosphorus Strait. Three replicates were taken in each sampling time at each sampling station. The index of dispersion was applied to all data to test the randomness. As the result of this analysis, random distribution could not be determined and most species of the benthic community showed aggregated distribution. Therefore, the three replicate quadrates sampled in each sampling sites were averaged and interpretations of the data were done using the averaged values.

In the present study, sampling periods were planned according to seasons, because seasonal data are needed to understand the dynamics of benthic community structure and are essential when assessing the potential biological effects of discharge. Hence, the source of changes were tried to analyze if they were originated from natural changes or the disturbance on the community.

The analysis of 180 quadrate samples yielded a total of 167537 individuals belonging to 85 taxa (Table 3.1). Hierarchic taxonomy of the identified species is given in the Appendix 1. These are distributed qualitatively among the taxonomic groups as follows: Crustacea 50.59% (43 taxa); Polychaeta 21.18% (18 taxa); Mollusca 14.12% (12 taxa); Nemertea 3.53% (3 taxa); Turbellaria 3.53% (3 taxa) and other groups Cnidaria (2 taxa), Oligochaeta (2 taxa), Pycnogonida (1 taxa) and Echinodermata (1 taxa) (Figure 3.1)

Individuals, on the other hand, these are distributed among the taxonomic groups quantitatively as follows: Crustacea 43.99% (73919 ind.); Mollusca 37.25% (62258 ind.); Polychaeta 11.06% (18490 ind.); Oligochaeta 5.79% (9681 ind.) and other groups Turbellaria (1899 ind.), Nemertea (799 ind.), Cnidaria (385 ind.), Pycnogonida (121 ind.) and Echinodermata (5 ind.) (Figure 3.2).
With regard to qualitative and quantitative dominance, Crustacea was the most important taxonomic group in the area investigated. In this sense, study area appeared to be colonized mainly by the crustaceans.

Before the present study the only research concerning the shallow water macrozoobenthic communities has been carried out by Topaloğlu and Kihara (1993). According to these authors, crustacean was qualitatively and quantitatively dominant group in the study area.

As seen in Table 2.1, biotopes of most of the sampling sites were characterized by *Mytilus galloprovincialis* communities. Literature data shows that polychaetes and crustaceans are the most important taxonomic groups of the *M. galloprovincialis* assemblages, contributing almost 50% of the total faunal species abundance (Bellan-Santini, 1969; Saldanha, 1974; Kocataş, 1978; Bellan, 1980; D'Anna et al., 1985; Topaloğlu and Kihara, 1993). The abundance of these groups varies from one area from the other seems to be dependent on the specific features of each study area (polluted/non-polluted; midlittoral/supralittoral), a fact was also reported by Thiel and Ullrich (2002) for purple mussel (*Perumytilus perpuratus*) assemblages. Saldanha (1974), for instance, recorded fewer polychaetes and more crustacean species on the coast of Portugal, while D'Anna et al. (1985) recorded exactly the opposite for Sicily. Kocataş (1978) found 35 polychaete and 32 crustacean species in the İzmir Bay, whereas Topaloğlu and Kihara (1993) reported 10 polychaete and 22 crustacean species in the same area of the present study.

Mollusca comprising 99.18% (386304.84 g) of the total wet weight (389506.0575 g) was dominant in biomass. Other groups Cnidaria, Turbellaria, Nemertea, Polychaeta, Oligochaeta, Pycnogonida, Crustacea and Echinodermata (3198.7241 g) composed of only \approx 1% of total wet weight (Figure 3.3). Only two species, *Mytilus galloprovincialis* and *Mytilaster lineatus*, comprising % 99.15 of the total biomass, were responsible for this predominance of molluscs. High abundance, relatively larger sizes and shell weights of these species caused an uneven distribution of biomass (Figure 3.3).



Figure 3.1. Qualitative dominance of systematic groups.



Figure 3.2. Quantitative dominance of systematic groups.



Figure 3.3. Dominance of biomass of systematic groups.

The abundance of the most dominant species found in all samplings of the area were; *Mytilus galloprovincialis* (22.98 %), *Mytilaster lineatus* (13.45%), *Echinogammarus olivii* (12.42%), and *Hyale perieri* (7.94%). In addition, the most dominant species by biomass were *Mytilus galloprovincialis* (91.33%) and *Mytilaster lineatus* (7.82%) (Table 3.1).

In this regard, it can be construed that two bivalves *Mytilus galloprovincialis* and *Mytilaster lineatus* dominated the benthic communities in the studied area. Large populations of these filter-feeding species naturally grow in this area. It has frequently (may be consistently) been noted in areas of grossly obvious nutrient overload that filter-feeding organisms that thrive on particulate organic matter, become relatively more dominant in the environment. (Schramm and Nienhuis, 1996). In addition, high nitrogen loading causes a benthic community dominated by filter feeders (Laws, 1983).

According to Soyer's frequency (F) classification, only 23 out of 85 species found can be classified as constant (F \geq 50%), 16 species as common (25% \leq F<50%) and 46 species as rare (F<25%). Among the constant species, *Platynereis dumerilii* (93.33%) ranked first, followed by *Nereis* (*Hediste*) *diversicolor* (91.67%), *Mytilus galloprovincialis* (91.67%), *Hyale perieri* (90%). The species with the highest frequency scores within the common category were *Gammarellus angulosus* (48.33%), *Pilumnus hirtellus* (46.67%) and *Balanus improvisus* (43.33%). The rare species found in only one sampling were *Etone picta*, *Orchestia stephenseni*, *Caprella acantifera*, *Lekanesphaera monodi*, *Jaera sp., Armadillidium* cf. *album*, *Idotea sp., Eriphia verrucoa* and *Pusillina inconspicua* (Table 3.1).

Table 3.1. List of species found in the four sampling periods at stations (F: Soyer's frequency index, NDA: numerical dominance by abundance, NDB: numerical dominance by biomass)

SPECIES	STATIONS	F (%)	NDA (%)	NDB (%)
CNIDARIA				
Actinia cf. equine (Linnaeus, 1758)	B1-B7, B9, B10, B12, B14, B15	50.00	0.2191	0.0342
Actinaria (sp.)	B15 B3, B5, B15	5.00	0.0107	0.0001
TURBELLARIA	- , - , -			
Macrostomida (sp.)	B1, B3, B4, B6, B8, B9, B12-B15	25.00	0.4244	0.0005
<i>Notoplana</i> sp.	B1-B7, B9, B12, B14, B15	51.67	0.2746	0.0057
Tricladida (sp.)	B6, B10, B11, B15	15.00	0.4345	0.0003
NEMERTEA				
Lineidae (spp.)	B1, B2, B5, B12, B14	8.33	0.0084	0.0001
Tetrastemma cf. coronatum (Quatrefages, 1846)	B1-B12, B14, B15	66.67	0.4274	0.0034
Emplectonema cf. gracile (Johnston, 1837)	B4, B9, B11, B12, B15	13.33	0.0292	0.0003
POLYCHAETA				
Namanereis sp.	B9, B13	3.33	0.0024	0.0001
Nereis (Hediste) diversicolor (O.F. Müller, 1776)	All stations	91.67	2.7785	0.0360
Perinereis cultrifera (Grube, 1840)	All stations except B11	58.33	0.1444	0.0294
Platynereis dumerilii (Audouin & Milne-Edwards, 1833)	All stations	93.33	3.2679	0.0994
Eumida sp.	B4, B13	3.33	0.0012	0.0001
Eteone picta Quatrefages, 1865	B1	1.67	0.0012	0.0001
Eulalia clavigera (Audouin & Milne Edwards, 1834)	B1-B5, B9	26.67	0.0304	0.0003
Grubeosyllis alvaradoi (San Martín, 1984)	B1-B10, B12, B13, B15	63.33	0.6297	0.0001
Syllis amica Quatrefages, 1865	B6, B9	3.33	0.0018	0.0001
Syllis columbretensis Campoy, 1982	B1, B5, B6	6.67	0.0066	0.0001
Syllis gracilis Grube, 1840	All stations except B13	73.33	0.4572	0.0010
Fabriciinae (sp.)	B4-B6, B8-B14	33.33	0.4113	0.0001
Polydora cf. cornuta Bosc,1802	B2-B4, B7-B9, B13-B15	20.00	0.1009	0.0002
Prionospio multibranchiata Berkeley & Berkeley, 1927	B14, B15	3.33	0.0024	0.0001
Polycirrus sp.	B1, B5, B15	8.33	0.0066	0.0001
Capitella capitata (Fabricius, 1780)	B1-B7, B9, B11, B13-B15 41.67		1.4636	0.0048
Euclymene oerstedi (Claparède, 1863)	B3, B5	5.00	0.0030	0.0001
Opheliidae (sp.)	B1-B10 B12	60.00	1.7274	0.0024
OLIGOCHAETA				
Enchytraeus buchholzi Vejdovsky, 1879	All stations	61.67	4.9386	0.0032
Lumbricillus rivalis (Levinsen, 1884)	B4-B6, B8-B11, B13-B15	35.00	0.8398	0.0006
PYCNOGONIDA				
Tanystylum conirostre (Dohrn, 1881)	B2, B7, B12, B15	21.67	0.0722	0.0001
CRUSTACEA				
Balanus improvisus Darwin, 1854	B2-B4, B6-B15	43.33	0.2262	0.0340
Ampithoe helleri Karaman, 1975	B1-B5, B9, B12 2		0.5897	0.0006
Ampithoe ramondi Audouin, 1826	All stations except B13	65.00	0.8882	0.0061
Microdeutopus gryllotalpa Costa, 1853	All stations except B15	55.00	0.7473	0.0012

Table 3.1. (Continued)

