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# Trophic state and oceanographic conditions of Amvrakikos Gulf: evaluation and monitoring

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#### ABSTRACT

The environmental state of estuaries is often compromised by various processes with direct and indirect ecological impacts such as eutrophication, harmful algal blooms, trophic interactions, and so on. In this paper, the eutrophication state of an important semi-enclosed embayment, the Amvrakikos Gulf, in Western Greece, for the first time will be examined, and the present situation will be compared with previous measurements. Field data used in this study were collected during four sampling cruises (April 2009 to March 2010) in the Amvrakikos Gulf. In compliance with the results, both the rivers' high discharges and the high evaporation rate lead to strong stratification of the water column throughout the year due to either salinity or temperature fluctuations. In combination with the limited communication with the open sea, this has resulted in different spatial and temporal dissolved oxygen distributions, as the western part of the gulf is seasonally hypoxic, while the eastern part is seasonally anoxic. The use of fertilizers, the load from fish farming, the phosphate geological layers, and other point and nonpoint source pollutions increase the nutrient pollution of the Amvrakikos Gulf. As a consequence, according to Carlson's trophic state index, the Amvrakikos Gulf varies from mesotrophic to eutrophic.

Keywords: Eutrophication; Stratification; Hypoxia; Anoxia; Trophic state index; Amvrakikos Gulf

#### 1. Introduction

To begin with, nutrient overloads are among the most important environmental problems. They may occur due to natural processes such as internal nutrient loading (e.g. sediment nutrient release) or to land-based activities and external nutrient loading (e.g. agricultural fertilizers and manure, sewage and industrial discharges, atmospheric releases from fossil fuel combustion, etc.). The increased human population growth, urbanization, agricultural and industrial expansions, and human activities are the dominant source of nutrients [1].

Additionally, the most important and biologically productive coastal zones include estuaries and transition zones where freshwater from land drainage mixes

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with sea water. Despite their importance, their water quality is often compromised by the overloading of nutrients and organic matter, the influx of pathogens, and the accumulation of chemical contaminants [2]. Direct and indirect ecological impacts of nutrient overenrichment in estuarine waters include production of plant-based organic matter, i.e. eutrophication, harmful algal blooms, changes in the trophic structure, trophic interactions, and trophodynamics of phytoplankton, zooplankton, and benthic communities. Consequently, this generally leads to ecological changes and economic losses [3-5]. Eutrophication is accompanied by an increased demand for oxygen [6]. Some of this increased oxygen demand is due to the greater respiration of the increased biomass of plants and animals, due to the high rates of microbial in both the water column and sediments that consume the organic matter produced by the greater plant production [7].

The Amvrakikos Gulf is a large (405 km<sup>2</sup>), semienclosed embayment, located between 38<sup>°</sup>51′ and 39<sup>°</sup>04′N, and 20<sup>°</sup>45′ and 21<sup>°</sup>11′E, in Western Greece (Fig. 1). It is 5–15 km wide and 35 km long. Its maximum depth is approximately 63 m, while the mean depth is equal to 26 m. The Gulf plays an important role in aquaculture and tourism, as well as being of great conservation value for wildlife and wetland vegetation. Furthermore, its northern part is also protected by the ecological network Natura 2000 and the Ramsar convention. The waters inside the gulf are strongly affected by runoff from the two rivers Arachthos and Louros which are located in the northern area. The Gulf, therefore, acts as a typical dilution basin from winter to early summer, while during summer and early autumn, evaporation becomes more important than freshwater input from rivers [8].

According to the literature, there is very little information about the eutrophication levels and the physical oceanography in the whole gulf, as the majority of published works deal with the environmental state of the lagoons in the northern part of the gulf. As a consequence, the main aim of this paper is, primarily, to determine the trophic state of the Amvrakikos Gulf and, secondly, to compare the present situation of the Gulf with the past, in order to obtain a conclusion about the environmental state progress.

#### 2. Materials and methods

#### 2.1. Water sampling

In order to determine the eutrophication levels of the Amvrakikos Gulf, four seasonal sampling cruises were organized during 2009–2010, in spring (10 April 2009), summer (16 July 2009), autumn (22 October 2009), and winter (19 March 2010). Physicochemical characteristics such as temperature, salinity, dissolved oxygen, pH, and ORP were determined *in situ* by using a conductivity – temperature – depth (TROLL



Fig. 1. Amvrakikos Gulf (NW Greece) and sampling stations (A1-A16).

