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## Benthic Hydrozoans as Potential Indicators of Water Masses and Anthropogenic Impact in the Sea of Marmara

NUR EDA TOPÇU<sup>1</sup>, LUIS FELIPE MARTELL<sup>1,2</sup>, IZZET NOYAN YILMAZ<sup>3</sup>  
and MELEK ISINIBILIR<sup>1</sup>

<sup>1</sup>Department of Marine Biology, Faculty of Aquatic Sciences, Istanbul University, Turkey

<sup>2</sup>University Museum of Bergen, Department of Natural History, University of Bergen, Norway

<sup>3</sup>Institute of Marine Sciences and Management, Istanbul University, Turkey

Corresponding author: [edatopcu@istanbul.edu.tr](mailto:edatopcu@istanbul.edu.tr)

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### Abstract

Changes in the abundance and distribution of marine benthic hydrozoan species are indicative of variations in environmental conditions in the marine realm. The comparative analysis of such assemblages can improve our understanding of environmental and ecological conditions in the Sea of Marmara, a strongly stratified and heavily populated inland sea connecting the Aegean and Black seas, on route of national and international maritime traffic. We compared the hydrozoan assemblages occurring in harbours with those developed at natural sites, as well as the assemblages associated with the Black Sea water mass *versus* the Mediterranean water mass in the vicinities of the Prince Islands, the north-easternmost section of the Sea of Marmara. Sampling took place at 12 stations, once in March 2015 and once in August 2015 in order to cover species with both warm and cold water affinities. Multivariate analyses showed that benthic hydroid assemblages with both affinities differed significantly between the heavily trafficked harbours of the Prince Islands (connected to the metropolis of Istanbul) and areas without human settlements and maritime traffic. In addition, highly distinct hydroid assemblages were found characterizing both areas with water of Mediterranean origin and areas with water from Black Sea origin. Based on our results, we discuss the potential for the use of these organisms as indicators of water masses and anthropogenic impact at the regional level.

**Keywords:** Hydroids; Prince Islands; harbours; Black Sea water; Mediterranean water; bioindicators.

### Introduction

Benthic hydrozoans are common components of bottom communities in tropical and temperate shallow waters, where they form distinct and often characteristic assemblages in different marine habitats (Boero, 1984). They have a high potential as monitor and indicator species of environmental conditions in the marine realm (Mergner, 1977), in part because of their ubiquity, but also due to other life-history traits such as the sessility of their colonies (which prevent migration due to changing conditions), their differential sensitivity to stress, and their rather rapid response to disturbances (Gili & Hughes, 1995). Morphological modifications in individual colonies of some benthic hydrozoans are indicative of turbulent conditions (e.g. da Silveira & Migotto 1991) and heavy metals pollution (Karbe, 1972; Theede *et al.*, 1979), while local changes in abundance and distribution of hydrozoan species have been linked to variations in intensity and speed of water flow (Riedl 1966). As a group, these organisms have been used as indicators of particular hydrodynamic conditions (Wedler, 1975; Mergner,

1972, 1977, 1987), as well as of climate change (Puce *et al.*, 2009; González-Duarte *et al.*, 2014), and anthropogenic impact in harbours (Megina *et al.*, 2013, 2016).

Despite their ecological importance and advocated potential as bioindicators, there are still only few studies focusing on hydrozoan assemblages from harbours *versus* assemblages on natural sites, and there is not enough research intended to evaluate the suitability of benthic hydrozoans as indicators of important oceanographic features such as water masses. In the Sea of Marmara in particular, research on hydrozoans has historically been focused on the production of species inventories (e.g. Ostroumoff, 1896; Demir, 1954), with recent studies reporting on the punctual presence of either pelagic (Isinibilir *et al.*, 2010; Isinibilir *et al.*, 2015a; Yılmaz *et al.*, 2017) or benthic (Albayrak & Balkıs, 2000; Marques *et al.*, 2000; Topçu *et al.*, 2016) taxa. As a result, sixty-two species of hydrozoans have been recorded so far in the Sea of Marmara (see Isinibilir & Yılmaz, 2016 for a check-list), although more can be expected as sampling effort increases in the region. The documented hydrozoan diversity in the neighbouring Aegean Sea was also not very high until

recently, especially along the Turkish coasts, but late reports highly increased the number of species, showing that the apparently low species richness was mainly due to limited research effort in the area (Isinibilir *et al.*, 2015b; Topçu *et al.*, 2017).