SPECIES	STATIONS	F (%)	NDA	NDB	
Riancolina algicola Della Valle 1893	B1 B3 B5	10.00	0.3134	0.0001	
Monocorophium insidiosum Crawford 1937	B1-B9 B12-B14	41.67	0.1731	0.0001	
Dexamine spinosa (Montagu 1813)	B1 B3-B7 B9 B13	15.00	0.0173	0.0001	
Apherusa chiereghinii Giordani-Soika 1950	B1 B3 B5-B7 B13	23 33	0.01/3	0.0001	
Gammarellus angulosus (Rathke, 1843)	B1-B8 B10-B13 B15	48.33	0.1427	0.0024	
Echinogammarus olivii (Milne-Edwards 1830)	All stations except B3	65 00 12 41		0.2846	
Hyale perieri (Lucas, 1849)	All stations	90.00	7.9403	0.0786	
Hyale pontica Rathke, 1837	B1-B7, B10-B12, B14, B15	50.00	1.9070	0.0107	
Parhyale cf. plumicornis (Heller, 1866)	B1, B2	8.33	0.4859	0.0037	
Ericthonius brasiliensis (Dana, 1855)	B1, B3-B6, B10	21.67	1.3191	1 0.0006	
Ericthonius punctatus (Bate, 1857)	B1, B5, B7	5.00	0.0149	0.0001	
Jassa marmorata (Holmes, 1903)	All stations	71.67	5.7617	0.0236	
Jassa ocia (Bate, 1862)	B1, B2, B6-B9, B11-B15	31.67	0.4423	0.0005	
Melita palmate (Montagu, 1804)	All stations	61.67	0.8344	0.0067	
Stenothoe tergestina Nebeski, 1881	All stations except B8	61.67	1.3143	0.0008	
Orchestia stephenseni Cecchini, 1928	B3	1.67	0.0006	0.0001	
Caprella acanthifera Leach, 1814	B13	1.67	0.0006	0.0001	
Caprella danilevskii Czerniavski, 1868	B1, B3, B5	5.00	0.1522	0.0001	
Caprella liparotensis Haller, 1879	B1, B3, B5, B15	16.67	0.2596	0.0004	
Caprella rapax Mayer, 1890	B1-B5, B12	28.33	0.1355	0.0002	
Dynamene bidentatus (Adams, 1800)	B1-B3, B5-B7, B9, B11, 15	41.67	0.8368	0.0035	
Lekanesphaera monodi (Arcangeli, 1934)	B4	1.67	0.0006	0.0001	
Sphaeroma serratum (Fabricius, 1787)	B1, B4, B6, B8-B11, B13,	30.00	0.6160	0.0367	
	B14				
Jaera nordmanni (Rathke, 1837)	B2, B3, B6, B7, B15	16.67	0.4972	0.0004	
Jaera sp.	B6	1.67	0.0006	0.0001	
Idotea balthica (Pallas, 1772)	All stations	70.00	0.4118	0.0564	
Idotea pelagica Leach, 1815	All stations except B11	73.33	1.4039	0.0019	
<i>Idotea</i> sp.	B5	1.67	0.0006	0.0001	
Synisoma capito (Rathke, 1837)	B1, B3-B6, B15	23.33	0.0310	0.0014	
Trichoniscus cf. provisorius Racovitza, 1908	B6, B8	3.33	0.0018	0.0001	
Armadillidium cf. album Dollfus, 1887	B6	1.67 0.00		0.0001	
Tanais dulongii (Audoum, 1826)	All stations except B7	75.00	3.9430	0.0112	
Leptochelia savignyi (Kroyer, 1842)	BI-B3, B6, B7, B9, B10, B12, B15	23.33	0.0191	0.0001	
Cumella nyamaga gurinica Bacescu, 1950	B12, B13 B1 B6	3 33	0.0024	0.0001	
Pisidia longimana (Risso 1816)	B1 B2 B4-B7	18 33	0.1343	0.0001	
Frinhia verrucosa (Forskål 1775)	B15	16.55	0.0006	0.0001	
Pilumnus hirtellus (Linnaeus 1761)	B1-B7 B9-B12 B14 B15	46 67	0.0495	0.0266	
	21 27, 27 212, 21 , 210	.0.07	0.0 .90	0.0200	
Xantho poressa (Olivi, 1792)	B4, B6	5.00	0.0018	0.0021	
Pachygrapsus marmoratus (Fabricius, 1787)	B2, B6, B10	6.67	0.0024	0.0001	
MOLLUSCA					
Acanthochitona fascicularis (Linnaeus, 1767)	B1, B3, B5, B15	11.67	0.0101	0.0006	
Lepidochitona cinerea (Linnaeus, 1767)	B1, B3, B5, B9	13.33	0.0113	0.0002	
Gibbula deversa Milaschewitsch, 1916	B1-B7, B10, B13-15	33.33	0.0848	0.0161	
Tricolia pullus pullus (Linnaeus, 1758)	B1-B7, B9, B15	38.33	0.1815	0.0057	
Pusillina inconspicua (Alder, 1844)	B4	1.67	0.0006	0.0001	
Rissoa splendida Eichwald, 1830	B2, B3, B5, B6, B9	10.00 0.0113 0		0.0007	
Rissoa cf. variabilis (Von Mühlfeldt, 1824)	B2, B15	3.33	3.33 0.0012 0.		
Setia sp.	B1, B4, B7, B9	10.00	0.0221	0.0001	
Odostomia eulimoides Hanley, 1844	B1-B3, B5, B7, B11, B12, B14, B15	35.00	0.4011	0.0027	
Myosotella myosotis (Draparnaud, 1801)	B7, B15	5.00	0.0024	0.0001	
Mytilaster lineatus (Gmelin, 1791)	All stations	88.33	13.4514	7.8196	
Mytilus galloprovincialis Lamarck, 1819	All stations	91.67 22.983		91.3317	
ECHINODERMATA					
Amphipolis squamata (Delle Chiaje, 1828)	B15	3.33	0.0030	0.0001	

Mean value of number of species, abundance and biomass on the sampling sites with respect to seasons were represented. The highest number of species for the stations B2, B14 and B15 were obtained in May; B8's in August; B1, B5, B7, B9 and B10's in November and B3, B4, B6, B11, B12 and B13's in February (Figure 3.4). The highest abundance values of the stations B4, B9, B12 and B13 were found in May; B1, B2, B3, B5, B6, B7, B8, B10, B11 and B14's in August and B15's in February (Figure 3.5). The highest biomass values of the stations B1, B3, B6, B7, B10, B11, B13, B14 and B15 were found in August; B5 and B9's in November and B2, B4, B8 and B12's in February (Figure 3.6).

Comparison of seasons in terms of the number of species, abundance and biomass are as follows. In May, the highest number of species (37) was found at B15 and the lowest (1) was found at B13; the highest abundance (54400 ind.m⁻²) was recorded at B4 and the lowest (25 ind.m⁻²) was recorded at B13; the highest biomass value (10862.98 g.m⁻²) was measured at B14 and the lowest (0.04 g.m⁻²) was measured at B13 again. In August, the highest number of species (36) was found at B1 and the lowest (16) was found at B11; the highest abundance (63083.33 ind.m⁻²) was recorded at B6 and the lowest (5041.67 ind.m⁻²) was recorded at B9; the highest biomass value (21978.50 g.m⁻²) was measured at B7 and the lowest (301.87 g.m⁻²) was measured at B9. In November, the highest number of species (42) was found at B1 and the lowest (13) was found at B8; the highest abundance (37708.33 ind.m⁻²) was recorded at B8 and the lowest (4575 ind.m⁻²) was recorded at B13; the highest biomass value (13701 g.m⁻²) was measured at B11 and the lowest (2097.32 $g.m^{-2}$) was measured at B10. In February, the highest number of species (43) was found at B6 and the lowest (11) was found at B9 and B10; the highest abundance (55275 ind.m⁻²) was recorded at B15 and the lowest (2558.33 ind.m⁻²) was recorded at B9; the highest biomass value (17551.39 g.m⁻²) was measured at B7 and the lowest (1.85 g.m⁻²) was measured at B9 (Figures 3.4, 3.5 and 3.6).



Figure 3.4. Changes in the number of species at sampling sites.



Figure 3.5. Changes in abundance at sampling sites.



Figure 3.6. Changes in biomass at sampling sites.

The Bray-Curtis cluster analysis was applied to the presence/absence data of the community to realize the differentiation of species composition between sampling sites (Figure 3.7). This analysis indicated that there were three station groups of $\approx 65 \%$ similarity degree possessing similar species composition in the study area. The first cluster (Group 1) embraced stations B8, B10, B11, B13 and B14. The second (Group 2) includes B2, B7, B12 and B15 and the third cluster (Group 3) includes B1, B3, B4, B5, B6 and B9. The one-way ANOSIM test (R=0.423 at a significance level of 0.4%) confirmed the significant differences between groups obtained from the cluster analysis.



Figure 3.7. Dendrogram produced with the BRAY-CURTIS – group average clustering technique based on the presence/absence data of the entire community.

Non-metric MDS ordination for the presence/absence data with descriptors of the biotope characteristics superimposed on each sampling site revealed that the reason of this spatial variation in the species composition may because of differences in biotope characteristics of the sampling sites (Figure 3.8).



Figure 3.8. Non-metric MDS ordination for the presence/absence data with descriptors of the biotope characteristics superimposed on each sampling site.

3.2. Environmental Condition Description

Spatial variations of major physical, chemical and biological water parameters including temperature, salinity, dissolved oxygen, nitrite and nitrate, total phosphate, silicate and fecal coliform among stations are presented in Figures 3.9-3.15 separately. During the course of the study, temperature showed a regular annual cycle, ranging from 5 to 23° C. The salinity, on the other hand, did not show regular cycle. The range of the salinity was 11-18.4 psu during the sampling period. In addition, dissolved oxygen varied from 5.44mg/L to 10.3mg/L, total phosphate from 0.05 μ M/L to 94.65 μ M/L, nitrite and nitrate from 0.08 μ M/L to 32.22 μ M/L and silicate from 1.45 μ M/L to 126.29 μ M/L. The fecal coliform values were ranged from 1-1000000000 CFU/100mL between sampling sites during the sampling seasons.



Figure 3.9. Spatial variation of temperature among sampling stations.



Figure. 3.10. Spatial variation of salinity among sampling stations.



Figure 3.11. Spatial variation of dissolved oxygen among sampling stations.



Figure 3.12. Spatial variation of nitrite and nitrate among sampling stations.



Figure 3.13. Spatial variation of phosphate among sampling stations.



Figure 3.14. Spatial variation of silicate among sampling stations.