Sampling stations	Sampling depths						
	0	5	10	20	25	40	50
A <sub>1</sub>	С	С	С				
$A_4$	C–T	С	C-T				
A <sub>8</sub>	C–T	С	C–T	C-T-H		C–T–H	
A <sub>14</sub>	C–T	С	C-T-H		C-T-H		C–T–H
A <sub>13</sub>							Н
A <sub>15</sub>	C–T						
Indexe C. Chile T. TD II.	ПС						

Table 1	
Water sampling stations and depths in the Amvrakikos Gulf, during 2009–201	0

Index: **C**: Chl-a **T**: TP **H**:  $H_2S$ .

9500) at each of the 16 sampling stations, with 1 m vertical interval. Simultaneously, the transparency of the water was determined with a Secchi disk at each sampling station. For the determination of chlorophyll-a, total phosphorus (TP), and hydrogen sulfide, water samples from six sampling stations were collected by using a 2.5 L free flow sampler (HYDRO-BIOS). Table 1 describes, in more detail, the sampling stations and depths for each of the three parameters.

#### 2.2. Methods of chemical analysis

Chlorophyll-a (chl-a) was determined according to the trichromatic method [9]. The water samples for chla were filtered immediately after sampling using Glass Microfibre filters GF/F (45 mm pore size) and pigment extraction took place in 90% acetone. Pigment optical density (absorbance) was determined with a spectrophotometer. The concentration was then estimated by using the equations of the trichromatic method.

The TP concentrations were determined spectrophotometric using the persulfate digestion method [9]. According to this method, all the samples were digested in an autoclave with sulfuric acid and ammonium persulfate in order to convert all phosphorus to orthophosphate. For the determination of phosphorus concentrations, a separate calibration curve had been constructed by using standards solutions, with different concentrations (from 0.00 to  $0.50 \text{ mg L}^{-1}$ ), through the persulfate digestion procedure. Moreover, at each measurement, two standard solutions, with a known concentration, were used as reference materials, in order to validate the persulfate digestion method.

The water samples for hydrogen sulfide were determined according to the iodometric method [9]. The samples were collected with minimum aeration and due to the fact that they were not analyzed immediately, zinc acetate and sodium hydroxide solutions were added in the bottle of the sample, before it was filled with the sample.

#### 2.3. Trophic state determination

In order to determine the trophic state of the Amvrakikos Gulf, the trophic state index of Carlson (TSI) [10] was used. The TSI uses algal biomass as the basis for trophic state classification and three variables were used to independently estimate algal biomass: chlorophyll pigments ( $\mu g L^{-1}$ ), Secchi disk depth (m, transparency), and TP concentrations  $(\mu g L^{-1})$ . The range of the index is approximately from 0 to 100, although theoretically it has no lower or upper boundaries. A range between 40 and 50 is usually associated with mesotrophic water conditions (moderate productivity), index values greater than 50 are associated with eutrophic conditions (highly productivity), while values less than 40 are associated with oligotrophic conditions (low productivity). Hypereutrophic, or excessively productive systems, have TSI values greater than 70. Higher numbers are associated with increased probabilities of encountering nuisance conditions, such as excessive macrophyte growth and algal scums [11]. The TSI was calculated using the following equations [12]:

TSI of chlorophyll = 
$$30.6 + 9.81 \ln(CHL)$$
 (1)

TSI of phosporus = 
$$4.15 + 14.42 \ln(\text{TP})$$
 (2)

TSI of transparency = 
$$60 - 14.41 \ln(SD)$$
 (3)

$$\overline{\text{TSI}} = \frac{\text{TSI}_{\text{Chl}-a} + \text{TSI}_{\text{TP}} + \text{TSI}_{\text{SD}}}{3}$$
(4)

## 3. Results

#### 3.1. Environmental pollution

The problems that contribute to the degradation and eutrophication conditions in the Amvrakikos Gulf vary in reference to the origin of the source and relate both to the gulf's particular morphology and to human activities which take place in the study area. As a typical semi-enclosed gulf, the only connection with the open Ionian Sea is a narrow strait (the Preveza Channel), which is about 600 m wide, 3 km long, and its mean depth is 8.5 m.