The Sea of Marmara offers a unique opportunity for the evaluation of the potential of benthic hydrozoans as indicators of environmental conditions. It is a semi-enclosed sea, connecting the Black Sea to the Aegean Sea via the Turkish straits system, and it is characterized by a two-layer stratification, with the brackish surface layer formed by the Black Sea water mass flowing into the Sea of Marmara through the Istanbul Strait (Bosphorus) and the more saline Mediterranean Sea water entering through the Çanakkale Strait (Dardanelles) and flowing approximately 20 m below the brackish layer (Beşiktepe *et al.*, 1994). The Turkish straits system serves as an ecological barrier, a biological corridor and an acclimatization zone for the biota of the Mediterranean and the Black Sea (Öztürk & Öztürk, 1996). These characteristics make the Sea of Marmara particularly interesting for the study of benthic hydrozoans in relation to clearly defined water masses. Within the Sea of Marmara, the Prince Islands (located at the north-easternmost sector) constitute a good example of a “natural laboratory” for the comparative study of benthic invertebrate communities, since they include both heavily trafficked small harbours and natural areas with high habitat heterogeneity and diversity of faunistic (e.g. octocoral) assemblages (Topçu & Öztürk, 2015), while at the same time they offer the opportunity to observe the typical change of the Marmara vertical profile from Black Sea habitats to Mediterranean ones, including the coralligenous. In this sense, the different anthropogenic pressure at the north and south of the islands allows for an interesting comparison between the fauna associated with harbours *versus* natural sites, while the effect of the water masses on the abundance and distribution of the species can be easily evaluated at the southern part of the archipelago. The aim of this study was therefore to investigate the assemblages of benthic hydrozoans associated with the typical water masses of the Sea of Marmara (Black Sea water mass *vs* Mediterranean water mass), and evaluate the specific characteristics of benthic hydroid assemblages developing in harbours in comparison with those developing in well-preserved, adjacent natural areas.

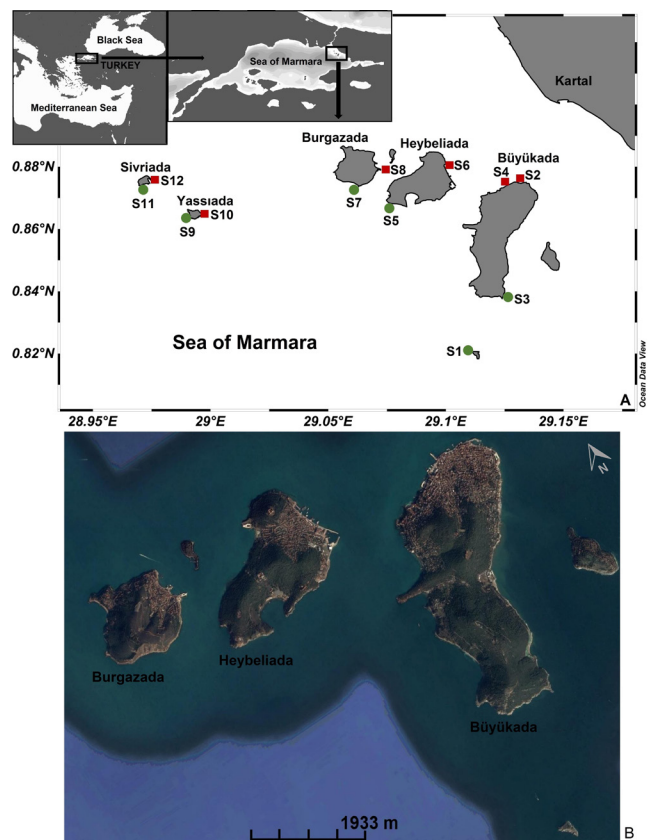
## Material and Methods

### Study Area and Sampling Design

The circulation in the Sea of Marmara is characterized by a strong two-layer stratification, with a Black Sea originated brackish upper layer flowing southwards and a lower Mediterranean layer flowing northwards. The two layers are separated by a permanent halocline, located at 15-30 m depth depending on the season and location (Beşiktepe *et al.*, 1994). In the lower layer, the salinity

is around 38.5, while the temperature is around 14-15°C throughout the year. In the upper layer, the salinity is around 20 at the north of the Sea of Marmara and the temperature varies between 8 and 24°C depending on the season.

The specific sampling area, the Prince Islands, are located in the northeast Sea of Marmara, very close to the highly populated coasts of Istanbul, between Kadıköy and Kartal (Fig. 1). The northern coasts of the larger islands (facing Istanbul) are urbanized and have several harbours with different purposes, while the southern coasts are mainly uninhabited and pristine. The two north-easternmost sampled islands, Yassiada and Sivriada, are small islands with harbours and some buildings used in past times, but they are currently uninhabited. The depth at the northern coasts of the Prince Islands is generally around 20 m and do not descend below 50 m in the area between Istanbul and the archipelago; therefore the Mediterranean water layer (below 20-30 m) is almost never reached in this part. The southern coasts are characterized by a descending profile that rapidly descends to 40-50 m. All sampling stations were characterized by a



**Fig. 1:** (A) Location of Prince Islands and sampling sites in the Sea of Marmara. Squares represent harbours and circles represent natural coasts. (B) Google Earth satellite picture of Büyükada, Heybeliada and Burgazada, showing the urbanization at the northern coasts and locations of the harbours, in contrast to the pristine southern coasts.

substrate composed of a mixture of rocks, sand and bioconcretions of dead shells/polychaete tubes/pebbles. S9 and S11 comprised dense facies of the yellow gorgonian *Eunicella cavolini*.

The sampling was designed to compare benthic hydrozoan assemblages:

(1) in the upper layers of natural coasts (southern coasts) versus the harbours (northern coasts) of the Prince Islands,

(2) in the upper layer (Black Sea waters) versus the lower layer (Mediterranean waters) of (southern) natural coasts.