Figure 3.15. Spatial variation of fecal coliform among sampling stations.

To be able to clarify the differences for physical, chemical and biological parameters between sampling sites, Mann-Whitney U test was applied. Thus, in terms of dissolved oxygen, nitrite and nitrate, total phosphate, silicate and fecal coliform, the significant differences between control and discharge stations were determined with the test. On the other hand, differences in the salinity and temperature measures between the control and discharge stations were not significant (Figures 3.16, 3.17). Superimposing physical, chemical and biological parameters parameters on the faunistic MDS plot revealed that nitrite and nitrate, phosphate, silicate and fecal coliform values were controlling factors for the differentiation of the control stations from the discharge stations (Figure 3.16).



Figure 3.16. MDS plots based on the fauna distribution (a) and when superimposing environmental parameters such as salinity (b), temperature (c), dissolved oxygen (d), nitrite and nitrate (e), phosphate (f), silicate (g) and fecal coliform (h).

In May, salinity values of control stations, which were varied between 18 and 18.4 psu, were higher than those of discharges, which were varied between 16.6 and 18.4 psu. Temperature values of control stations, which were varied between 16 and 18°C, were also higher than those of discharges, which were varied between 15 and 17°C. In addition, dissolved oxygen values of control stations, which were varied between 7.12 and 9.01 mg/L, were higher than those of discharges, which were varied between 5.63 and 8.38mg/L. There was significant difference in each variable of salinity (p=0.014) and dissolved oxygen (p= 0.018) between two groups of stations according to Mann-Whitney U test (Figures 3.16, 3.18). Despite slight difference was observed in the box plot of temperature data, there was no significant difference between control and discharge stations (p= 0.064) (Figure 3.17).



Figure 3.16. The box plot of control and discharge stations produced from salinity data of May (p=0.014)



Figure 3.17. The box plot of control and discharge stations produced from temperature data of May (p=0.064).



Figure 3.18. The box plot of control and discharge stations produced from dissolved oxygen data of May (p=0.018).

Nitrite and nitrate values of control stations, which were varied between 1.54 and 4.33μ M/L were slightly lower than those of discharges which were varied between 0.86 and 7.39 μ M/L in May. Phosphate values of control stations, which were varied between 0.02 and 0.41 μ M/L, were distinctly lower than those of discharges, which were varied between 0.05 and 58.68 μ M/L. Silicate values of control stations, which were varied between 1.45 and 4.26 μ M/L, were also lower than those of discharges, which were varied between 3.33 and 126.29 μ M/L. Mann-Whitney *U* test revealed that there was significant difference in each variable of phosphate (*p*=0.003) and silicate (*p*=0.059) between control and discharge stations (Figures 3.20, 3.21). However, there was not significant difference in nitrite and nitrate (*p*=0.814) (Figure 3.19).



Figure 3.19. The box plot of control and discharge stations produced from nitrite and nitrate data of May (p=0.814).



Figure 3.20. The box plot of control and discharge stations produced from phosphate data of May (p=0.003).



Figure 3.21. The box plot of control and discharge stations produced from silicate data of May (p=0.059).

Fecal coliform values of control stations, which were varied between 2 and 480 CFU/100mL, were sharply lower than those of discharges, which were varied between 180 and 100000000 CFU/100mL. Mann-Whitney U test showed that there was highly significant difference between control and discharge stations (Figure 3.22).



Figure 3.22. The box plot of control and discharge stations produced from fecal coliform data of May (p=0.001).

In August, salinity values of control stations, which were varied between 17.2 and 17.6 psu, were slightly higher than those of discharges, which were varied between 13.9 and 17.7 psu. Temperature of control stations were the same (22°C) and were slightly lower than those of discharges, which were varied between 22 and 23°C. Dissolved oxygen values of control stations, which were varied between 6.19 and 6.93mg/L, were higher than those of discharges, which were varied between 5.44 and 6.82 mg/L. Mann-Whitney U test showed that there was significant difference in dissolved oxygen (p=0.001) between control and discharge stations (Figure 3.25). However, there was no significant difference in salinity (p=0.066) and temperature (p=0.126) between these two groups of stations (Figures 3.23, 3.24).



Figure 3.23 The box plot of control and discharge stations produced from salinity data of August (p=0.066)



Figure 3.24. The box plot of control and discharge stations produced from temperature data of August (p=0.126)



Figure 3.25. The box plot of control and discharge stations produced from dissolved oxygen data of August (p=0.01).

Nitrite and nitrate values of control stations, which were varied between 0.08 and 0.58 μ M/L were distinctly lower those that of discharges, which were varied between 0.11 and 23.43 μ M/L in August. Phosphate values of control stations, which were varied between 0.12 and 0.21 μ M/L, were distinctly lower than those of discharges, which were varied between 0.11 and 94.65 μ M/L. Silicate values of control stations, which were varied between 1.94 and 3.53 μ M/L, were also lower than those of discharges, which were varied between 2.36 and 34.73 μ M/L. Mann-Whitney *U* test revealed that there was a significant difference in each of nitrite and nitrate (*p*=0.018), phosphate (*p*=0.026) and silicate (*p*=0.059) between control and discharge stations (Figures 3.26-3.28).



Figure 3.26. The box plot of control and discharge stations produced from nitrite and nitrate data of August (p=0.018).



Figure 3.27. The box plot of control and discharge stations produced from phosphate data of August (p=0.026).



Figure 3.28. The box plot of control and discharge stations produced from silicate data of August (p=0.059).

In August, fecal coliform values of control stations, which were varied between 25 and 210 CFU/100mL, were distinctly lower than that of discharges, which were varied between 10000 and 620000 CFU/100mL. There was a significant difference (Mann-Whitney U test, p=0.001) between control and discharge stations (Figure 3.29).



Figure 3.29. The box plot of control and discharge stations produced from fecal coliform data of August (p=0.001).

In November, salinity values of control stations, which were varied between 17.1 and 18.2 psu, were slightly higher than those of discharges, which were varied between 16.9 and 17.9 psu. Temperature of control stations, which were varied between 12-13.5°C, were very close to that of discharges, which was 13°C. In addition, dissolved oxygen values of control stations, which were varied between 7.85 and 9.20mg/L, were higher than those of discharges, which were varied between 6.93 and 8.88mg/L. There was a significant difference (p=0.018) in dissolved oxygen between two groups according to Mann-Whitney U test (Figure 3.32). However, there was no significant difference in each of salinity (p=0.399) and temperature (p=0.171) between control and discharge stations (Figures 3.30 and 3.31).



Figure 3.30. The box plot of control and discharge stations produced from salinity data of November (p= 0.399)



Figure 3.31. The box plot of control and discharge stations produced from temperature data of November (p=0.171).



Figure 3.32. The box plot of control and discharge stations produced from dissolved oxygen data of November (p=0.018).

Nitrite and nitrate values of control stations, which were varied between 0.41 and 1 μ M/L were lower than that of discharges which were varied between 0.50 and 4.47 μ M/L in November. Phosphate values of control stations, which were varied between 0.30 and 0.45, were clearly lower than those of discharges, which were varied between 0.46 and 8.55 μ M/L. Silicate values of control stations, which were varied between 1.80 and 3.12 μ M/L, were also lower than that of discharges, which were varied between 2.64 and 5.58 μ M/L. Mann-Whitney *U* test revealed that there was a significant difference in each of nitrite and nitrate (*p*=0.013), phosphate (*p*=0.005) and silicate (*p*=0.007) between control and discharge stations (Figures 3.33-3.35).



Figure 3.33. The box plot of control and discharge stations produced from nitrite and nitrate data of November (p=0.013).



Figure 3.34. The box plot of control and discharge stations produced from phosphate data of November (p=0.005).



Figure 3.35. The box plot of control and discharge stations produced from silicate data of November (p=0.007).

In November, fecal coliform values of control stations, which were varied between 39 and 240, were clearly lower than those of discharges, which were varied between 10000 CFU/100mL and 1000000000 CFU/100mL. There was significant difference (Mann-Whitney U test, p=0.001) again between control and discharge stations in this sampling period (Figure 3.36).



Figure 3.36. The box plot of control and discharge stations produced from fecal coliform data of November (p=0.001).

In February, salinity values of control stations, which were varied between 17.2 and 17.6 psu, were higher than those of discharges, which were varied between 11.0 and 17.9 psu. Temperature values of control stations, which were varied between 6 and 7°C, were higher than those of discharges, which were varied between 5 and 7°C. Dissolved oxygen values of control stations, which were varied between 8.65 and 11.7mg/L, were slightly

higher than those of discharges, which were varied between 8.65 and 10.30 mg/L. Mann-Whitney U test revealed that there was a significant difference in temperature (p=0.015) between control and discharge stations. However, there was no significant difference in salinity (p=0.075) and dissolved oxygen values (p=0.213) between these two groups of stations (Figures 3.37-3.39).



Figure 3.37. The box plot of control and discharge stations produced from salinity data of February (p=0.075).



Figure 3.38. The box plot of control and discharge stations produced from temperature data of February (p=0.015).



Figure 3.39. The box plot of control and discharge stations produced from dissolved oxygen data of February (p=0.213).

Nitrite and nitrate values of control stations, which were varied between 0.54 and 3.63μ M/L, were remarkably lower than those of discharges which were varied between 6.89 and 32.22μ M/L in February. Phosphate values of control stations, which were varied between 0.10 and 0.33μ M/L, were clearly lower than that of discharges, which were varied between 0.81 and 10.85μ M/L. Silicate values of control stations, which were varied between 2.83 and 14.10 μ M/L, were also lower than those of discharges, which were varied between 11.17 and 65.21μ M/L. Mann-Whitney U test revealed that there was significant difference in each variable of nitrite and nitrate (*p*=0.002), phosphate (*p*=0.001) and silicate (*p*=0.009) between control and discharge (Figures 3.40-3.42).



Figure 3.40. The box plot of control and discharge stations produced from nitrite and nitrate data of February (p=0.002).



Figure 3.41. The box plot of control and discharge stations produced from phosphate data of February (p=0.001).



Figure 3.42. The box plot of control and discharge stations produced from silicate data of February (p=0.009).