According to former studies, the main problem of the water quality resulted from the limited renewal of the gulf's deepest water due to the permanent pycnocline in the water column [13]. Balopoulos and Papageorgiou [14] show that the current speeds in the Amvrakikos Gulf are very low. The highest speeds were observed in the region of Aktio (entrance), where the average speed was equal to  $0.14 \,\mathrm{m\,s^{-1}}$ , and in the spring of 1988, it was equal to  $0.974 \,\mathrm{m \, s^{-1}}$ . In the remaining gulf, the highest and average speeds were always lower than 0.189 and  $0.02 \text{ m s}^{-1}$ , respectively. In combination with the construction of the new harbor near the entrance of the gulf during the 1970s, which reduced the inlet's dimensions, this resulted in the limited inflow of the rich Ionian water, a process which can take place only under particular conditions of salinity and density.

As it has already been mentioned above, the Amvrakikos Gulf receives freshwater input from two drainage basins of Louros and Arachthos. Their water discharges vary from season to season, however, their mean annual discharges are about 70 and  $19 \text{ m}^3 \text{ s}^{-1}$ , respectively [15]. The water quality of the Arachthos river can be characterized as better than that of Louros. With reference to the nutrient concentrations in the Arachthos river, the nitrate concentrations vary from 300 to  $1,300 \,\mu\,\text{L}^{-1}$  and phosphorus from 5 to  $60 \,\mu\text{g}\,\text{L}^{-1}$ , while in the Louros River, the nitrates and phosphorus concentrations vary from 900 to 1,600  $\mu$ g L<sup>-1</sup> and from 8 to  $210 \,\mu g \, L^{-1}$ , respectively [13]. Also, apart from these two major drainage basins, Amvrakikos is a recipient of a net of irrigation and drainage canals, and various pumping stations, which receive water from farming, rich in nutrients. All of these have resulted in faster transportation of pollution in the gulf since the concentrations of water in fertilizers, pesticides, insecticides, and other wastes are quite high. Taking all the above into account, apart from the fact that they boost the stratification of the water column and as a result limit the mixing and the oxygenation of the deepest layer, they reinforce the eutrophication problems as they drain large amounts of nutrients throughout the year.

#### 3.2. Temporal and spatial distribution of stratification

Seasonal distribution of temperature, salinity, and density in the Amvrakikos Gulf is shown in Fig. 2.

According to this, the water column is strongly stratified almost throughout the year, with the exception of autumn, due to either salinity or temperature. In spring (Fig. 2(a)), the water column can be divided into three different layers, according to the density. The surface layer (surface-3 m), where the density was almost stable and equal to 14-15, was characterized by low salinity (25-27%), while near the entrance, it increased to 34.6% and the temperature was equal to 15-16°C. Below this layer, the pycnocline existed until the depth of 10 m and, inside it, both density and salinity increased along with the depth, as they ranged from 16 to 27, and 25 to 36%, respectively, temperature was equal to 15-16°C. The third layer took place under the pycnocline and the three parameters, density, salinity, and temperature were stable and equal to 27–28, 36%, 15°C, respectively.

In summer, particularly in July, as the evaporation was stronger, in the upper layer, the salinity ranged from 28‰ in the eastern part of the gulf to 36‰ in the western part. Moreover, due to high solar radiation, the temperatures (24–26°C) were higher than in spring. Due to this variability in salinity values, the density in the surface layer (Fig. 2(b)) was different between the eastern (18.5) and western part (23) of the gulf too. In the pycnocline (between 6.5 and 10 m depth), the salinity increased from 31 to 36%, while the temperature decreased from 24 to 18°C. As a result, the density varied with the depth as its values ranged from almost 19 in the upper layer of pycnocline to 27 at its end. Below this layer, both salinity and temperature were almost stable and equal to 38% and 16°C, respectively, and the density was almost the same as it was in spring.

