MONITORING THE MARINE ENVIRONMENT OF THERMAIKOS GULF

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Abstract
In this study, the quality of the marine environment of Thermaikos Gulf was appraised by measuring physical, chemical and biological parameters of the water column and the seabed. Water and sediment samples were seasonally collected from three sampling stations located at the inner part of Thermaikos Gulf. Specific physical-chemical characteristics (temperature, salinity, density along with pH and dissolved oxygen) throughout the water column were evaluated by conducting in situ measurements during the sampling campaigns. In situ processing of the water density data enabled the determination of the water column stratification. Afterwards, water samples were collected from the different strata: surface, pycnocline and bottom, to assess relevant variations of the chemical and the biological characteristics of the water masses. The studied chemical parameters included ammonium nitrogen, nitrites, nitrates, phosphates and total phosphorus and the biological ones phytoplankton and protozooplankton species composition, abundance and biomass. Sediment samples were collected with a standard VanVeen grab from each sampling station. Benthic organisms (macro-invertebrates) were sorted, enumerated under major taxa, and identified up to species levels to assess ecological quality status applying the BENTIX biotic index. Sediment composition and organic content were also assessed. The obtained results are discussed with regards to seasonal and spatial variability and water column stratification.

Keywords: Thermaikos Gulf, monitoring, nutrients, phytoplankton, protozooplankton, benthic organisms

1. INTRODUCTION

The European Union Marine Strategy Framework Directive (MSFD) 2008/56/EC establishes an integrated framework for the achievement or maintenance of the good environmental status in the marine systems by the year 2020 the latest. This Directive stipulates detailed procedures for its implementation including the development of marine strategies by Member States, in order to protect and preserve the marine environment. The program of measures shall take into account relevant measures required under the European legislation, in particular the Water Framework Directive (WFD) 2000/60/EC, the Council Directive 91/271/EEC concerning urban waste-water treatment and the Bathing Water Directive (BWD) 2006/7/EC of the European Parliament and of the Council concerning the management of bathing water quality (MSFD, 2008/56/EC). The BWD (Directive 2006/7/EC) aims to protect health and (marine) environment based on scientific knowledge under a holistic approach that is integrated into all other European measures protecting the quality of all waters through the WFD. WFD introduces the concepts of the ecological and chemical status for the European water bodies. The ecological status is based on biological quality elements supported by hydromorphological and physico-chemical environmental characteristics, and is divided into five classes (‘High’, ‘Good’, ‘Moderate’, ‘Poor’, ‘Bad’). According to the normative guidelines of the WFD, good ecological status is achieved when biological communities present are close to those that would be present with minimal anthropogenic disturbance (2000/60/EC). According to guidelines of the MSFD, qualitative descriptors to be used in assessing the ecological or environmental status include biodiversity, non-indigenous species, exploited fish and shellfish, food webs, human-induced eutrophication, sea-floor integrity, hydrographical conditions, contaminants and contaminants in fish. All in all, the MSFD aims to be based upon an ecosystem-based approach that has a holistic view on the management and protection of marine ecosystems, focusing on ensuring sustainable use of the seas, and providing safe, clean, healthy and productive marine waters (Borja et al., 2008; Borja et al., 2010).

Phytoplankton is the biological element of WFD most closely related to eutrophication and a primary indicator for the assessment of water quality, as it forms the basis of food webs and exhibits high reproduction rates and immediate response to environmental changes. Different phytoplankton attributes are considered essential for the appraisal of ecological status, including species composition, abundance and biomass, as well as frequency and intensity of phytoplankton blooms (WFD, 2000/60/EC). Amongst quality descriptors, phytoplankton biomass, in terms of chlorophyll-α, although it is a gross metric, it is simple and records the responses of phytoplanktonic communities to nutrient enrichments (Tsirtsis and Karydis, 1998; Garmendia et al., 2013). However, chlorophyll-α has been reported as being highly variable, thus also showing higher disagreement with the final classification of the water bodies (Borja et al., 2004). On the other hand, the use of benthic indices has been shown to provide valuable elements for the integrated quality status assessment of water bodies (Borja et al., 2014). Composition and abundance of benthic invertebrate fauna has been proved to be a biological quality element that can be reliably used for the classification of water bodies due to responsiveness to major environmental or anthropogenic changes. Macro-benthic animals are relatively sedentary (they are affected by environmental/ anthropogenic conditions), have relatively long life-spans, consist of different species that demonstrate variable tolerances to chemical stresses and have a substantial role in sediment processes, e.g., enhancing the flow of nutrients and materials between the sediments and the water column, and vice versa, through bioturbation and bioirrigation (Borja et al., 2000). For these reasons macrobenthic communities are listed among quality descriptors for the implementation of MSFD (MSFD, 2008/56/EC). In this context, the classification of ecological status is implemented using indices based on sensitivity/ tolerance of various species. In European waters the most frequently applied indices are AMBI and M-AMBI that have been developed using the data from the coastal marine areas and are mainly used to assess the organic enrichment (Pitacco et al., 2018). However, for the Mediterranean Sea, and especially the eastern basin, the most appropriate index is BENTIX, originally developed in the Aegean Sea (Simboura and Zenetos, 2002), as revealed by the intercalibration procedure of the EU members (Simboura and Reizopoulou, 2008). Accordingly, the BENTIX index, is the official tool for the ecological status assessment in Greece and Cyprus sedimentary bottoms.