Six sites at the north (harbours) and six sites at the south of the islands were thus selected for sampling, with the sites at the south sampled at two depth layers. Upper layer samplings were performed at 1 – 6 m depth while lower layer samplings were performed at 30 – 35 m depth. The sampling took place once in March 2015 and once in August 2015 in order to cover species with both warm and cold water affinities. In each station, benthic hydroid colonies from the bottom and fragments of diverse substrates supporting or susceptible to support hydrozoan colonies (e. g. algae, invertebrates, etc.) were collected by two trained SCUBA divers using visual collection techniques (Boero & Fresi, 1986; Megina *et al.*, 2013). One of the divers picked up all hydroid colonies or their potential substrates from two randomly placed transects of 6 m<sup>2</sup>. The other diver conducted a visually-oriented collection in order to obtain an efficient representation of hydroid species diversity in shallow coastal benthic habitats (Piraino *et al.*, 2013), selectively collecting hydroid colonies or their potential substrates from a single, larger homogeneous bottom belt (5m x 10 m length = 50 m<sup>2</sup>) at the targeted depth zone.

Samples were fixed immediately after collection in a 10% formalin-seawater solution. All hydrozoans were sorted and identified in the laboratory to the lowest taxonomic level with the aid of specialized scientific literature (Bouillon *et al.*, 2004, 2006). The identity of the taxa not previously recorded for the area was confirmed through a comparison with specimens included in the Hydrozoa Collection, University of Salento, Italy (accession numbers for relevant species as in Gravili *et al.*, 2015) and the University Museum of Bergen, Norway (accession numbers ZMBN 10781, 10788, 10789, 13283, and 13597). For most of the species, hydroid abundance was calculated by counting the number of polyps in each site (see Megina *et al.*, 2013, González-Duarte *et al.*, 2014); however, for large colonies ( $\geq 10$  cm high) of *Obelia dichotoma*, *Kirchenpaueria pinnata* and *Bougainvillia muscus* the number of polyps was estimated by averaging the total number of polyps in 15 randomly selected colonies and multiplying this number by the total number of colonies observed in the station (< 1% of the entries in the abundance matrix).

## Statistical Analysis

Species richness, total hydroid abundance and diversity (as measured by Simpson's index 1-D) between the two layers and the two levels of anthropogenic impact (harbours vs natural sites) for both warm-water and cold-water assemblages were compared by non-parametric Kolmogorov-Smirnov tests performed with GraphPad Prism version 6.01 for Windows.

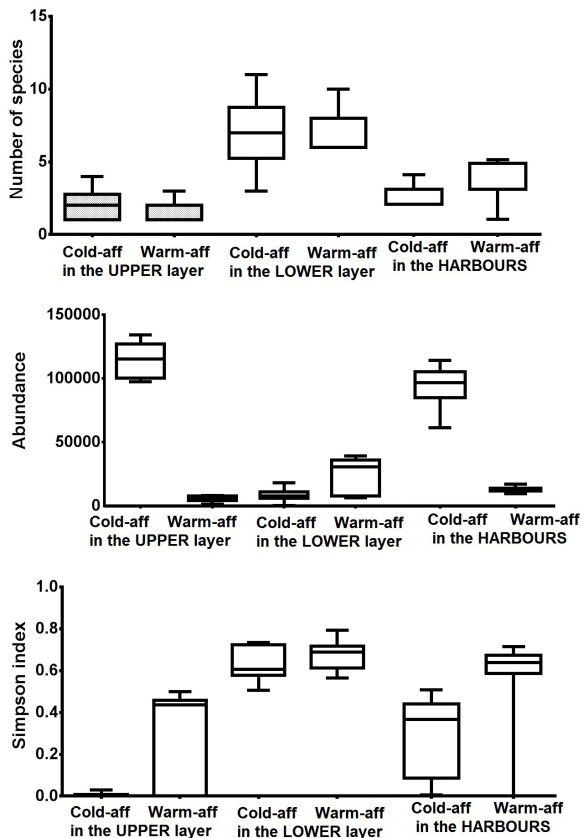
To test for differences in assemblage composition, sample by species matrices were calculated for abundances of species at all sites. The abundance matrices were square-root transformed and the samples were compared on the basis of species abundances using the Bray-Curtis similarity index. Non-Metric multidimensional scaling (nMDS) analyses were performed to visualize changes in the assemblages. Differences in the hydrozoan assemblages according to the tested factors were identified using permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001); while a similarity percentage analysis (SIMPER) was performed to determine the contribution of each taxon to the observed similarities and dissimilarities between groups. Based on the above-mentioned sampling design that contemplates the differences between the northern and southern coasts of the islands, two different experimental designs were employed in the analysis, each including two factors: Design 1) factors "anthropogenic impact" (fixed, two levels: not impacted [southern coasts – upper layer] and harbours [northern coasts – upper layer]) and "affinities" (fixed, two levels: cold and warm); and Design 2) factors "water mass" (fixed, 2 levels: upper layer of southern -not impacted- coasts [Black Sea water] and lower layer [Mediterranean water]) and "affinities" (fixed, two levels: cold and warm). All the above analyses and diversity index computations were carried out with the Primer v6 software (Clarke & Gorley, 2006).