In February, fecal coliform values of control stations, which were varied between 4 and 210 CFU/100mL, were clearly lower than that of discharges, which were varied between 650 and 310000 CFU/100mL. The same as the previous sampling periods, there was a significant difference (Mann-Whitney U test, p=0.001) between control and discharge stations (Figure 3.43).



Figure 3.43. The box plot of control and discharge stations produced from fecal coliform data of February (p=0.001).

The non-metric MDS plots applied to the all physical, chemical and biological parameters for entire data and seasonal data separately. All MDS plots showed distinct groups corresponding to the control and the discharge stations (Figures 3.44-3.48). The stress values were all less than 0.1 and as Clark (1993) suggested this emphasize excellent representation of community data in ordination space. The one-way ANOSIM test confirmed the significant dissimilarity between control and discharge stations. Global *R* and significance level scores derived from the ANOSIM tests given in Table 3.2. According to SIMPER analysis the fecal coliform was the most important parameter in distinguishing two groups of stations; control and discharge (Table 3.3).



Figure 3.44. MDS ordination of entire environmental parameters data for the factor level [control (C) and discharge (D) stations].



Figure 3.45. MDS ordination of environmental parameters data of May sampling for the factor level [control (C) and discharge (D) stations].



Figure 3.46. MDS ordination of environmental parameters data of August sampling for the factor level [control (C) and discharge (D) stations].



Figure 3.47. MDS ordination of environmental parameters data of November sampling for the factor level [control (C) and discharge (D) stations].



Figure 3.48. MDS ordination of environmental parameters data of February sampling for the factor level [control (C) and discharge (D) stations].

Table 3.2. Global test scores of the ANOSIM analysis for environmental parameters.

Global test	Whole Samplings	May	August	November	February
Global R	0.937	0.852	0.672	0.975	0.951
Significance level (%)	0.1	0.1	0.1	0.1	0.1

Measured Parameters	Percentage Cover				
	Whole Samplings	May	August	November	February
Fecal Coliform	83.46 (May data)	100	99.97	99.80	99.65
Other parameters	16.54	0	0.03	0.20	0.35

Table 3.3. SIMPER analysis for environmental parameters contributing to dissimilarities between control and discharge stations.

Consequently, rather high values of nitrite and nitrate total phosphate and silicate were found in discharge stations. Deposition of organic material or organic enrichment in a marine area is often accompanied by the introduction of inorganic nutrients (Pearson and Rosenberg, 1978). In this sense, high values of nitrite and nitrate, total phosphate and silicate indicating nutrient-rich conditions suggest that sewage discharges caused organic enrichment in discharge stations. Organic enrichment is the best documented disturbance affecting marine macrobenthos in coastal areas. Pearson and Rosenberg (1978) proposed that organic enrichment of benthic environments due to sewage may result in a series of non-linear changes in the abundance, biomass and the diversity of benthic organisms, in both spatial and temporal patterns. Further studies of effects of organic enrichment on marine communities support the community change described by Pearson and Rosenberg (1978) (Mirza and Gray, 1981; Essink, 1984; Whitlatch and Zajac, 1985; Pearson et al., 1986; Weston, 1990).

3.3. Community Descriptive Analyses

Average number of species, found in each sampling, varies from 1 to 43. Average value of total abundance, on the other hand, varies from 25 to 63083.33 ind./m². In addition, average value of total biomass varies from 0.37 to 21995.82 g/m² among each sampling. Comparison of the four season's samples on the basis of their abundance by Kruskal Wallis test showed significant differences among them (H= 3, p=0.0086). Also comparison of biomass values showed significant differences among seasons (H=3, p=0.0003). However, comparison of the number of species revealed no significant differences among seasons (H=3, p=0.4656) (Figures 3.49-3.51).



Figure 3.49. The box plot of sampling seasons produced from number of species data.



Figure 3.50. The box plot of sampling seasons produced from abundance data.



Figure 3.51. The box plot of sampling seasons produced from biomass data.

The range of variation over all samplings of the indices was: for species richness (*d*) $0 \le d \le 7.42$, for the Shannon diversity (*H'*) $0 \le H' \le 3.94$ and for evenness $0 \le J \le 0.76$. Comparison of the four seasons' samples by Kruskal-Wallis test showed no significant difference in each of species richness (H=3, r=0.4707), diversity (H=3, r=0.3654) and evenness (H=3, r=0.3429) among seasons (Figures 3.52-3.54).



Figure 3.52. The box plot of sampling seasons produced from species diversity (H') data.



Figure 3.53. The box plot of sampling seasons produced from species richness (d) data.



Figure 3.54. The box plot of sampling seasons produced from evenness (J) data.

When control and discharge stations were compared with regard to dominance of number of species and biomass (wet weight), no significant difference between group per centages were observed (Figures 3.55-3.56 and 3.59-3.60). On the other hand, significant difference related with the dominance of abundance was obtained between sampling groups (Figures 3.57, 3.58). While Mollusca was quantitatively dominant group comprising 45% of the individuals for the control stations, Crustacea was quantitatively dominant group comprising 51% of the individuals for the discharge stations. In control stations, Mollusca was followed by Crustacea comprising of 36% the individuals, Polychaeta comprising of 15% the individuals, Oligochaeta comprising of 2% the individuals and other groups Cnidaria, Turbellaria, Nemertea, Pchnogonida and Echinodermata. In discharge (target) stations, Crustacea was followed by Mollusca comprising of 31% the individuals, and other groups Cnidaria, Turbellaria, Nemertea, Pchnogonida and Echinodermata.











Figure 3.57. Quantitative dominance of systematic groups at control stations.


Figure 3.58. Quantitative dominance of systematic groups at discharge stations.



Figure 3.59. Dominance of biomass of systematic groups at control stations.



Figure 3.60. Dominance of biomass of systematic groups at discharge stations.

The most important difference between the control and the discharge stations was the differentiation in per centage abundance of the crustaceans and molluscs. High abundance of opportunistic crustaceans such as *Echinogammarus olivii*, *Hyale perieri* and *Tanais dulongii* found in discharge stations and high abundance of two bivalves *Mytlus galloprovincialis* and *Mytilaster lineatus* in control stations responsible for this differentiation (Table 3.1).

The range of variation of the number of species, abundance, biomass, species richness, diversity and evenness of control and discharge stations are given in Table 3.4. Comparison of the samplings of the control and discharge stations by Mann-Whitney U test revealed a significant difference in each of the number of species (p=0.0000), the abundance (p=0.0020), the species richness (p=0.0000) and the diversity (p=0.0000). However, there was no significant difference in biomass (p=0.4373) and evenness (p=0.0592) between the control and discharge stations (Figures 3.61-3.66).

The number of species, total abundance, species richness and diversity values of the discharge stations 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). With this regard, decreasing these community measures indicated disturbed community in discharge stations in the present study.

Community descriptive measures	Control Stations	Discharge Stations		
Shannon diversity (H')	2.61≤ <i>H</i> '≤3.94	$1.33 \le H' \le 3.69$		
Evennes (<i>J</i>)	$0.50 \le J \le 0.76$	$0.36 \le J \le 0.73$		
Margalef's Species richnes (d)	5.33≤ <i>d</i> ≤6.55	$0 \le d \le 7.42$		
Number of species	$22 \leq$ number of species ≤ 42	$1 \le$ number of species ≤ 43		
Abundance (ind.m ⁻²)	$7200 \le$ abundance ≤ 55275	25≤ abundance ≤63083.33		
Biomass (g.m ⁻²)	$623.55 \leq biomass \leq 15793.30$	$0.04 \le biomass \le 21978.50$		

Table 3.4. Range variations of community descriptive measures at the control and discharge stations.







Figure 3.62. The box plot of the control and discharge stations produced from number of species data.



Figure 3.63. The box plot of the control and discharge stations produced from biomass data.







Figure 3.65. The box plot of the control and discharge stations produced from species diversity data.



Figure 3.66. The box plot of the control and discharge stations produced from evenness data.

3.4. Ecological Quality Assessment – Biological Indices

The Bentix scores of control stations, which were varied between 2.40 and 5.37, were distinctly higher than that of discharges, which were varied between 2 and 3.13 (Figure 3.67). According to BENTIX, station B3 was classified as normal/pristine and possessed high ecological quality status (ECoQ) in majority of its samplings. Station B1 was classified as slightly polluted, transitional and possessed good ECoQ in majority of its samplings. Station B5 was classified as normal/pristine and possessed high ECoQ in August and February, but classified as slightly polluted - transitional and possessed good ECoQ in November and May. Stations B2, B4, B6 and B15 were classified as moderately polluted and possessed moderate ECoQ in majority of their samplings. Stations B7, B9, B10, B11, B13 and B14 were classified as heavily polluted and possessed poor ECoQ in all of their samplings. In addition, station B8 and B12 were also classified as heavily polluted and possessed poor ECoQ in majority of their samplings (Table 3.5). All stations, classified as heavily polluted and possessed 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 possessed moderate ECoQ. All other stations, classified as normal/pristine, slightly polluted – transitional and moderately polluted and possessed high, good and moderate ECoQ were not directly affected by sewage discharge (control stations).

The stations were classified according to physically stressed community type by using their BENTIX scores in order to get realistic assessment on the ecological quality status for the sampling sites under the physical effects such as currents, strong waves and mussel harvesting activities. When the sampling sites were classified with this approach, some differences in their ecological quality status were observed compared to other classification type (Table 3.5). Especially stations B1 and B4, which were under the effects of artificial waves caused by maritime traffic, rise upper level of pollution classification and ecological quality status.



Figure 3.67. BENTIX index trend in the study area.

With regard to BENTIX scores, ecological quality status of the discharge stations worse than that of controls. BENTIX index is a very robust and adequately effective tool in classifying benthic communities into ecological quality classes. Its robustness lies in the fact that it is largely habitat type and sample size independent and thus has a potential for global application. Its effectiveness in discriminating between ecological classes is based on its ability to reflect the faunal composition in relation with the resistance of species to disturbance factors (Simboura and Zenetos, 2002). Although so far it has been used for soft-bottom communities, Bentix appears to work successful also in the present study. In this sense, it can be said that the macrozoobenthic communities of the discharge stations, with low BENTIX scores and worse ecological quality status, were affected by pollution.