In Northern Greece, Thermaikos Gulf is a marine ecosystem of high complexity due to the various activities taking place in the greater area. Thermaikos Gulf is the final receiver of the discharges of Axios, Aliakmon, Loudias and Gallikos Rivers, as well as of the effluents of two municipal wastewater treatment plants of Thessaloniki, with Axios River having the highest contribution of freshwater input into the gulf. However, Thermaikos is not only affected by the discharges of the watersheds of rivers, but also by the discharges of numerous industrial activities located along the coast. The anthropogenic pressures that originate from agricultural, industrial, commercial, marine and aquaculture activities have resulted in elevated concentrations of nutrients in the water column and accumulation of trace elements in sediments (Friligos et al., 1997; Nikolaidis et al., 2006).

Moreover, the exchanges with the open Aegean Sea waters across the southern boundary are an additional factor that affects the stratification, circulation and renewal of the Gulf (Krestenitis et al., 2012). The complexity of Thermaikos Gulf system, the variability of environmental factors and the specific circulation and renewal dynamics of the system require the development of a quality monitoring scheme that takes into account physical, chemical and ecological quality elements (in the context of the European legislation). To this aim, the present study focuses on the quality assessment of the marine environment of Thermaikos Gulf, using physical, chemical and biological/ecological elements of the water column and the seabed.

2. MATERIALS AND METHODS

2.1 Oceanographic surveys

Within the framework of this study, water and sediment samples that were seasonally (July, October and December 2017) collected from three sampling stations located at the inner part Thessaloniki Bay, inner and outer Thermaikos Gulf. The characteristics of the sampling stations (coordinates of each station, corresponding depth and description of the area) are presented in Table 1. As it is shown in Figure 1, the station S1 is located at the discharge point of the Municipal Wastewater Treatment Plant of Thessaloniki (WWTPT) outlet, the station S2 is located at northern Thessaloniki bay, while the station S3 is located at the discharge point of the Municipal Wastewater Treatment Plant of Michaniona-AINEIA.

Table 1. Characteristics of sampling stations

Sampling Station	Longitude	Latitude	Description	Depth (m)
S1	40.55237285	22.8536432	Station at the outlet of the WWTP of Thessaloniki (WWTPT)	25
S2	40.61821578	22.94157171	Inner Thessaloniki bay	13
S3	40.46212649	22.82078561	Station at the outlet of the WWTP of Michaniona (AINEIA)	30

The physical oceanographic parameters were recorded by means of a CTD (SBE 19) profiler. Apart from the standard sensors (conductivity, temperature, pressure), the instrument is equipped with auxiliary sensors for dissolved oxygen and pH. Raw data were properly processed (low-pass filtering, alignment, cell thermal effects removal) and corrected, to assure the accuracy of the derived parameters. The processed data were averaged over depth-bins of 0.25 m. During sampling, in situ processing of the water density data enabled the preliminary determination of the water column stratification. Afterwards, water samples were collected from three levels of the water column (surface, pycnocline and bottom) with a Niskin-type water sampler, in order to investigate the variations of the measured chemical and biological parameters over the water column depth.

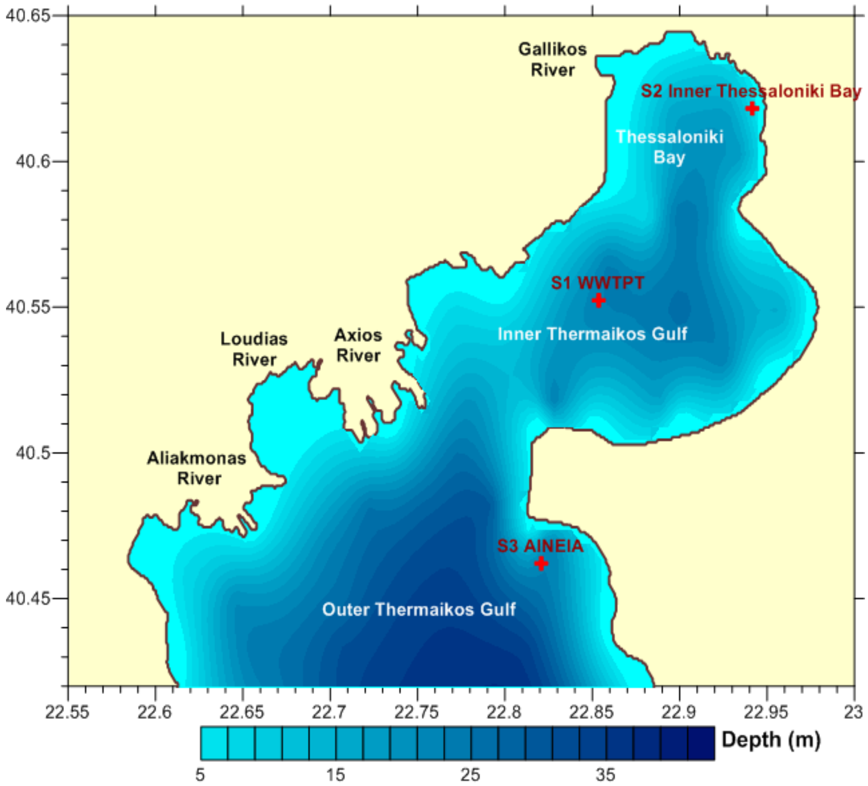

Figure 1. Map of sampling stations, rivers and sub-regions in Thermaikos Gulf.