## Results

Forty-one hydrozoan taxa were found along the coasts of Prince Islands (Table 1). To our knowledge, 17 represent first records for the Sea of Marmara, with 7 of them being also first records for the Turkish coastal area. In the case of *Eudendrium* sp., *Halecium* sp. 1 and sp. 2, Campanulinidae sp., *Campanulina* sp., *Cuspidella* sp., Corynidae sp. 1 and sp. 2, and Anthoathecata sp., identification to species level was impossible, either because of the small amount of material available or because of the lack of characters essential for identification. Species richness of cold-affinity hydrozoans was not significantly different from that of warm-affinity hydrozoans, neither between the studied water masses nor in the unimpacted sites or the harbours (Fig. 2). Conversely, the abundance of hydrozoans in both cold and warm-affinity assemblages were significantly different from each other in all cases

**Table 1.** Species found at Prince Islands coasts according to water masses (the upper layer (Black Sea waters) *versus* the lower layer (Mediterranean waters)), sites (natural *versus* impacted (harbours) and sampling time (March [M] or August [A]). First records for the Sea of Marmara are in bold. Species recorded for the first time in Turkey are marked with \*.

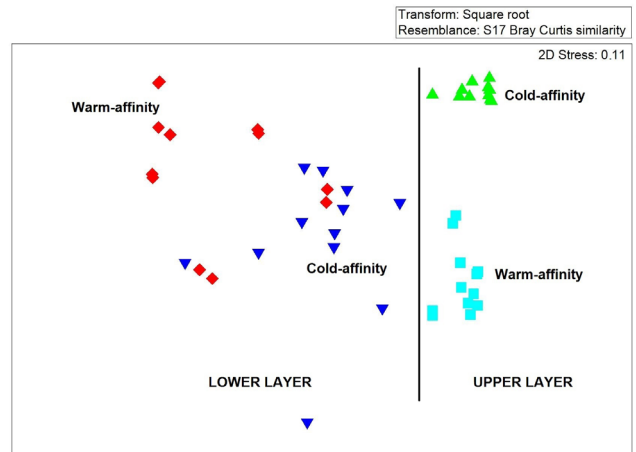
Species	Upper Layer			Sampling time
	Natural	Impacted	Lower layer	
<i>Aglaophenia octodonta</i> Heller, 1868				M-A
<b><i>Amphinema dinema</i></b> (Péron & Lesueur, 1810)*				M-A
<i>Antennella secundaria</i> (Gmelin, 1791)				M-A
<i>Anthoatecata</i> sp.				A
<i>Bougainvillia muscus</i> (Allman, 1863)				M-A
<b><i>Campanularia hincksii</i></b> Allder, 1856				M
<i>Campanulina</i> sp.				A
<i>Campanulinidae</i> sp.				M
<b><i>Clytia viridicans</i></b> (Leuckart, 1956) *				M
<i>Clytia gracilis</i> (Sars, 1850)				M
<i>Clytia hemisphaerica</i> (Linnaeus, 1767)				M-A
<b><i>Clytia linearis</i></b> (Thorneley, 1900)				M-A
<b><i>Clytia paulensis</i></b> (Vanhöffen, 1910)				M-A
<i>Coryne eximia</i> Allman, 1859				M
<i>Corynidae</i> sp.1				M
<i>Corynidae</i> sp.2				A
<i>Cuspidella</i> sp.				A
<i>Ectopleura larynx</i> (Ellis & Solander, 1786)				M
<b><i>Eudendrium armatum</i></b> Tichomiroff, 1890				M
<b><i>Eudendrium glomeratum</i></b> Picard, 1952				M
<b><i>Eudendrium racemosum</i></b> (Cavolini, 1785)				M
<i>Eudendrium</i> sp.				A
<i>Filellum serpens</i> (Hassall, 1848)				M-A
<i>Halecium beanii</i> (Johnston, 1838)				M-A
<i>Halecium</i> sp.1				M
<i>Halecium</i> sp.2				A
<b><i>Halecium tenellum</i></b> Hincks, 1861				M
<i>Kirchenpaueria halecioides</i> (Alder, 1859)				M
<b><i>Kirchenpaueria pinnata</i></b> (Linnaeus, 1758)				M-A
<b><i>Lafoeina tenuis</i></b> Sars, 1874				M
<i>Laomedea calceolifera</i> (Hincks, 1871)				M-A
<i>Laomedea flexuosa</i> Alder, 1857				A
<i>Nemertesia tetrasticha</i> Meneghini, 1845				A
<i>Obelia dichotoma</i> (Linnaeus, 1758)				M-A
<b><i>Plumularia setacea</i></b> (Linnaeus, 1758)				A
<b><i>Podocoryna exigua</i></b> (Haeckel, 1879)*				A
<b><i>Proboscidactyla ornata</i></b> (McCrary, 1859) *				M
<b><i>Racemoramus panicula</i></b> (G. O. Sars, 1874) *				M
<i>Sertularella polyzonias</i> (Linnaeus, 1758)				M-A
<b><i>Stylactis inermis</i></b> Allman, 1872*				M
<b><i>Zanclaea giancarloii</i></b> Boero, Bouillon & Gravili, 2000*				M