In autumn, specifically in October, stratification was not as strong as it was in summer and spring of the same year (Fig. 2(c)). This is because of the fact that in this season, winds with higher speeds than those in summer dominated. Therefore, the rivers did not discharge large volumes of water, the solar radiation was not as high as it was in summer months, and thus the upper layer started to mix with the deep waters. As a result, the surface layer extended to a greater depth, almost until to a depth of 20 m, while the salinity varied from 34-36‰ and the temperature was equal to 19-20°C. The pycnocline occupied the area from a depth of 22 m until 24 m and the density values changed only by two units (from 26 to 28), while in reference to salinity and temperature inside it, there were only small variations. The bottom layer, however, was characterized by the same features as it was in summer.

In winter, the stratification was different in the eastern area than in the rest of the gulf. In the wes-



Fig. 2. Vertical profiles of sigma-t, temperature and salinity, along transect  $A_1 - A_{14}$  (stations  $A_1 - A_2 - A_3 - A_4 - A_5 - A_8 - A_{12} - A_{14}$ ).

tern and central gulf, the water column could be divided into three different layers (Fig. 2(d)). The surface layer extended to a depth of 4 m and was characterized by relatively stable density and salinity values (14–15 and 21–22‰, respectively) as well as low temperatures. Below this, the pycnocline was detected between a depth of 4 and 8 m where the density increased from 16 to 26. Inside the pycnocline, the temperature did not vary significantly, as its values ranged between 14 and 15°C, however, salinity increased from 20 to 35%. On the other hand, in the western part of the gulf, there was only one layer from the surface at a depth of 8 m, the pycnocline. Due to higher river discharges, the salinities near the surface were much lower than in the other seasons but generally the salinity ranged from 17 to 25% and the temperature was equal to  $14-15^{\circ}$ C. Finally, in the layer below the pycnocline (both in the eastern and the western area), another one existed below the after layer which was characterized by stable density, salinity, and temperature conditions (27–27.5, 37‰, and 16°C, respectively).

#### 3.3. $Oxygen - H_2S$

Regarding the DO distribution, the Amvrakikos Gulf can be separated into two parts: the western and the eastern, with important differences between each other during the year. In the western area, the oxygen levels decreased in depth throughout the year. The water column was well oxygenated during winter and spring (Fig. 3(a)), as the minimum oxygen saturation level was more than 30% (DO>3 mg L<sup>-1</sup>). This area was directly affected by the Ionian waters, and in combination with the small depth (<35 m) of the area, it led to the waters column mixing and thus to the oxygenation of the water column. On the other hand, in summer and autumn, hypoxic conditions  $(< 2 \text{ mg L}^{-1})$  occurred below the depth of 17 and 21 m, respectively, as the oxygen saturation levels varied

from 28 to 3% near the bottom. A possible reason for this is that in summer, the pycnocline was very strong and thus the bottom waters were isolated from the oxygen-rich surface water, which was combined with the decomposition of organic matter and led to the decrease of dissolved oxygen levels below the  $2 \text{ mg L}^{-1}$ , but never close to zero levels.

In the eastern area, the water column consisted of three different layers throughout the year. In the upper layer, which extends from the surface to a depth of 7 m, the oxygen saturation levels ranged from 115 to 155% while the oxygen levels were always above  $7.5 \text{ mg L}^{-1}$  and both of them increased in depth, only in summer and winter (Fig. 3(b)). This is due to photosynthesis, a process which causes oxygen production from the phytoplankton, due to high photosynthetic activity, and water from the Louros river, rich in oxygen. In the second layer, the oxycline formed between the depths of 7 and 20 m and the oxygen concentrations decreased in depth, while in



Fig. 3. Vertical distribution of dissolved oxygen in mg  $L^{-1}$  (upper) and oxygen saturation in % (lower) in (a) western (st. A<sub>4</sub>) and (b) eastern (st. A<sub>14</sub>) study area.

the third layer, which extended below the depth of 20 m, the oxygen levels were less than  $2 \text{ mg L}^{-1}$  during the whole year. Anoxic conditions occurred only in this area in summer and autumn, because the water column was stratified, due to the salinity-controlled strong pycnocline, almost throughout the year. Moreover, this third layer was isolated from rich in oxygen waters for long periods of time. Therefore, in combination with the decomposition of organic matter, it could be characterized as an isolated water mass with very low oxygen concentrations.