The chemical status of Thermaikos Gulf was appraised by performing chemical analyses including nitrites (APHA-AWWA-WEF, 1999), nitrates (Grasshoff et al., 1999), ammonium nitrogen (Hach, 2013), phosphates (APHA-AWWA-WEF, 1999), total phosphorus (APHA-AWWA-WEF, 1999) and silica (APHA-AWWA-WEF, 1999; Grasshoff et al., 1999).

2.2 Biological parameters/Phytoplankton and Protozooplankton

Fresh water subsamples (250 ml) were placed at portable refrigerator and subsamples (250 ml) were immediately fixed with Lugol's iodine. Fresh and preserved water samples were examined under a light inverted microscope (Nikon SE 2000), and species were identified using appropriate taxonomic keys. Unicellular planktonic organism counts were performed using the sedimentation method of Utermöhl (1958). Briefly, at least 400 plankton individuals were counted in samples, when possible, in sedimentation chambers of 3 mL, 10 mL or 25 mL, depending on the total abundance in each sample. The dimensions of 30 individuals (cells, or colonies) of each dominant species (comprising of $\geq$ 10 % of the total plankton in terms of abundance and biomass) were measured using the relevant tools of a digital microscope camera (Nikon DS-L1). Mean cell, or colony volume estimates were calculated using appropriate geometric formulae according to Hillebrand et al. (1999).

2.3 Zoobenthos and sediment composition

Two replicate sediment samples were collected with a standard VanVeen grab (0.1 m^2) from each sampling station and period, whereas a third one was collected for sediment composition and organic content analyses. Overall, 18 biological and 9 sediment samples were obtained. Sediment samples were dried out to assess the organic content (H_2O_2 method) and the granulometric composition (siphonometric menthod) applying the Folk's system of sediment classification (Folk et al., 1970). Each biological sample was sieved on board (mesh-opening 1 mm) and preserved in 10% formalin - seawater solution. In the laboratory all living specimens were sorted out from each biological sample under a binocular stereoscope, counted, and identified at species level using relevant identification keys for each major taxa, and a microscope, when appropriate. The species/abundance data matrix was analyzed by standard biocoenotic methods to estimate biodiversity, and by multivariate methods based on Bray-Curtis distances to assess the similarity of zoobenthic communities' structure and possible spatial or seasonal effects, using the PRIMER software package (Clarke and Gorley, 2006). Also, the BENTIX biotic index was applied to assess the ecological quality status of sampling stations, using the freeware software developed and provided by the National Centre for Marine Research (www. cloudfs.hcmr.gr/index.php).

3. RESULTS

3.1 Physical and Chemical parameters

Very strong stratification, based on both salinity and temperature profiles, was observed in all stations in July, supporting the stability of the water column (Figure 2). The temperature difference between the surface and bottom was around 10°C in the inner Thermaikos Gulf (S1), while the smaller difference was measured in S3 (<3°C). The stations of the inner Gulf (S1 and S2) showed similar distributions in autumn (~21°C) and winter (~13°C). On the contrary, the outer station S3 revealed strong thermocline at 15 m in autumn, where the temperature was reduced by 4°C indicating the possible intrusion of colder waters from the Aegean Sea. This finding agrees with Hyder et al. (2002) and Krestenitis et al. (2012), who showed that this area is the passage of northern Aegean waters supporting the renewal of the Gulf. The same station showed significantly higher temperature values (~16°C) in comparison to the two northern stations (~13°C) in winter. Salinity was very low at the surface of S1 and S2 stations in July (<36). Summer values were lower than winter values in all cases. Moreover, in all cases, salinity was higher in the S3 station, which is usually out of the effect of the riverine waters that are discharged at the western Gulf. Especially in autumn, the salinity distribution of S3 station was not homogenous but increased at 15m from 37 to 39 (Aegean waters). This station is also characterized by warmer and saltier waters along the entire water column during winter.