In addition, diversity index scores of control stations, which were varied between 0.50 and 0.76, were higher than that of discharges, which were varied between 0 and 0.70 (Figure 3.68). According to Shannon diversity index (H'), stations were divided in two groups. Stations B1, B4, B6 and B15 were classified as moderately polluted in majority of their samplings. Stations B7, B8, B9, B10, B11, B12, B13 and B14, on the other hand, were classified as heavily polluted in majority of their samplings. Station B2 was classified as moderately polluted in August and November. Station B3 was classified as moderately polluted in August and February, but classified as heavily polluted in May and November. Station B5 was classified as

moderately polluted in May and November, but classified as heavily polluted in August and February (Table 3.5). All stations, classified as heavily polluted were discharge stations. Although station B6 was also discharge station, it was classified as moderately polluted according to Shannon diversity index (H'). All other stations, classified as moderately polluted, and stations B2, B3 and B5 were control stations (Table 3.5).



Figure 3.68. Shannon-Wiener diversity index trend in the study area.

In terms of community diversity, control stations seem better than discharges. The Shannon-Wiener index of diversity is one of most commonly used diversity indices in the assessment of pollution in marine benthic communities (Simboura and Zenetos, 2002). However, the use and interpretation of this index (and other diversity indices) has been subjected to much debate (Clarke and Warwick, 1994; Jennings and Reynolds, 2000). Although, decrease or increase in community diversity could not be used as a curtain indicator of the health or stability of the ecosystem (Nybakken, 1997; Simboura and Zenetos, 2002), it is known that the pollution perturbed benthic communities possess low diversity values (Hyland et al., 2000). Relatively low diversity values of discharge stations hence provide evidence for pollution effect on macrozoobenthic communities.

St.	Sampling Months	H'	J	Pollution Classification based on H'	BENTIX	ECoQ-1	Pollution Classification based on BENTIX-1	ECoQ-2	Pollution Classification based on BENTIX-2
	May	3.30	0.64	Moderately polluted	4.5	High	Normal/Pristine	High	Normal/Pristine
DI	August	2.93	0.57	Heavily polluted	4.3	Good	Slightly polluted- transitional	High	Normal/Pristine
BI	November	3.41	0.63	Moderately polluted	4.4	Good	Slightly polluted- transitional	High	Normal/Pristine
	February	3.94	0.76	Moderately polluted	4.3	Good	Slightly polluted- transitional	High	Normal/Pristine
	May	3.15	0.62	Moderately polluted	3.5	Good	Slightly polluted- transitional	Good	Slightly polluted- transitional
DJ	August	2.69	0.54	Heavily polluted	2.7	Moderate	Moderately polluted	Good	Moderately polluted
D2	November	2.83	0.63	Heavily polluted	3.2	Moderate	Moderately polluted	Good	Slightly polluted- transitional
	February	3.29	0.67	Moderately polluted	2.8	Moderate	Moderately polluted	Good	Moderately polluted
	May	2.95	0.62	Heavily polluted	4.6	High	Normal/Pristine	High	Normal/Pristine
D2	August	3.26	0.64	Moderately polluted	4.8	High	Normal/Pristine	High	Normal/Pristine
Б3	November	2.64	0.58	Heavily polluted	4	Good	Slightly polluted- transitional	High	Normal/Pristine
	February	3.50	0.68	Moderately polluted	4.5	High	Normal/Pristine	High	Normal/Pristine
	May	2.92	0.60	Heavily polluted	3.4	Moderate	Moderately polluted	Good	Slightly polluted. transitional
D4	August	2.63	0.54	Heavily polluted	3.4	Moderate	Moderately polluted	Good	Slightly polluted. transitional
В4	November	2.69	0.54	Heavily polluted	3.4	Moderate	Moderately polluted	Good	Slightly polluted. transitional
	February	2.70	0.54	Heavily polluted	3.8	Good	Slightly polluted. transitional	Good	Slightly polluted. transitional
	May	3.09	0.60	Moderately polluted	4.4	Good	Slightly polluted- transitional	High	Normal/Pristine
R5	August	2.85	0.58	Heavily polluted	5.4	High	Normal/Pristine	High	Normal/Pristine
103	November	3.13	0.60	Moderately polluted	4	Good	Slightly polluted- transitional	High	Normal/Pristine
	February	2.85	0.56	Heavily polluted	5	High	Normal/Pristine	High	Normal/Pristine
	May	3.44	0.68	Moderately polluted	3.1	Moderate	Moderately polluted	Moderate	Slightly polluted. transitional
R6	August	3.25	0.65	Moderately polluted	2.4	Poor	Heavily polluted	Poor	Heavily polluted
100	November	3.17	0.61	Moderately polluted	2.5	Moderate	Moderately polluted	Moderate	Moderately polluted
	February	3.45	0.64	Moderately polluted	2.8	Moderate	Moderately polluted	Moderate	Moderately polluted
	May	3.08	0.66	Moderately polluted	2.3	Poor	Heavily polluted	Poor	Heavily polluted
B 7	August	2.54	0.55	Heavily polluted	2.4	Poor	Heavily polluted	Poor	Heavily polluted
51	November	2.76	0.56	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.70	0.55	Heavily polluted	2.3	Poor	Heavily polluted	Poor	Heavily polluted

Table 3.5. Shannon-Wiener diversity, evenness and BENTIX index scores with ecological quality status and pollution classification of the samplings.

Table 3.5. (Continued)

St.	Sampling Months	H'	J	Pollution Classification based on H'	BENTIX	ECoQ-1	Pollution Classification based on BENTIX-1	ECoQ-2	Pollution Classification based on BENTIX-2
	May	2.71	0.73	Heavily polluted	2.8	Moderate	Moderately polluted	Moderate	Moderately polluted
	August	2.98	0.68	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
B8	November	1.33	0.36	Azoic to heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.39	0.60	Heavily polluted	2.4	Poor	Heavily polluted	Poor	Heavily polluted
	May	2.13	0.62	Heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
RQ	August	2.53	0.58	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
D >	November	3.69	0.70	Moderately polluted	2.3	Poor	Heavily polluted	Poor	Heavily polluted
	February	1.79	0.50	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	May	2.22	0.67	Heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
B10	August	2.40	0.55	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
DIU	November	2.49	0.54	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	1.97	0.55	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
	May	2.23	0.65	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
R11	August	2.19	0.55	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	November	2.21	0.53	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.11	0.48	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
	May	1.58	0.36	Heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
R12	August	2.20	0.49	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
DIZ	November	2.31	0.54	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.77	0.57	Heavily polluted	2.5	Moderate	Moderately polluted	Moderate	Moderately polluted
	May	0	0	Azoic to heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
R13	August	2.02	0.50	Heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
в13	November	3.05	0.69	Heavily polluted	2.1	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.68	0.58	Heavily polluted	2	Poor	Heavily polluted	Poor	Heavily polluted
	May	2.39	0.52	Heavily polluted	2.4	Poor	Heavily polluted	Poor	Heavily polluted
B14	August	2.55	0.56	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	November	2.52	0.55	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted
	February	2.62	0.60	Heavily polluted	2.2	Poor	Heavily polluted	Poor	Heavily polluted

Table 3.5. (Continued)

St.	Sampling Months	H'	J	Pollution Classification based on H'	BENTIX	ECoQ-1	Pollution Classification based on BENTIX-1	ECoQ-2	Pollution Classification based on BENTIX-2
B15	May	2.61	0.50	Heavily polluted	2.4	Poor	Heavily polluted	Poor	Heavily polluted
	August	3.14	0.64	Moderately polluted	2.8	Moderate	Moderately polluted	Moderate	Moderately polluted
	November	3.08	0.65	Moderately polluted	3.2	Moderate	Moderately polluted	Good	Slightly polluted- transitional
	February	3.05	0.62	Moderately polluted	2.8	Moderate	Moderately polluted	Moderate	Moderately polluted

As mentioned before, BENTIX scores were calculated according to the scores of three ecological groups. These groups are; G1 includes species which are sensitive to disturbance in general, G2 includes species which were tolerant to disturbance and G3 includes the first order opportunistic species. In the present study, *Capitella capitata, Echinogammarus olivii* and *Tanais dulongii* were determined as the first order opportunistic species.

The per centages of sensitive species had the highest value among other groups at all samplings of B1, B3 and B5 and the per centages of tolerant species had the highest value among other groups at all samplings of B2, B4, B7, B10, B12 and B15; November and February samplings of B6; May, August and February samplings of B8; May, November and February of B9; May, August and February of B11; November and February of B13 and May, August and November of B14. The highest per centages of first order opportunistic species, on the other hand, were found at May and August samplings of B6, November sampling of B8, August sampling of B9, November sampling of B11, May and August samplings of B13 and February sampling of B14. In the present study, discharge stations were characterized by the high ratio of first order opportunistic species. Relatively high abundance of sensitive species (GI) was found mostly at control stations (especially at stations B1, B3 and B5). Pollution tolerant species (GII) were recorded either at control and discharge stations but mostly at stations classified as moderately polluted according to BENTIX (Table 3.6).