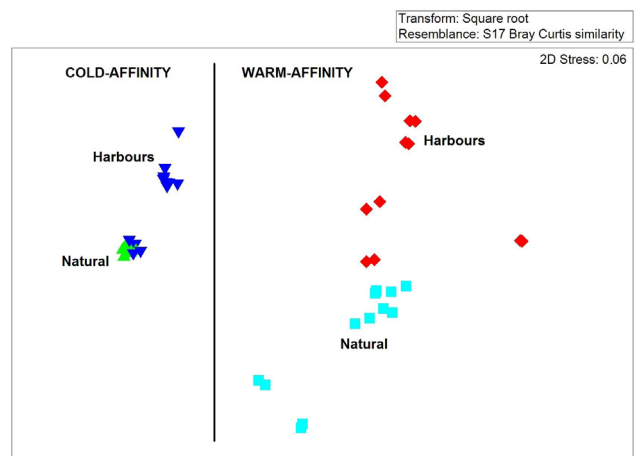
**Fig. 2:** Box and whiskers plot of species richness, abundances and Simpson indices; of cold- and warm-affinity hydrozoan assemblages in the lower layer, and in the upper layer of natural coasts and harbours.

(in the lower layer *versus* upper layer, and in the unimpacted sites *versus* harbours, respectively), cold-affinity hydrozoans being generally more abundant. Diversity, species richness and abundance of hydrozoans in the upper and lower layers were significantly different from each other, with Simpson diversity indices being much higher in the lower layer. Species richness, abundance and diversity were also significantly different between the unimpacted sites and the studied harbours, except for species richness in the cold-affinity assemblage.

The PERMANOVA comparisons revealed that the assemblages in the upper and lower layers were significantly different from each other, with the cold-water assemblages being also significantly different from the warm-water ones (Table 2; Table S1). In addition, the nMDS plot showed the groupings of upper and lower layer samples with a fair goodness of fit (Fig. 3). The SIMPER analyses suggest that the average similarity in the group of “upper layer” is rather high (80 %) and the main species responsible for this similarity is *Obelia dichotoma* with a contribution of 87 % (Table 3). The group of “lower layer” revealed only a fair similarity (44 %), due mainly to *Bougainvillia muscus* that had a relatively high



**Fig. 3:** MDS of the samples based on the cold/warm affinity hydroid species composition of the upper and lower layers.



**Fig. 4:** MDS of the samples based on the cold/warm affinity hydroid species composition of natural coasts and harbours.

abundance at some sites. The two groups of seawater layers had strong dissimilarity (84%).

The hydrozoan assemblages in the natural, not impacted sites were significantly different from the assemblages developed in the harbours (PERMANOVA comparisons, Table 2). In turn, the corresponding nMDS plot reflects with a well goodness of fit the distinction between cold and warm assemblages, but that of localities was less evident (Fig. 4). The “harbours” were grouped together with a similarity of 67% according to the SIMPER analysis, mainly due to *Obelia dichotoma*, which typically occurs in the harbours (Table 4). The two tested groups had a dissimilarity of only 46%. The group of cold-affinity hydrozoan assemblages had a strong similarity (85%) regardless of the impact of each site, due mainly to *Obelia dichotoma* which contributed 91 % of the similarity alone. The group of “warm-affinity” hydrozoan assemblages had a less strong similarity (62%), associated to

**Table 2.** Results of the PERMANOVA testing for the effects of (A) the seawater layer (*upper [Black Sea originated] and lower [Mediterranean originated]*) and affinity (*cold and warm*) and (B) anthropogenic impact (*not impacted versus harbours*) and affinity (*cold and warm*) on the hydrozoan assemblages.

PERMANOVA		(A)			
Source	df	SS	MS	Pseudo-F	P(perm)
Layer	1	38307	38307	35.769	0.0001
Affinity	1	18404	18404	17.185	0.0001
Layer x Affinity	1	24871	24871	23.223	0.0001
Res	44	47121	1070.9		
Total	47	1.287E5			
Pairwise comparisons					
Within level 'Upper' of factor 'Layer'			Within level 'Lower' of factor 'Layer'		
Groups	t	P(perm)	Groups	t	P(perm)
Cold, Warm	9.2267	0.0001	Cold, Warm	2.5088	0.0001
Within level 'Cold' of factor 'Affinity'			Within level 'Warm' of factor 'Affinity'		
Groups	t	P(perm)	Groups	t	P(perm)
Upper, Lower	5.6639	0.0001	Upper, Lower	5.2539	0.0001
PERMANOVA		(B)			
Source	df	SS	MS	Pseudo-F	P(perm)
Impact	1	16407	16407	30.336	0.0001
Affinity	1	61229	61229	113.21	0.0001
Impact x Affinity	1	4608.4	4608.4	8.5208	0.0001
Res	44	23797	540.84		
Total	47	1.0604E5			
Pairwise comparisons					
Within level 'Cold' of factor 'Affinity'			Within level 'Warm' of factor 'Affinity'		
Groups	t	P(perm)	Groups	t	P(perm)
Natural, Harbour	4.2036	0.0004	Natural, Harbour	4.4536	0.0001
Within level 'Natural' of factor 'Impact'			Within level 'Harbour' of factor 'Impact'		
Groups	t	P(perm)	Groups	t	P(perm)
Cold, Warm	9.2267	0.0001	Cold, Warm	6.9163	0.0001

*Kirchenpaueria pinnata*, the abundance of which highly increased in the warm period. The two groups of affinities had a dissimilarity of 80 % due to the change in the dominant species.