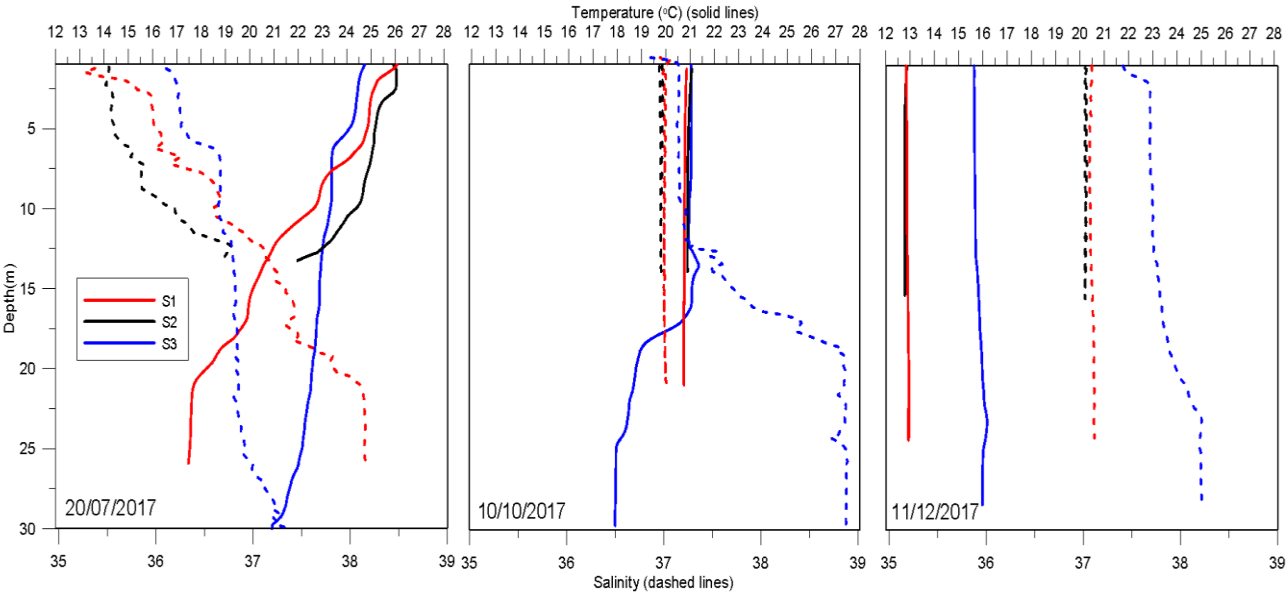

Figure 2. Vertical temperature and salinity profiles of the three Thermaikos stations in July (left), October (middle) and December (right).

The chemical quality of Thermaikos Gulf was mainly based on the seasonal monitoring of nutrients and included the inorganic forms of nitrogen, the orthophosphates and the total phosphorus. The highest concentration of ammonium nitrogen, equal to 1.12 μmol/L NH_4^+, was recorded during the third sampling campaign of December 2017 in the surface sample collected from the sampling station S1 (data not shown). Moreover, relatively high concentration of nitrites, 0.31 μmol/L, was obtained in the same sample, possibly denoting the presence (or the beginning) of reducing conditions in the regional marine environment. The increased concentrations of nitrites were accompanied by a significant reduction of nitrates, as it is shown in Figure 3.

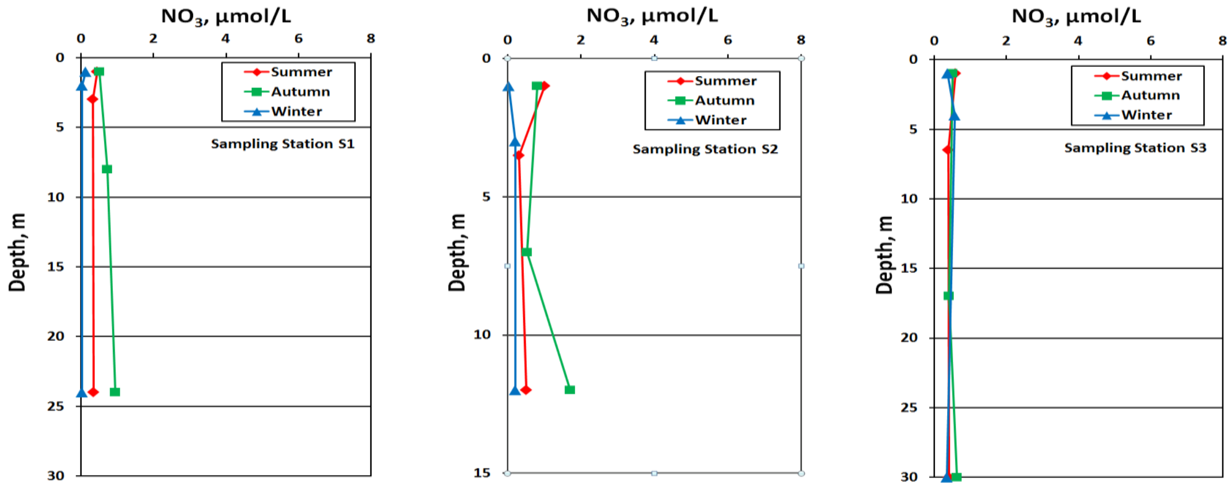

Figure 3. Nitrates concentration results.

The samples collected from the inner part of Thermaikos Gulf indicated rather low concentrations in nutrients of nitrogen and phosphorus. However, during the winter sampling campaign, the red tide phenomenon was observed on the sea surface; the seawater transparency was low, while the total phosphorus concentration was significantly higher compared to the corresponding one measured during the summer period (Figure 4).

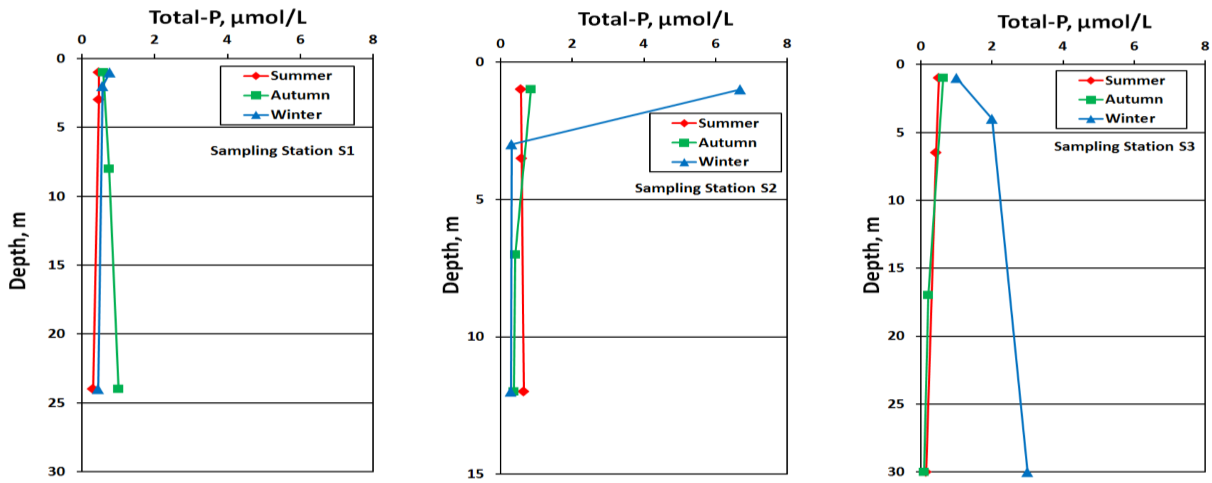

Figure 4. Total phosphorus concentration results.