64	St SAMPLING MONTHS					
51.	MAY	AUGUST	NOVEMBER	FEBRUARY		
	G I: 62.88	G I: 57.64	G I: 60.34	G I: 57.52		
	G II: 15.99	G II: 21.56	G II: 19.86	G II: 25.94		
1	G III: 21.13	G III: 20.80	G III: 19.80	G III: 16.54		
	G.I: 38 57	G I: 18 68	G I: 30.14	G I: 21 22		
2	G II: 38.57	G II: 18:08	G II: 59 80	G II: 68 60		
2	C III. 17.29	C III: 12.57	C III: 10.07	C III: 10.09		
	0 111. 17.58	0111.15.57	G III. 10.97	G III. 10.08		
	G I: 68.85	G I: 70.34	G I: 49.33	G I: 61.57		
3	G II: 17.25	G II: 14.36	G II: 27.91	G II: 28.94		
	G III: 16.90	G III: 15.29	G III: 22.77	G III: 9.49		
	G I: 36.08	G I: 34.79	G I: 34.70	G I: 44.33		
4	G II: 58.06	G II: 56.46	G II: 59.11	G II: 50.91		
	G III: 5.87	G III: 8.75	G III: 6.19	G III: 4.76		
	G I: 59.05	G I: 84.27	G I: 50.50	G I: 75.25		
5	G II: 20.42	G II: 10.62	G II: 28.86	G II: 16.88		
	G III: 20.53	G III: 5.11	G III: 20.64	G III: 7.87		
	C I: 29 17	G I: 0 41	G I: 12.00	G I: 10.16		
(G I. 26.17 C II: 24.55	G I. 9.41 C II. 21.95	G I. 12.09 C II: 62.62	С Ц. 41.11		
0	G II. 34.33 C III. 27.29	G II. 51.85 C III. 59.75	G II. 02.02 C III. 25.20	G II. 41.11 C III. 20.72		
	G III: 37.28	G III: 58.75	G III: 25.29	G III: 39.75		
	G I: 6.30	G I: 8.81	G I: 5.42	G I: 6.68		
7	G II: 86.90	G II: 89.35	G II: 93.59	G II: 91.17		
	G III: 6.81	G III: 1.84	G III: 0.99	G III: 2.15		
	G I: 19.97	G I: 3.39	G I: 5.66	G I: 9.63		
8	G II: 53.87	G II: 57.43	G II: 21.90	G II: 64.59		
	G III: 26.16	G III: 39.18	G III: 72.44	G III: 25.78		
	G I: 0.12	G I: 2.64	G I: 8.42	G I: 4.23		
9	G II: 66.23	G II: 36.86	G II: 71.41	G II: 85.02		
	G III: 33.65	G III: 60.50	G III: 20.17	G III: 10.75		
	G I: 0.96	G I: 2 03	G I: 4 76	G1:126		
10	G II: 72 60	G II: 65.68	G II: 170	G II: 72 63		
10	G III: 72.00 G III: 26.44	G III: 32 29	G III: 72.55 G III: 22.68	G III: 72.05 G III: 26.12		
	C I: 4 41	C I: 5 57	C I: 4 (2	C I: 2 27		
11	G I: 4.41 G II: 59.74	G I: 5.57	G I: 4.63	G I: 3.27 G II: 72 (7		
11	G II: 58./4	G II: 83.35	G II: 44.68	G II: 73.67		
	G III: 36.85	G III: 11.07	G III: 50.69	G III: 23.06		
	G I: 0.54	G I: 4.44	G I: 5.13	G I: 13.65		
12	G II: 97.84	G II: 93.09	G II: 93.84	G II: 84.26		
	G III: 1.62	G III: 2.47	G III: 1.03	G III: 2.09		
	G I: 0.00	G I: 1.15	G I: 1.28	G I: 0.47		
13	G II: 0.00	G II: 30.26	G II: 66.12	G II: 86.17		
	G III: 100.00	G III: 68.59	G III: 32.60	G III: 13.36		
	G I: 8 79	G I: 5 74	G1: 5.13	G I 4 45		
14	G II: 53 17	G II: 59 13	G II: 48 85	G II: 43.02		
17	G III. 38.04	G III: 35 13	G III: 46.02	G III: 52 53		
	C I: 0.05	C I: 21 12	C I: 21 14	C I: 19.05		
15	C H, 94 20	G I. 21.12 C II: 70.00	G I. 51.14 C II. (2.5)	G H. 55 49		
15	G II: 84.29 C III: 5.7(G II: /0.00 G III: 7.00	G II: 03.30 G III: 5.20	G II: 55.48		
1	G III: 5.76	G III: 7.88	G III: 5.30	G III: 25.57		

Table 3.6. Ecological group per centages for each sampling period of the stations.

3.5. ABC and Rarefaction Curves

The partial dominance curves for each seasonal sampling demonstrate the effects of pollution on examined communities (Figures 3.69-3.76). ABC plots belonging to all sampling seasons of B1 and B3; May samplings of B2, B4, B5, B6, B7 and B10; August samplings of B2, B4, B8, B9 and B12; November samplings of B5, B9 and B15 and February samplings of B2, B5, B6 and B8 indicated unpolluted conditions with the biomass curve above the abundance curve of its entire length. Only August sampling of B5, November and February samplings of B7 and May and August samplings of B15 were grossly disturbed according to Warwick's interpretation of ABC plots. The ABC plots of all other samplings indicated "moderately polluted" conditions, with the biomass and abundance curves quite closely coincident. In conclusion, by this analysis most of the discharge stations would be classified as grossly and moderately polluted whilst most of the control stations would be classified as unpolluted with some exceptions in the present study.



Figure 3.69. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B1 and B2.



Figure 3.70. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B3 and B4.



Figure 3.71. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B5 and B6.



Figure 3.72. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B7 and B8.



Figure 3.73. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B9 and B10.



Figure 3.74. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B11 and B12.



Figure 3.75. Partial dominance curves (abundance/biomass comparison) for the samplings of stations B13 (except May sampling) and B14.



Figure 3.76. Partial dominance curves (abundance/biomass comparison) for the samplings of station B15.

Rarefaction curves provide a measure of species diversity which is robust to sample size effects, permitting comparison between communities where, for example, densities of animals are very different. This method may also provide clues about the pollution status of the communities. Steeper and elevated curves indicate more diverse communities which are relatively less affected by pollution (James et al., 1981). In the present study, these curves were applied to seasonal data separately.

In May, the curves of B1, B2, B3, B5, B6 and B7 stations were the steepest and the most elevated in all curves of stations. Therefore, they can be accepted as the cleanest stations in the sampling sites. In addition, the stations, B4, B12 and B14, represented by steeper curves, seemed more diverse and less affected by pollution than other stations, the most disturbed ones (Figure 3.79). In August, the stations B1, B2, B3, B4, B5, B6 and B15, represented by steeper curves, seemed more diverse and less affected by pollution than other stations (Figure 3.80). In November, steeper curves indicated the stations B1, B4, B5, B6, B7, B9 and B15. It could be suggested that these stations were unpolluted (clear) compared to other sampling sites (Figure

3.81). Finally, in February stations B1, B2, B3, B4, B5 and B6 had the steeper curves than other stations (Figure 3.82).



Figure 3.77. Rarefaction curves comparing the stations in May sampling.



Figure 3.78. Rarefaction curves comparing the stations in August sampling.



Figure 3.79. Rarefaction curves comparing the stations in November sampling.



Figure 3.80. Rarefaction curves comparing the stations in February sampling.

3.6. Comparative Analyses of Distribution Patterns in Discharge and Control Stations

Multidimensional scaling (MDS) analysis (Figures 3.83-3.87) applied to the entire data and seasonal data separately showed distinct groups corresponding to the control and discharge stations. However, the abundance data of B13 in May (not found any macrozoobenthic species in two replicates) was not included in MDS analysis, since all sampling periods of the stations except May of B13 were grouped very close to each other at the one side of the plot when May of B13 are included.

The MDS configuration that resulted from the entire abundance matrix showed a separation of stations into two different groups (control and discharge). Samples of the discharge stations are located in the right side of the figure whereas those of the control stations are positioned towards the right (Figure 3.83). The stress value of two dimensional MDS configuration is 0.17, indicating good ordination of samples (Clark and Warwick, 1994). 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 (control and discharge) was confirmed.



Figure 3.81. MDS ordination based on the entire abundance data (logarithmic transformed) for the factor level [control (C) and discharge (D) stations].

SIMPER analysis of the transformed entire abundance data allow the examination of the species which contribute to the dissimilarity between control and discharge stations. The control stations showed an average similarity of 36.75%. According to analysis, five species, *Mytilaster lineatus*, *Hyale pontica*, *Mytilus galloprovincialis*, *Platynereis dumerili* 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 is 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*, *Ericthonius brasiliensis*, Opheliidae (sp.) and *Stenothoe tergestina* (Table 3.7). It is quite clear that only a few species are important in characterizing and differentiating stations.

Table 3.7. SIMPER analysis differences in average abundances or per centage cover per species contributing to dissimilarities between all samplings of control and discharge stations. A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	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

To be able to assess the difference between control and discharge stations, the multidimensional scaling was applied separately to each sampling period of abundance data. In May, two separated groups were obtained (Figure 3.84). First group embraced the control stations (B1, B2, B3, B4, B5 and B15) and some of discharge stations (B6 and B7) and second group including stations B8, B9, B10, B11 and B13. MDS ordination includes two ungrouped stations. These are two discharge stations B12 and B14, which appears in the middle of the figure, seems to differ considerably from all the other samples. ANOSIM analysis (global R=0.433 at a significance level of 0.9%) confirmed significant differences

between groups obtained from the plot. As a consequence, the MDS analysis, with stress value of 0.1, showed almost well defined separation of control and discharge stations.



Figure 3.82. MDS ordination based on the abundance data (logarithmic transformed) of May sampling for the factor level [control (C) and discharge (D) stations].

The first group (control stations) showed an average similarity % 36.11. As identified by SIMPER analysis, five species, *Mytilaster lineatus*, *Hyale pontica*, *Mytilus galloprovincialis*, *Platynereis dumerilii* and *Nereis* (*Hediste*) *diversicolor* contributed 80% of the similarity in control stations and the discharge stations reached an average similarity of 20.85%, with four species, *Mytilus galloprovincialis*, *Echinogammarus olivii*, *Enchytraeus buchholzi* and *Hyale perieri* covering 80%. The control stations had an average dissimilarity of 89.42% with the discharge stations. These groups were separated from each other with nine species, *Mytilaster lineatus*, *Mytilus galloprovincialis*, *Hyale pontica*, *Jassa marmorata*, *Nereis* (*Hediste*) *diversicolor*, *Platynereis dumerilii*, *Idotea pelagica* Opheliidae (sp.) and *Enchytraeus buchholzi* (Table 3.8)

Table 3.8. SIMPER analysis differences in average abundances or per centage cover per species contributing to dissimilarities between May samplings of control and discharge stations. A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	Control	Discharge
Mytilaster lineatus	26.89	5.64
Mytilus galloprovincialis	13.02	28.67
Hyale pontica	12.10	0
Idotea pelagica	11.06	1.99
Platynereis dumerilii	11.00	0.44
Nereis (Hediste) diversicolor	8.27	0.25
Echinogammarus olivii	6.01	22.80
Enchytraeus buchholzi	1.94	11.01
Hyale perieri	8.05	17.00

The affinity of August samples is shown in Figure 3.85. The MDS ordination, with a stress value of 0.12, indicates a separation of the samples into two main groups. The first group includes all samples of control stations and two discharge stations (B7 and B12) whereas the second group includes some of discharge stations (B6, B8, B10, B11 and B14). The stations B9 and B13 are out of these groups. The performance of a one-way ANOSIM test gave global R of 0.547 at a significance level of 0.1%. Thus, the two groups were separated. In other words, differentiation between control and discharge stations was defined.