## Discussion

The scarcity of records of hydrozoan species in the Sea of Marmara contrasts sharply with the general view of the Mediterranean Sea as a well-studied region in terms of its hydrozoan diversity (Bouillon *et al.*, 2004). Both the number of species documented and the number of studies focused on hydrozoans are lower in the Sea of Marmara than in comparable regions in the western and central

Mediterranean Sea (Isinibilir & Yılmaz, 2016; Gravili *et al.*, 2013), and the lack of research effort in the area is evidenced by the fact that almost every study dealing with hydrozoans in the area includes at least one new record or documents the extension of the distribution range of a species (*confer* Isinibilir & Yılmaz, 2016 and references therein). Our survey, despite the relatively small area covered, represents an increase higher than 20% in the number of hydrozoan species reported for the Sea of Marmara, and greatly elevates the number of recorded species in the area. Not surprisingly, most of the new records observed in our study fill up distributional gaps for well-known, typical Mediterranean species such as *Campanularia hincksii*, *Clytia linearis*, *Clytia paulensis*, *Lafoeina*

**Table 3.** Results of the SIMPER analyses showing similarities within groups of layer (upper and lower) across affinities (warm and cold) and similarities within groups of affinities across all layer groups; dissimilarities between pairs of groups and the species mostly responsible for the observed differences.

<b>Group Upper: Average similarity: 79.77</b>					<b>Group Cold: Average similarity: 67.70</b>				
Species	Av.Sim	Sim/SD	Contrib%	Cum.%	Species	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Obelia dichotoma</i>	69.39	2.83	86.99	86.99	<i>Obelia dichotoma</i>	54.5	1.43	80.51	80.51
<i>Kirchenpaueria pinnata</i>	9.99	0.51	12.53	99.52	<i>Bougainvillia muscus</i>	9.77	0.82	14.43	94.94
<b>Group Lower: Average similarity: 44.05</b>					<b>Group Warm: Average similarity: 56.12</b>				
Species	Av.Sim	Sim/SD	Contrib%	Cum.%	Species	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Bougainvillia muscus</i>	16.7	2.06	37.92	37.92	<i>Obelia dichotoma</i>	24.91	1.02	44.38	44.38
<i>Obelia dichotoma</i>	10.02	1	22.75	60.67	<i>Kirchenpaueria pinnata</i>	10.01	0.51	17.84	62.22
<i>Nemertesia tetrasticha</i>	5.84	0.51	13.26	73.93	<i>Bougainvillia muscus</i>	7.23	0.94	12.89	75.11
<i>Sertularella polyzonias</i>	3.54	0.52	8.03	81.96	<i>Nemertesia tetrasticha</i>	5.93	0.52	10.56	85.67
<i>Halecium beanii</i>	2.54	0.35	5.77	87.73	<i>Sertularella polyzonias</i>	3.19	0.47	5.68	91.35
<i>Filellum serpens</i>	1.74	0.59	3.94	91.68					
<b>Groups Upper &amp; Lower: Average dissimilarity = 84.33</b>					<b>Groups Cold &amp; Warm: Average dissimilarity = 73.59</b>				
Species	Av.Diss	Diss/SD	Contrib%	Cum.%	Species	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>Obelia dichotoma</i>	32.2	1.28	38.18	38.18	<i>Obelia dichotoma</i>	35.65	1.22	48.44	48.44
<i>Bougainvillia muscus</i>	10.87	2.12	12.89	51.07	<i>Nemertesia tetrasticha</i>	7.03	0.73	9.56	58
<i>Nemertesia tetrasticha</i>	7.84	0.7	9.3	60.37	<i>Kirchenpaueria pinnata</i>	4.92	0.79	6.68	64.68
<i>Halecium beanii</i>	5.92	0.66	7.02	67.39	<i>Sertularella polyzonias</i>	4.2	0.68	5.7	70.38
<i>Sertularella polyzonias</i>	5.69	0.77	6.74	74.13	<i>Halecium beanii</i>	3.96	0.56	5.38	75.76
<i>Kirchenpaueria pinnata</i>	5.29	0.74	6.27	80.4	<i>Bougainvillia muscus</i>	3.61	0.91	4.91	80.67
<i>Antennella secundaria</i>	2.49	0.5	2.95	83.35	<i>Antennella secundaria</i>	1.96	0.46	2.67	83.34
<i>Filellum serpens</i>	2.42	0.82	2.87	86.22	<i>Anthoatecata sp.</i>	1.87	0.3	2.54	85.88
<i>Anthoatecata sp.</i>	2.2	0.3	2.61	88.83	<i>Filellum serpens</i>	1.62	0.7	2.21	88.09
<i>Campanulina sp.</i>	1.58	0.63	1.87	90.7	<i>Campanulina sp.</i>	1.29	0.63	1.75	89.83
					<i>Eudendrium glomeratum</i>	0.83	0.38	1.13	90.96