The results obtained from the third sampling station, at the outer part of Thermaikos Gulf, demonstrated that the concentrations of nutrients were significantly lower compared to the rest sampling stations. However, the concentration of nitrates varied between 0.40 and 0.62 µmol/L, considerably higher compared to the results of 2014 (HCMR, 2015). Still, much higher concentrations, exceeding 2.0 µmol/L, were recorded some years ago (Samanidou et al., 1989). The intensification of sampling and measurements is needed, in order to extract safe conclusions on the quality characteristics of this area.

3.2 Phytoplankton

Species composition

Overall, 87 phytoplankton and 13 protozooplankton taxa were identified in the water samples during the investigation. Within the phytoplankton community, diatoms were recorded with the highest number of species reaching 43 identified taxa, followed by dinophytes (32) and haptophytes (5). For each of the rest phytoplankton taxonomic groups (cryptophytes, chlorophytes, dicthyophytes, euglenophytes, raphidiophytes, xanthophytes) less than 5 representatives were identified. The protozooplankter *Noctiluca scintillans* (Figure 5d) with its rare relative *Spatulodinium pseudonoctiluca*, responsible for the frequent red tides in Thessaloniki Bay, were recorded in every sample of the inner Gulf (S1 and S2).

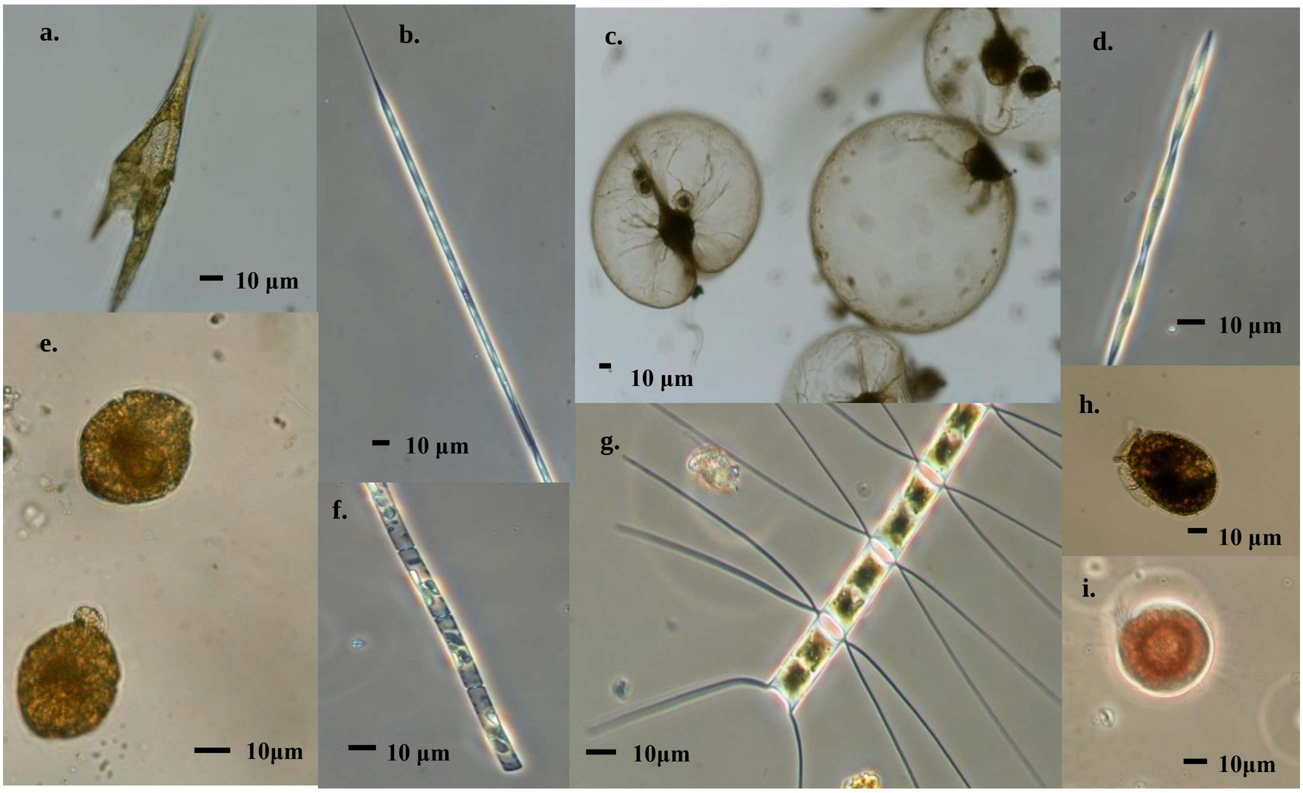

Figure 5. Light micrographs (phase contrast) of phytoplankton and protozooplankton taxa in the water samples from Thermaikos Gulf in June, October and December 2017. a. *Ceratium furca* b. *Rhizosolenia setigera* c. *Noctiluca scintilans* d. *Pseudonitzschia pungens* e. *Gonyaulax* cf. *fragilis* f. *Leptocylindrus danicus* g. *Chaetoceros* sp. h. *Dinophysis* cf. *acuminata* i. *Mesodinium rubrum.*