Figure 3.83. MDS ordination based on the abundance data (logarithmic transformed) of August sampling for the factor level [control (C)and discharge (D) stations].

The first group showed an average similarity 38.55 %. According to SIMPER analysis, four species alone (*Mytilus galloprovincialis, Mytilaster lineatus, Hyale perieri* and *Ampithoe ramondi*) were responsible for 80% of the similarity. The second group, on the other hand, reached an average similarity persentage of 43.88%. *Mytilus galloprovincialis, Echinogammarus olivii* and *Tanais dulongii* alone covered 80% of this similarity. As regards the divergence among groups, the first group had an average dissimilarity percentage of 70.30% with second group. The species *Echinogammarus olivii, Mytilus galloprovincialis, Enchytraeus buchholzi, Mytilaster lineatus, Tanais dulongii, Hyale perieri, Platynereis dumerilii, Ericthonius brasiliensis, Microdeutopus gryllotalpa, Capitella capitata and Melita palmata contributed 80% of dissimilarity percentage between two groups (Table 3.9).*

Table 3.9. SIMPER analysis differences in average abundances or per centage cover per species contributing to dissimilarities between August samplings of control and discharge stations. A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	Control	Discharge
Echinogammarus olivii	1.94	21.06
Mytilus galloprovincialis	42.33	42.94
Enchytraeus buchholzi	0.29	7.93
Mytilaster lineatus	18.35	4.89
Tanais dulongii	0.34	9.32
Hyale perieri	8.62	6.40
Platynereis dumerilii	5.36	8.18
Ericthonius brasiliensis	1.89	0
Microdeutopus gryllotalpa	0.41	6.88
Capitella capitata	0.11	5.65
Melita palmata	3.44	0.51

The MDS configuration in Figure 3.86 shows the grouping of the stations according to their faunal affiliations in November. Three major groups were formed with a stress value of 0.11. Stations B1, B3 and B5, which are control stations, were grouped together in the first group; stations B2, B4, B6, B7, B9, B10, B12 and B15, which were most of discharge stations and some of control stations, formed a second group and stations B8, B11, B13 and B14, which are all discharge stations, was separated from the rest as the third group. The performance of a one-way ANOSIM test gave global R=0.312 at a significance level of 0.08%, so the separation of the three groups was confirmed. Consequently, the



MDS demonstrated that discharge stations separated into two groups segregated from control stations.

Figure 3.84. MDS ordination based on the abundance data (logarithmic transformed) of November sampling for the factor level [control (C) and discharge (D) stations].

The first group showed an average similarity of 73.97%. As identified by SIMPER analysis, three species, *Mytilaster lineatus*, *Nereis (Hediste) diversicolor* and Opheliidae (sp.) were responsible for 80%. The second group reached an average similarity of 46.72, with three species, *Mytilus galloprovincialis, Hyale perieri* and *Mytilaster lineatus* covering 80%. Finally, the third group reached an average similarity of 38.68%, with two species *Echinogammarus olivii* and *Mytilus galloprovincialis* being responsible for 80%. As regards the divergence between groups (Table 3.10), the first group had an average dissimilarity percentage of 73.40% with second group (8 species contributed 80%) and 90.81 with third group (7 species contributed 80%) while the dissimilarity percentage between groups (second and third) was 72.96% (five species contributed 80%).

Table 3.10. SIMPER analysis differences in average abundances or per centage cover per species contributing to dissimilarities between November samplings of control and discharge stations (1:control stations, 2:control+discharge stations, 3:discharge stations). A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	1-2	1-3	2-3
Mytilus galloprovincialis	3.24	40.78	30.71
Mytilaster lineatus	48.46	9.06	3.76
Nereis (Hediste) diversicolor	21.75	1.31	1.70
Hyale perieri	6.93	29.23	0.49
Jassa marmorata	0.14	7.84	3.51
Opheliidae (sp.)	7.00	0.25	0
Dynamene bidentatus	9.17	0.29	0.01
Platynereis dumerilii	4.84	2.43	1.02
Echinogammarus olivii	0.01	0.97	53.77

Two main groups are obtained by MDS in February. While first group embraced B13, B9 and B10, which are some of discharge stations, second group including the remaining stations (Figure 3.87). Stations B1, B3 and B5 in the second group compose of a subgroup. ANOSIM analysis (R= 0.268 at a significance level of 3%) confirmed significant differences between groups obtained from the plot. Although the MDS plot demonstrated a mixed group of control and discharge stations, the divergence between control and discharge is noteworthy.



Figure 3.85. MDS ordination based on the abundance data (logarithmic transformed) of February sampling for the factor level [control (C) and discharge (D) stations].

The first group showed an average similarity of 21.95%. As identified by SIMPER analysis, only two species were responsible for 80% of the similarity in the first group. The second group reached an average similarity of 50.40% with seven species covering of 80% of the similarity and finally, the third group (sub-group of the second group) reached an average similarity of 40.08% with four species being responsible 80% of similarity. As regards the divergence between groups (Table 3.11), the second group had an average dissimilarity percentage of 82.38% with third group, and 91.97% with the first group (seven species contributed 80%), while the dissimilarity percentage between the fist and the third group was 91.93 % (12 species contributed to 80%).

Table 3.11. SIMPER analysis differences in average abundances or per centage cover per species contributing to dissimilarities between February samplings of control and discharge stations (1: control stations, 2: sub-group of the control stations, 3: discharge stations). A cut-off of a cumulative % dissimilarity of 80% was applied.

Species	1-2	1-3	2-3
Mytilaster lineatus	33.31	11.20	32.05
Biancolina algicola	15.38	0	0
Nereis (Hediste) diversicolor	0.12	1.58	11.63
Stenothoe tergestina	8.24	8.20	3.83
Opheliidae (sp.)	1.96	0.74	3.85
Enchytraeus buchholzi	11.74	13.14	3.53
Platynereis dumerilii	0.06	2.78	3.05
Mytilus galloprovincialis	0.15	40.98	0.89
Echinogammarus olivii	0.04	14.64	0
Hyale perieri	0.68	12.31	0.68
Lumbricillus rivalis	13.31	0.52	0
Tanais dulongii	10.58	7.26	0.02
Fabriciinae (sp.)	6.33	0	0

Consequently, the main discriminator species with the highest contribution to the dissimilarity between control and discharge stations were *Mytilus galloprovincialis*, *Mytilaster lineatus*, *Nereis* (*Hediste*) *diversicolor*, *Platynereis dumerilii*, *Echinogammarus olivii*, *Hyale perieri*, *Hyale pontica*, *Tanais dulongii* and *Enchytraeus buchholzi*. Among them *M. lineatus*, *N. diversicolor*, *P. dumerilii* and *H. pontica* were more abundant in control stations, whilst *M. galloprovincialis*, *E. olivii*, *H. perieri*, *T. dulongii* and *E. buchholzi* were more characteristic for the discharge stations.

Contrary to the present study, *Nereis (Hediste) diversicolor* was reported as a characteristic species of polluted areas (Anger, 1975). This species found also on the edge of a grossly polluted area by Ghirardelli and Pignatti (1968) and in a polluted harbor area by Tulkki (1968). In addition according to Smyth et al. (1974) it was numerous and widely distributed on polluted shores. According to Sanders et al. (1972) and Grassle and Grassle (1974), *Platynereis dumerilii* is not an opportunistic species but a dominant secondary colonizer. It was, however, classified as first order opportunistic species by Simboura and Zenetos, 2002. These findings may clarify the high abundance of this species, characteristic to the control stations, in some samplings of discharge stations.

There are few available studies concerning the pollution tolerance of *Echinogammarus olivii* and *Tanais dulongii*, which were described as characteristics of discharge stations in the present study. *Tanais dulongii* appears to be tolerant to 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). *Echinogammarus olivii*, on the other hand, was classified sensitive or indifferent to disturbance by Simboura and Zenetos (2002). However, this species was one of the first order opportunistic species and a characteristic to the discharge stations in the present study.

Bellan- Santini (1981) proposed that the ratio of the abundance (or dominance) 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* reflects the degree of pollution (the value is higher under increased pollution), at least for the western Mediterranean Sea. *Jassa* species (*J. marmorata* and *J. ocia*) seem to be indifferent to disturbance, always present in low or moderate densities with non-significant variations with time, as they cannot be considered as tolerant by any degree of pollution in the present study. On the other hand, one of the *Hyale* species (*H. perieri*), which is one of the characteristic species of the discharge stations, appears to be tolerant to disturbance or stress whose populations respond to pollution by an increase of density. However, the other *Hyale* species (*H. pontica*) appears to be sensitive to disturbance and was found as a characteristic to the control stations.

High numbers of oligachaetes are generally considered indicator of very poor water quality (Brinkhurst and Jamieson, 1971). One of the two oligochaetes (*Enchytraeus buchholzi*) found in the present study in the present study.

4. CONCLUSIONS

The present study has provided the knowledge of pollution effects on benthic life in the upper infralittoral rocky habitats of the Bosphorus Strait, the identification of humaninduced stresses on hard substrate macrozoobenthic communities, assessment of ecosystem health and ecological quality status of the hard substrate biocoenoses and the initial base for biomonitoring.