*tenuis*, *Halecium tenellum*, *Podocoryna exigua*, *Kirchenpaueria pinnata*, *Plumularia setacea*, and the large species of genus *Eudendrium*; all taxa already documented in the neighbouring Aegean and/or Black seas (Marinopoulos, 1979; Çınar *et al.*, 2014; Isinibilir *et al.*, 2015b); while other records (e.g. *Clytia viridicans*, *Stylactis inermis*, *Amphinema dinema*, *Zanclaea giancarloii*) constitute range extensions for species previously collected somewhere else in the eastern Mediterranean (Adriatic, Ionian, or Levantine coasts) but not reported hitherto from the Aegean or the Black seas.

Conditions similar to those of higher depths in the Mediterranean Sea can be found in shallower waters in the Sea of Marmara due to a combination of regional oceanographic features (high turbidity preventing daylight at very shallow depths, temperatures below 20 m

lower than temperatures at similar depths elsewhere in the Mediterranean) (Çoban-Yıldız *et al.*, 2000), and this has been advocated as one of the main explanations for the presence of deep-water cnidarians species at shallow depths in the area (Topçu & Öztürk, 2015). The same phenomenon may be involved in the maintenance of populations of *Racemoramus panicula* and *Proboscoidactyla ornata* around the Prince Islands. To our knowledge, the only previous record of polyps of *P. ornata* in the Mediterranean Sea is that of Brinckmann & Vannucci (1965) who found it in high numbers in depths of ca. 100 m in the Bay of Naples, but we found several polyps growing on tubes of *Branchiomma sp.* off Burgazada at shallower depths (around 30 m). The unexpected finding of colonies of *R. panicula* at 30 m off Büyükada and Sivriada, on the other hand, contrasts with the typically deep distribution



**Table 4.** Results of the SIMPER analyses showing similarities within groups of anthropogenic impact (natural coasts and harbours) across affinities (warm and cold) and similarities within groups of affinities across all anthropogenic impact; dissimilarities between pairs of groups and the species mostly responsible for the observed differences.

<b>Group Natural: Average similarity: 79.77</b>					<b>Group Cold: Average similarity: 84.59</b>					
Species	Av.Sim	Sim/SD	Contrib%	Cum.%	Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
<i>Obelia dichotoma</i>	69.39	2.83	86.99	86.99	<i>Obelia dichotoma</i>	299.85	76.94	4.57	90.96	90.96
<i>Kirchenpaueria pinnata</i>	9.99	0.51	12.53	99.52	<b>Group Warm: Average similarity: 62.50</b>					
<b>Group Harbour: Average similarity: 67.32</b>					Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Species	Av.Sim	Sim/SD	Contrib%	Cum.%	<i>Kirchenpaueria pinnata</i>	59.62	28.23	1.4	45.17	45.17
<i>Obelia dichotoma</i>	32.1	1.02	47.69	47.69	<i>Obelia dichotoma</i>	33.75	24.55	0.99	39.28	84.45
<i>Kirchenpaueria pinnata</i>	18.24	0.92	27.09	74.77	<i>Aglaophenia octodonta</i>	23.81	7.8	0.52	12.49	96.94
<i>Aglaophenia octodonta</i>	14.82	0.85	22.01	96.78	<b>Groups Cold &amp; Warm: Average dissimilarity = 79.75</b>					
<b>Groups Natural &amp; Harbour: Average dissimilarity = 45.60</b>					Species	Av.Diss	Diss/SD	Contrib%	Cum.%	
Species	Av.Diss	Diss/SD	Contrib%	Cum.%	<i>Obelia dichotoma</i>	54.06	3.98	67.78	67.78	
<i>Aglaophenia octodonta</i>	15.57	1.33	34.14	34.14	<i>Kirchenpaueria pinnata</i>	11.39	1.75	14.29	82.07	
<i>Obelia dichotoma</i>	12.23	1.61	26.83	60.97	<i>Aglaophenia octodonta</i>	7.82	0.76	9.8	91.87	
<i>Kirchenpaueria pinnata</i>	8.69	0.58	19.06	80.03						
<i>Bougainvillia muscus</i>	3.54	0.66	7.76	87.79						
<i>Laomedea flexuosa</i>	2.25	0.4	4.93	92.72						

of this species in temperate regions (70-5200 m, but mostly below 500 m) (Schuchert *et al.*, 2017).