In July, after the "dirty sea" phenomenon the mucilage forming species *Gonyaulax* cf. *fragilis* and *Chaetoceros* spp. were observed in the samples (Figure 5). During the winter sampling (on December 2017) a large extent red tide was conspicuous in the inner Gulf. The autotrophic ciliate *Mesodinium rubrum* (Figure 5i) was accountable for the phenomenon due to its extremely high abundance (>10000 cell/mL) that was measured in the water samples.

Species number, phytoplankton abundance and biomass

The number of identified species was comparable among the 3 sites following the same trend in terms of increasing depth; higher number of species was observed in the surface samples than the pycnocline and the bottom samples. Diatoms and dinoflagellates were the most diverse taxonomic groups with the first to dominate in richness almost in all samples apart from the occasional dominance of the latter in S1 and S2. The phytoplankton abundance was higher and frequently indicative for bloom formation in the inner gulf (sites S1 and S2) contrary to S3. Characteristically, the maximum measured phytoplankton abundance in S3 was 6 times lower than the maximum in S1 and 12 times lower than in S2. Similarly, the phytoplankton biomass was higher in S1 and S2 than S3 (Figure 6).

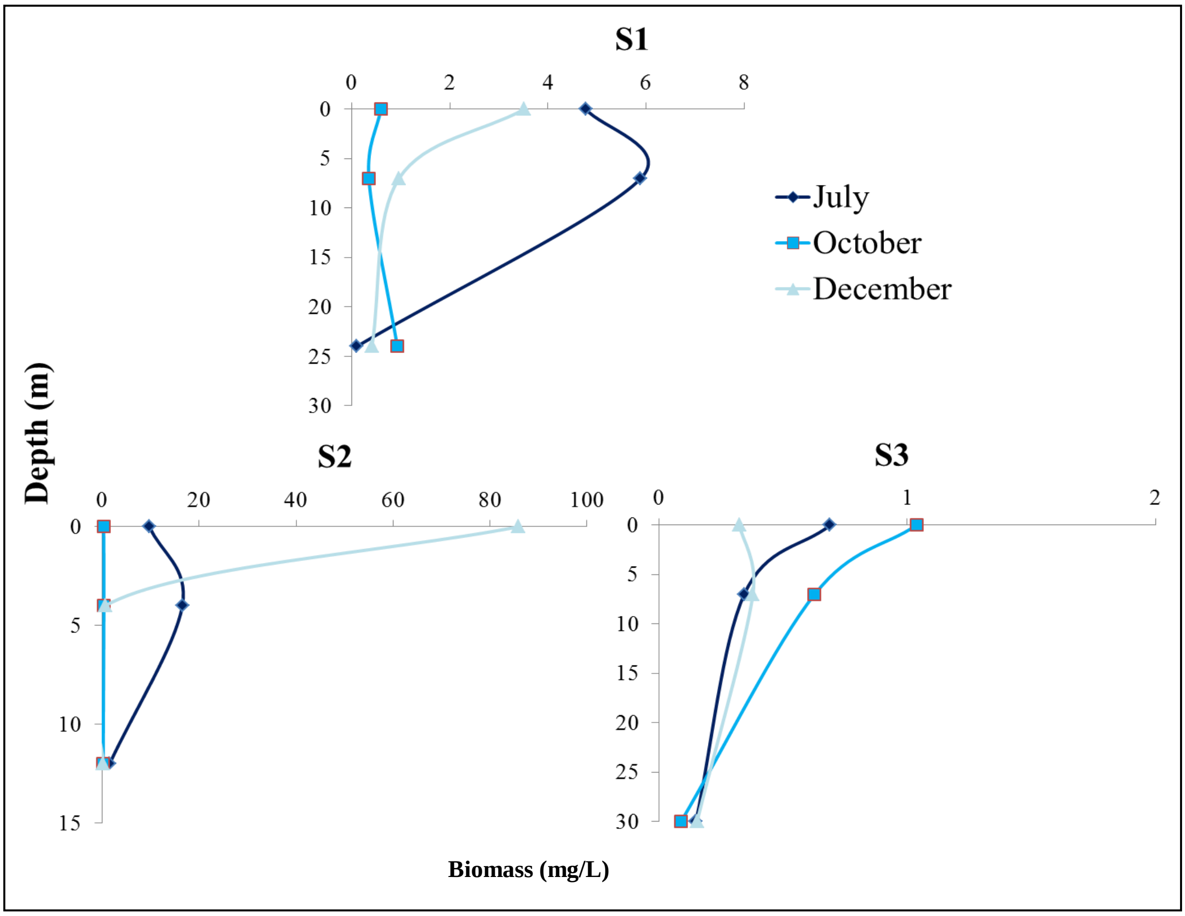

Figure 6. Phytoplankton biomass (mg/L) in each depth and in each site (S1, S2, S3) of Thermaikos Gulf. The dark blue depicts the phytoplankton biomass in July 2017, the ciel blue the biomass in October 2017 and the light ciel blue the biomass in December 2017.