The quantitative approach used in the present study not only confirmed the trends observed in descriptive work, but also enabled questions to be answered about differences in community structure between the control and discharge stations. Analyses of the data, collected from the study area, revealed clear differences between the control and discharge stations. The clear separation of two station groups provided strong evidence for the adverse effects of sewage on macrozoobenthic communities. The results from all univariate, graphical/distributional and multivariate analyses and faunistic composition suggested that benthic ecosystem was more or less damaged in discharge stations. The typical characteristics of the benthic communities exposed to pollutants such as the prevalence and high dominance of the opportunistic species, low number of species, low diversity and multi-metric benthic index scores and low total faunal abundance were encountered in most of these stations. On the contrary, it could be said that benthic communities was appeared to be healthier in control stations, characterized by the high number of species, high total faunal abundance, high diversity and multi-metric benthic index scores. It can be construed that the effects of pollution on these communities was quite low.

In conclusion, there is now almost adequate information about the effects of sewage discharges on shallow water hard substratum macrozoobenthic communities, although open questions. Now we have the initial baseline for further biomonitoring studies. It is evident that the further research is needed to understand in greater detail the potential environmental impacts of sewage discharges in the Bosphorus Strait.

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APPENDIX A

Hierarchic taxonomy of the identified species:

Phylum PLATYHELMINTHES Classis TURBELLARIA Ordo MACROSTOMIDA **Macrostomida (sp.)**

Ordo POLYCLADIDA Subordo ACOTYLEA Superfamilia PLANOCEROIDEA Familia LEPTOPLANIDAE *Notoplana* sp.

Phylum NEMERTEA Classis ANOPLA Ordo HETERONEMERTEA Familia LINEIDAE Lineidae (spp.)

Classis HOPLONEMERTEA Subordo MONOSTILIFERA Familia TETRASTEMMATIDAE *Tetrastemma* cf. coronatum (Quatrefages, 1846)

Familia EMPLECTONEMATIDAE *Emplectonema* cf. gracile (Johnston, 1837)

Phylum ANNELIDA Classis POLYCHAETA Subclassis PALPATA Ordo ACICULATA Subordo PHYLLODOCIDA Familia NEREIDIDAE *Namanereis* sp. *Nereis (Hediste) diversicolor* (O.F. Müller, 1776) *Perinereis cultrifera* (Grube, 1840) *Platynereis dumerilii* (Audouin & Milne-Edwards, 1833)

Familia PHYLLODOCIDAE

Eumida sp. *Eteone picta* Quatrefages, 1865 *Eulalia clavigera* (Audouin & Milne Edwards, 1834)

Familia SYLLIDAE

Grubeosyllis alvaradoi (San Martín, 1984) *Syllis amica* Quatrefages, 1865 *Syllis columbretensis* Campoy, 1982 *Syllis gracilis* Grube, 1840

Ordo CANALIPALPATA Subordo SABELLIDA Familia SABELLIDAE *Fabriciinae* (sp.)

Subordo SPIONIDA Familia SPIONIDAE *Polydora* cf. *cornuta* Bosc,1802 *Prionospio multibranchiata* Berkeley & Berkeley, 1927

Subordo TEREBELLIDA Familia TEREBELLIDAE *Polycirrus* sp.

Subclassis SCOLECIDA Ordo CAPITELLIDA Familia CAPITELLIDAE *Capitella capitata* (Fabricius, 1780)

> Familia MALDANIDAE *Euclymene oerstedi* (Claparède, 1863)

Ordo OPHELIIDA Familia OPHELIIDAE **Opheliidae (sp.)**

Classis CLITELLATA Subclassis OLIGOCHAETA Ordo HAPLOTAXIDA Subordo TUBIFICINA Familia ENCHYTRAEIDAE *Enchytraeus buchholzi* Vejdovsky, 1879 *Lumbricillus rivalis* (Levinsen, 1884)

Phylum ARTHROPODA Subphylum CHELICERATA Classis PYCNOGONIDA Ordo PANTOPODA Familia AMMOTHEIDAE *Tanystylum conirostre* (Dohrn, 1881)

Subphylum CRUSTACEA Classis MAXILLOPODA Subclassis THECOSTRACA Infraclassis CIRRIPEDIA Superordo THORACICA Ordo SESSILIA Subordo BALANOMORPHA Superfamilia BALANOIDEA Familia BALANIDAE Balanus improvisus Darwin, 1854

Classis MALACOSTRACA Subclassis EUMALACOSTRACA Superordo PERACARIDA Ordo AMPHIPODA Subordo GAMMARIDEA Familia AMPITHOIDAE *Ampithoe helleri* Karaman, 1975 *Ampithoe ramondi* Audouin, 1826

> Familia AORIDAE *Microdeutopus gryllotalpa* Costa, 1853

Familia BIANCOLINIDAE *Biancolina algicola* Della Valle, 1893

Familia COROPHIIDAE Monocorophium insidiosum Crawford, 1937

Familia DEXAMINIDAE *Dexamine spinosa* (Montagu, 1813)

Familia EUSIRIDAE Apherusa chiereghinii Giordani-Soika, 1950

Familia GAMMARELLIDAE *Gammarellus angulosus* (Rathke, 1843)

Familia GAMMARIDAE *Echinogammarus olivii* (Milne-Edwards, 1830)

Familia HYALIDAE *Hyale perieri* (Lucas, 1849) *Hyale pontica* Rathke, 1837

Parhyale cf. plumicornis (Heller, 1866)

Familia ISCHYROCERIDAE *Ericthonius brasiliensis* (Dana, 1855) *Ericthonius punctatus* (Bate, 1857) *Jassa marmorata* (Holmes, 1903) *Jassa ocia* (Bate, 1862)

Familia MELITIDAE *Melita palmata* (Montagu, 1804)

Familia STENOTHOIDAE Stenothoe tergestina Nebeski, 1881

Familia TALITRIDAE Orchestia stephenseni Cecchini, 1928

Subordo CAPRELLIDEA Infraordo CAPRELLIDA Superfamilia CAPRELLOIDEA Familia CAPRELLIDAE *Caprella acanthifera* Leach, 1814 *Caprella danilevskii* Czerniavski, 1868 *Caprella liparotensis* Haller, 1879 *Caprella rapax* Mayer, 1890

Ordo ISOPODA Subordo FLABELLIFERA Familia SPHAEROMATIDAE *Dynamene bidentatus* (Adams, 1800) *Lekanesphaera monodi* (Arcangeli, 1934) *Sphaeroma serratum* (Fabricius, 1787)

Subordo ASELLOTA Superfamilia JANIROIDEA Familia JANIRIDAE Jaera nordmanni (Rathke, 1837) Jaera sp.

Subordo VALVIFERA Familia IDOTEIDAE *Idotea balthica* (Pallas, 1772) *Idotea pelagica* Leach, 1815 *Idotea* sp. *Synisoma capito* (Rathke, 1837) Subordo ONISCIDEA Infraordo LIGIAMORPHA Sectio SYNOCHETA Superfamily TRICHONISCOIDEA Familia TRICHONISCIDAE *Trichoniscus* cf. *provisorius* Racovitza, 1908

Sectio CRINOCHETA Superfamilia ARMADILLOIDEA Familia ARMADILLIDIIDAE *Armadillidium* cf. album Dollfus, 1887

Ordo TANAIDACEA Subordo TANAIDOMORPHA Superfamilia TANAOIDEA Familia TANAIDAE *Tanais dulongii* (Audouin, 1826)

Superfamilia PARATANAOIDEA Familia LEPTOCHELIDAE *Leptochelia savignyi* (Kroyer, 1842)

Ordo CUMACEA Familia NANNASTACIDAE *Cumella pygmaea euxinica* Bacescu, 1950

Superordo EUCARIDA Ordo DECAPODA Subordo PLEOCYEMATA Infraordo ANOMURA Superfamilia GALATHEOIDEA Familia PORCELLANIDAE *Pisidia longimana* (Risso, 1816)

Infraordo BRACHYURA Sectio EUBRACHYURA Subsectio HETEROTREMATA Superfamilia XANTHOIDEA Familia MENIPPIDAE *Eriphia verrucosa* (Forskål, 1775)

> Familia PILUMNIDAE *Pilumnus hirtellus* (Linnaeus, 1761) Familia XANTHIDAE

Xantho poressa (Olivi, 1792)

Subsectio THORACOTREMATA Superfamilia GRAPSOIDEA Familia GRAPSIDAE *Pachygrapsus marmoratus* (Fabricius, 1787)

Phylum MOLLUSCA Subphylum PLACOPHORA Classis POLYPLACOPHORA Ordo NEOLORICATA Subordo ACANTHOCHITONINA Familia Acanthochitonidae *Acanthochitona fascicularis* (Linnaeus, 1767)

Subordo ISCHNOCHITONINA Familia ISCHNOCHITONIDAE *Lepidochitona cinerea* (Linnaeus, 1767)

Subphylum CONCHIFERA Classis GASTROPODA Subclassis PROSOBRANCHIA Ordo ARCHAEOGASTROPODA Superfamilia TROCHOIDEA Familia TROCHIDAE **Gibbula deversa** Milaschewitsch, 1916

Superfamilia TURBINOIDEA Familia PHASIANELLIDAE *Tricolia pullus pullus* (Linnaeus, 1758)

Ordo MESOGASTROPODA Superfamilia RISSOIDEA Familia RISSOIDAE *Pusillina inconspicua* (Alder, 1844) *Rissoa splendida* Eichwald, 1830 *Rissoa* cf. *variabilis* (Von Mühlfeldt, 1824) *Setia* sp.

Subclassis HETEROBRANCHIA Ordo HETEROSTROPHA Superfamilia PYRAMIDELLOIDEA Familia PYRAMIDELLIDAE Odostomia eulimoides Hanley, 1844

Subclassis PULMONATA

Ordo ARCHAEOPULMONATA Familia ELLOBIIDAE *Myosotella myosotis* (Draparnaud, 1801)

Classis BIVALVIA Subclassis PTERIOMORPHIA Superfamilia MYTILOIDA Familia MYTILIDAE *Mytilaster lineatus* (Gmelin, 1791) *Mytilus galloprovincialis* Lamarck, 1819

Phylum ECHINODERMATA Subphylum ASTEROZOA Clasis STELLEROIDEA Subclasis OPHIUROIDEA Ordo OPHIURIDA Subordo GNATHOPHIURINA Familia AMPHIURIDAE *Amphipolis squamata* (Delle Chiaje, 1828)