Hydrogen sulfide is produced only in areas which are characterized by anoxic conditions. Seasonal measurements show that hydrogen sulfide was detected only in autumn, below the depth of 20 m and during the other seasons, it was equal to  $0 \text{ mg L}^{-1}$ . The maximum concentration was detected in the deeper, eastern area (A<sub>13</sub> and A<sub>14</sub>) where the hydrogen sulfide concentration was equal to  $1.6 \text{ mg L}^{-1}$  near the bottom (at a depth of 50 m), whereas in the central area (A<sub>8</sub>) near the bottom (40 m depth), it was equal to  $0.8 \text{ mg L}^{-1}$ . However, in both areas at a depth of 20 m, the hydrogen sulfide concentrations ranged from 0.2 to  $0.4 \text{ mg L}^{-1}$ . This can also confirm that the Amvrakikos Gulf was only seasonally anoxic and not throughout the year.

# 3.4. TP distribution

From TP measurements in the gulf, the vertical concentrations were different near the entrance than in the rest of the gulf. Near the entrance (st.  $A_1$  and

 $A_4$ ), the concentrations were higher in the surface than those at a depth of 10 m. The reason for this was probably the various activities in the city of Preveza, as domestic waste and the influence of navigation, resulted in an increase in the concentrations of nutrients. Also, in the area, there are many marinas where due to tourism, in the summer months; they gather boats and vachts worsening the local problem. In the rest of the gulf, the TP concentrations increased in depth. The maximum surface TP concentration was measured in the northeastern area (st.  $A_{14}$ - $A_{15}$ ), in spring and in winter 1.37 and  $1.88 \,\mu M \,L^{-1}$ , respectively, probably because of the Arachthos river discharges which are rich in nutrients. In summer and in autumn, the maximum TP concentrations were detected in the central gulf area (st.  $A_8$ ) at a depth of 10 m (1.16 and  $0.75 \,\mu\text{ML}^{-1}$ , respectively). However, TP concentrations near the bottom were always almost three to four times higher than those in the surface, as it is shown in Fig. 4, and the highest value, equal to  $5.1 \,\mu\text{M}\,\text{L}^{-1}$ , was detected at a depth of 50 m (st. A<sub>13</sub>). This could possibly be due to the fact that the bottom waters are affected by the bottom sediments which are characterized by high concentrations of nutrients.

The internal phosphorus loading to a marine system from the sediments depends on hydrodynamic and biotic mechanisms that transport dissolved phosphorus from the sediments to the water. There are many different mechanisms which may be involved in the exchange of phosphorus across the sediment–water interface (sediment release), such as oxidation–reduction (redox), interactions dependent



Fig. 4. Distribution of TP ( $\mu$ M L<sup>-1</sup>) during April 2009 to March 2010.

on oxygen supply, mineral solubility and sorptive mechanisms, the metabolic activities of bacteria and fungi, and turbulence from physical and biotic activities [16,17]. However, bacterial metabolism of organic matter is one of the primary mechanisms by which organic phosphorus is converted into phosphate in the sediments creating the reducing conditions required to release phosphate into the water [17,18].

### 3.5. Chlorophyll-a

Concerning to chl-a distribution, there were many differences between the entrance of the gulf and the rest of it. In the western part of the gulf, in winter and spring, chl-a concentrations decreased in depth and their maximum values were detected near the surface (14.4 and  $3.3 \,\mu g \, L^{-1}$ , respectively). On the other hand, during summer and autumn, they were almost the same in the entire water column (less than  $1 \,\mu g \, L^{-1}$ ). In the rest of the gulf, the situation was different. Specifically, in winter and in spring, the

maximum concentrations were detected at a depth of  $5 \text{ m} (2.2 \,\mu\text{g L}^{-1} \text{ in spring and } 10.4 \,\mu\text{g L}^{-1} \text{ in winter})$ , while in the rest of the year, they reached a maximum at a depth of  $10 \text{ m} (0.73 \,\mu\text{g L}^{-1} \text{ in autumn and } 2.4 \,\mu\text{g L}^{-1} \text{ in summer})$ .

It is common for phytoplankton blooming to take place in spring, when there are high concentrations of nutrients because of high river discharges. In our study, according to Fig. 5, the maximum chl-a concentrations were detected in winter probably due to the fact that the highest discharges of the two rivers in the study area (Louros and Arachthos) took place in winter. Thus, they discharged into the gulf's waters with high nutrient concentrations. This, in combination with a long period of sunlight and high temperatures, led to phytoplankton blooming in winter.