The maximum phytoplankton biomass during the investigation (the extremely high value of 86 mg L^{-1}) was recorded in the surface sample of S2 simultaneously with the extensive red tide in the inner Gulf (on December 2017). The phytoplankton biomass was also high (>5 mg/L) in the surface and pycnocline samples of S1 and S2 on July 2017 (Figure 6). Conversely, the phytoplankton biomass in S3 was typical for oligotrophic marine environments with exception of the surface sample on October 2017 where the measured biomass was 1 mg/L.

3.3 Zoobenthos

According to the granulometric composition, the sediment is characterized as muddy in S1 and S3, and as sandy-mud in S2. The sediment composition was seasonally stable, with the exception of the December period, where the proportion of sand increased in S1 and S3 shifting the characterization of the sea-bottom as sandy-mud, as opposed to S2, where the proportion of clay increased and the sediment became muddy. At the same time, increased amounts of biogenic fragments were also observed in S2. The organic content showed spatial divergences with increased values in S2 (0.1465 ± 0.023) and lower, but inter-se similar, in S1 and S3 (0.1174 ± 0.022 and 0.1104 ± 0.025, respectively). Slight seasonal variations were also detected, but without following a similar trend between stations.

Overall 2,945 macro-invertebrate specimens were collected identified to 207 species. Polychaetes and molluscs were the most speciose groups, followed by crustaceans. The above groups prevailed also in abundance. Zoobenthic diversity and abundance showed significantly ($p<0.01$) higher values in S2 (mean S = 56, mean N = 347/0.1m^2) and decreased ones in S3 (mean S = 29, mean N = 80.33) and S1 (mean S = 16, mean N = 57.66), in particular. The most dominant and frequent species were the polychaetes *Ditrupa arietina*, *Magelona mirabilis*, *Nepthys hystricis*, *Notomastus latericeus*, *Sternaspis scutata*, the ophiuran *Amphiura chiajei* and the bivalve *Kurtiella bidentata*. Multivariate analyses discriminated samples primarily according to their geographic origin ($R = 1$ $p < 0.05$) and secondary (within each station group) according to the season of sampling ($R = 0.72$ $p < 0.05$); the latter case mostly due to the divergence of the samples collected in winter (Figure 7). This biotic pattern was mainly correlated with the amount of clay and the organic content of the sediments (Spearman $\rho = 0.625$).

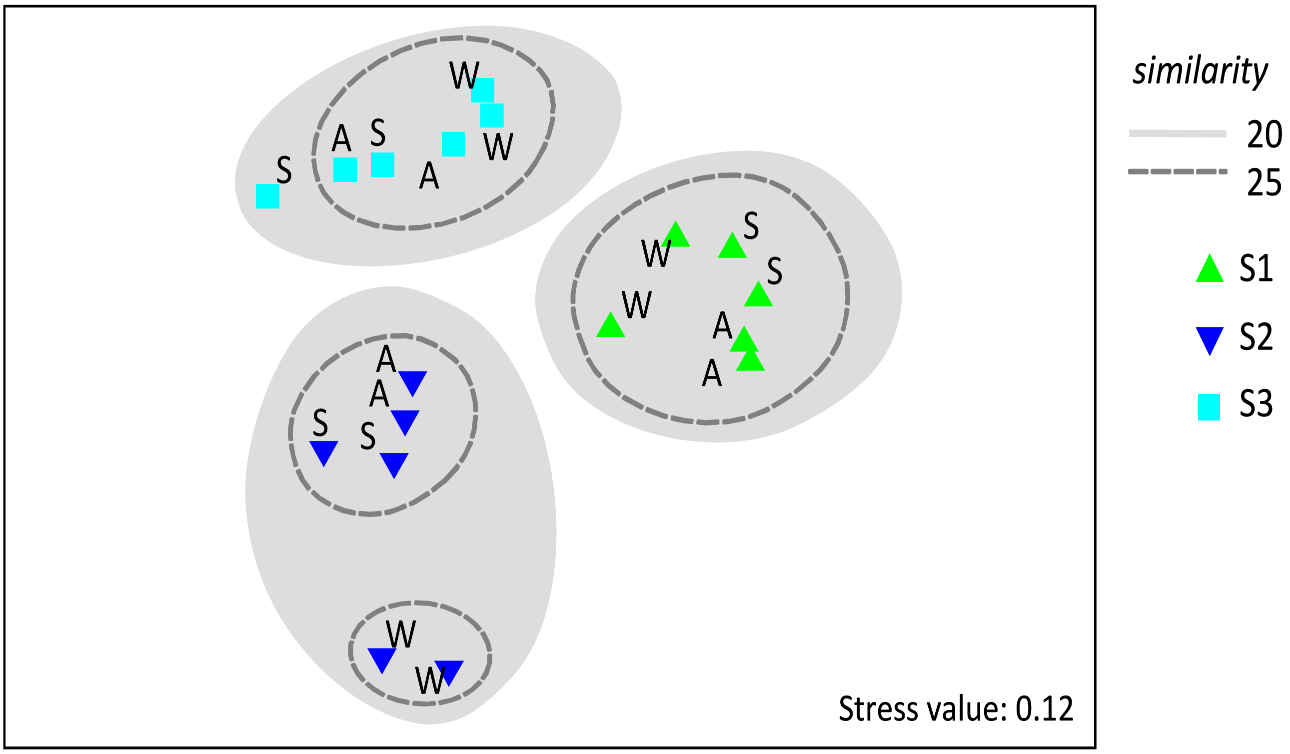

Figure 7. Non-metric multidimensional scaling ordination of zoobenthic samples from Thermaikos Gulf stations (S1-S3), based on Bray-Curtis similarity index calculated from square-root transformed numerical abundance data of macro-invertebrate species. S = summer sampling, A = autumn sampling, W = winter sampling.