The studied hydrozoan fauna reflected exceptionally well the double-layered structure of the Sea of Marmara, with highly distinctive assemblages, characteristic of the Black Sea and the Mediterranean Sea water masses. Low diversity and species richness, combined with high abundances were consistently observed in the upper layer, effectively signalling the water masses originating from the Black Sea and flowing into the Sea of Marmara through the Istanbul Strait. In turn, high diversity and species richness together with relatively low abundances were characteristic of the lower layer, indicating water masses originating from the Aegean Sea and flowing into the Sea of Marmara through the Çanakkale Strait. The effects of the strong stratification of the Sea of Marmara have been previously documented in such diverse taxa as diplomonads (Lara *et al.*, 2009), foraminiferans (Kirci-Elmas, 2013), and octocorals (Topçu & Öztürk, 2015), but direct observations regarding benthic hydrozoans have not hitherto been performed. Variations in abundance and

distribution of *Obelia dichotoma* corresponded particularly well with the studied water masses, so the species became a useful aid in the identification of the influence of Black Sea or Mediterranean waters in each particular site. Colonies of *Obelia* spp. are common throughout the entire Mediterranean Sea (Bouillon *et al.*, 2004), but they reach remarkably high abundances in the Bosphorus strait and the Black Sea (e. g. Wijsman *et al.*, 1999; Albayrak & Balkis, 2000; Revkov *et al.*, 2002), which is congruent with the observed pattern of high numbers of dominant, large colonies of *Obelia dichotoma* in the upper layer of the Sea of Marmara (Black Sea water masses) and scarcer, smaller colonies of the same species in the lower layer (Mediterranean water masses).

The differences between impacted and natural sites were also clearly indicated by the composition of the hydrozoan assemblages in the area. The harbour assemblages were characterized by the dominance of few large species (*Obelia dichotoma*, *Kirchenpaueria pinnata*, *Bougainvillia muscus* and *Aglaophenia octodonta*). Again, the hydroid *Obelia dichotoma* was in large part

responsible for the differences observed between natural and impacted (harbour) sites. This is a common and abundant species often encountered in disturbed sites and fouling communities in harbours (Megina *et al.*, 2013), where it predominates over other species (Standing, 1976; Witt *et al.*, 2004). While the colonies of *O. dichotoma* may also be common in natural sites, their abundance and frequency tends to be higher in harbours and marinas (Megina *et al.*, 2013), similarly to the observed pattern for this species and *Bougainvillia muscus* in the Sea of Marmara. According to Megina *et al.* (2013), for example, *B. muscus* was a characteristic species in West Mediterranean harbours, besides being completely absent from comparable natural sites, but in the present study colonies of this species were observed in both impacted and natural sites although with different abundance. Populations of benthic hydrozoans from harbours and natural areas, although belonging to the same species, could however represent different genetic pools adapted to different environmental conditions, as has been shown for other benthic invertebrates in the Mediterranean (Turon *et al.*, 2003), so the observed differences between natural and impacted sites based on hydrozoan assemblages could actually be larger than previously thought.

Seasonality was also a major factor determining the specific composition and abundance of the observed hydrozoan assemblages in both layers and natural and impacted sites. Comparatively higher competition for space between hydrozoans and algae (*Ulva* spp. in particular) was expected during the warm season in the upper layer stations, when the algae proliferate tremendously at shallow sites resulting in lower abundances of benthic hydroids. For the lower layer in the Sea of Marmara, however, we were not expecting to find a large influence of seasonality in the studied assemblages because the temperature there is almost constant throughout the year (Beşiktepe *et al.*, 1994). However, cold and warm affinity hydrozoan assemblages in the lower layer were significantly different. That was probably a biased result, due to the excessive increases of *O. dichotoma* and *B. muscus* following an unexpected event of mass mortality that impacted the Prince Islands coasts (Topçu *et al.*, 2016).

In general, considering both the upper and lower layers and the different natural / harbour habitats sampled, the hydroid assemblages constituted useful indicators of environmental conditions in the study area. The multivariate structure of the assemblages diverged significantly and the differences seem to be more influenced by the quantitative (abundance) and not the qualitative (specific) composition, with few species being exclusive of one habitat or the other. The benthic assemblages developing in harbours and natural areas, as well as those characteristic of the upper and lower layers in the Sea of Marmara, exhibit some particular and interesting characteristics that would make them a good bioindicator group, helping to reduce the effort needed for the comparison of different types of habitats and providing a useful tool for both scientists and policy-makers.

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## Appendix 1

Results of the Kolmogorov-Smirnov (K-S) test. S: species richness; N: abundances; 1-D: Simpson index; ns: no-significant.

Hydrozoan assemblages		DI	K-S "D"	p value	P value summary
Cold water vs warm water	in the upper layer (natural coasts)	S	0,08333	> 0,9999	ns
		N	1	< 0,0001	****
		1-D	0,6667	0,0097	**
	in the lower layer	S	0,25	0,8475	ns
		N	0,6667	0,0097	**
		1-D	0,4167	0,2485	ns
Upper layer vs lower layer	in the harbours	S	0,5	0,0996	ns
		N	1	< 0,0001	****
		1-D	0,8333	0,0005	***
	cold-affinity	S	0,8333	0,0005	***
		N	1	< 0,0001	****
		1-D	1	< 0,0001	****
Natural coasts vs harbours	warm-affinity	S	1	< 0,0001	****
		N	0,75	0,0023	**
		1-D	1	< 0,0001	****
	cold-affinity	S	0,4167	0,2485	ns
		N	0,6667	0,0097	**
		1-D	0,8333	0,0005	***
warm-affinity	S	0,6667	0,0097	**	
	N	1	< 0,0001	****	
		1-D	0,8333	0,0005	***