The majority of macroinvertebrates were classified into the tolerant category, being positively correlated with organic enrichment, with the exception of S1 in winter, where the sensitive species group prevailed (Table 2). The BENTIX biotic index ranged from 2.51 to 3.66, and accordingly, the ecological quality status of the stations ranged from moderate (S1, S2) to good (S3) (Table 2). The ecological quality status of S1 improved in winter, whereas remained seasonally stable in S2 (moderate) and S3 (good). However, the percentage of species not assigned to any ecological category overpasses 10% in some cases, and so these specific results should be viewed with caution.

4. DISCUSSION AND CONCLUSIONS

Although denser (colder and saltier) waters, possibly originated from the North Aegean, were detected in the outer Gulf during the fall measurements, they were not observed in the northern areas, indicating weak renewal of the inner Gulf. More brackish waters were detected in the drier summer months although the river discharge rates are usually smaller. A possible explanation is the operation of the power generation dams, which exist along the Aliakmonas river, leading to increased outflows toward the sea (Krestenitis et al., 2012). Krestenitis et al. (2012), based on 5 long cruise period (1994-2007) showed a general decrease of Gulf's salinity. On the contrary, the current measurements, almost 10 years later, showed higher salinity values, supporting the inverse of the decreasing trend found in older expeditions.

Table 2. Number of species (S), numerical abundance (N), ecological groups (GS = Sensitive species group, GT = tolerant species group, NA = not assigned species), BENTIX index, and ecological quality (EcoQ) status assessment per seasonal sampling and station (S1 – S3) in Thermaikos Gulf.

Sampling		S	N	Ecological Category			BENTIX	EcoQ
Station	Season			GS	GT	NA		
S1	Summer 2017	18	80	18.75%	71.25%	10.00%	2.55	MODERATE
	Autumn 2017	20	117	15.05%	80.34%	4.62%	2.51	MODERATE
	Winter 2017	30	145	48.97%	35.86%	15.17%	3.66	GOOD
S2	Summer 2017	93	623	32.74%	60.67%	6.58%	3.18	MODERATE
	Autumn 2017	73	635	34.96%	59.53%	5.51%	3.29	MODERATE
	Winter 2017	72	279	30.47%	56.99%	12.54%	2.96	MODERATE
S3	Summer 2017*	48	193	32.12	54.40	13.47	3.02	GOOD
	Autumn 2017*	44	111	33.33	53.15	13.51	3.06	GOOD
	Winter 2017	32	114	39.47	57.01	3.51	3.51	GOOD

* >90% silt limits were used

The chemical status of both the inner and outer part of Thermaikos did not present remarkable variations during summer and autumn, compared to earlier studies (HCMR, 2007; HCMR, 2015). In particular, total phosphorus concentration values were close to those reported earlier for summer and autumn (HCMR, 2007; HCMR, 2015). However, during the winter sampling, extremely high concentrations of phosphates and total phosphorus were recorded in the surface samples, probably related to the observed red tide phenomenon. The measured concentrations were higher than the threshold for the good water quality (Dasenakis et al., 2015). In addition, rather high phosphorus concentration was recorded in the outer part of the Gulf, especially in the sub-surface samples. So far, comparable concentrations were only recorded many years ago (Samanidou et al., 1989), implying the need for consecutive and more extensive monitoring of Thermaikos quality.

The occasional dominance of dinoflagellates in S1 and S2, the frequent phytoplankton blooms concurrently with the high phytoplankton biomass (>0.7 mg/L, Bozatzidou 2013) in all samples of S1 and S2 indicate a less than good water quality according to phytoplankton. Furthermore, the protozooplankton abundance in these sites was indicative of red tide formations. These characteristics demonstrate the eutrophic character of the inner Gulf, which disagrees with the requirement for normal abundance and diversity of marine food webs elements, in order to establish good environmental status (MSFD, 2008/56/EC). On the other hand, the species composition and the phytoplankton abundance and biomass in S3 were indicative for higher than good water quality except for the surface sample on October 2017.

The benthic fauna showed increased abundance but similar diversity values with previous studies (HCMR, 2015). Polychaetes were the most dominant taxon, mainly represented by opportunistic species and thus, indicating the prevalence of slightly disturbed environmental conditions. The structure of zoobenthic communities differed among the three study sites, and especially between the innermost and the outer Thermaikos stations (i.e. S2 vs S3). A typical seasonal pattern, i.e. divergence of winter samples, was assessed in all stations, being more profound in S1 and S2 where colder water masses occurred compared to the outer Gulf station (S3). The above biotic patterns derived from the combined effect of three main environmental parameters: temperature, clay and sediment's organic content. According to the biological quality element of macro-invertebrates, the water quality status of Thermaikos was assessed as moderate in the inner gulf stations (S1, S2), and as good in the outer station (S3); however, water quality improved in S1 (the station in the intermediated part of the Gulf) during the winter sampling reaching good status. These results are in agreement with the phytoplankton monitoring, and generally conform to previous studies and the national monitoring program (HCMR, 2015).

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