As we can see in Fig. 5(c), near the bottom of station  $A_8$ , from autumn to winter, another chl-a maximum in water column was detected, which was equal to  $4.6 \,\mu g \, L^{-1}$  in autumn and  $5.1 \,\mu g \, L^{-1}$  in winter. As we had no other information or measurements, we



Fig. 5. Spatial and temporal Chl-a distribution in stations (a) A<sub>1</sub>, (b) A<sub>4</sub>, (c) A<sub>8</sub>, and (d) A<sub>14</sub>.

can only assume that this second maximum was probably due to anoxygenic photosynthetic bacteria (green and purple sulfur bacteria) which require anaerobic conditions for photosynthetic activity [19].

#### 3.6. Transparency

As far as the Secchi disk transparency is concerned, its values vary from season to season. During spring, the water transparency increased from north to southwest. In the majority of the sampling stations, its values ranged from 2-3 m, with the exception of the northern part of the gulf where it was less than 1.5 m (st. A<sub>10</sub>). However, near the entrance of the gulf, the mean water transparency was 3.5 m, and this is possibly due to the Ionian water impact.

The spatial distribution of water transparency was different during the summer, as it decreased from east to west. In the eastern part of the gulf (st.  $A_{13}$ -st.  $A_{16}$ ), it reached its maximum values (equal to 4–5 m) while the minimum values were detected in the western sampling stations where the transparency was less than 3 m. This maximum in transparency values in the eastern part of the gulf was probably due to the fact that during summer, the Arachthos river discharges were reduced and as a result, the river's sediment material was limited.

In autumn, the Secchi disk transparency in the central and eastern part of the Amvrakikos Gulf was always higher than 3 m at all stations and its spatial distribution was the same during the summer sampling. On the other hand, in the western part of the gulf and mainly near the entrance of the gulf (st. A<sub>1</sub>), the water transparency reached its maximum value, almost 7 m. This high value near the entrance of the gulf was due to the fact that in this area, the speed of the currents was always very high (almost  $1 \text{ m s}^{-1}$ ) and thus the water column was characterized by high transparency. Furthermore, during winter, the water transparency was limited in the whole gulf as it ranged only from 1 to 2 m, with the exception of an area in the southwestern part of the gulf (st.  $A_4$ ) where reached 2.5 m. The minimum values that were measured in the study area in winter were probably affected by the sediment material from the Arachthos and Louros rivers which discharged in the north part of the Amvrakikos Gulf.

#### 3.7. Trophic state index

Summarizing the appropriate measurements from the study area in order to calculate the TSI, we formed the TSI diagram (Fig. 6). From the observation of Fig. 6(a), it is clearly visible that there are important differences between the three variables. Specifically, according to TP concentrations and transparency, the Amvrakikos Gulf can be characterized as a mesotrophic and seasonally eutrophic gulf, where nonalgal particulates and color dominant light attenuation are present. According to chl-a measurement, however, the Amvrakikos Gulf is an oligotrophic and seasonally mesotrophic gulf. However, taking into account the total TSI index (Eq. (4)), the trophic state of the study area varies from season to season (Fig. 6(b)). As a result, from spring to summer, the gulf is characterized by mesotrophic conditions, during autumn oligotrophic conditions dominated, while during winter, the Amvrakikos Gulf was marginally eutrophic.

Osgood [20] describes, in his paper, that in order to deal with the variability among the three values, you should use the TSI (diff), which describes the largest difference among the three TSI values. According to Osgood's classification, the best way to determine the trophic conditions in environments with high TSI (diff) is to use the TSI (TP). As a result, according to TP-TSI, we can assume that the Amvrakikos Gulf is



Fig. 6. Seasonal variations of trophic state index in Amvrakikos Gulf between 2009 and 2010 (E—eutrophic, M—mesotrophic, and O—oligotrophic).

mesotrophic from spring to autumn and a seasonally eutrophic gulf in winter, where nonalgal particulates and color dominant light attenuation are present.

This TSI takes into consideration only the measurements from the epilimnion, where those variables (Secchi depth, chl-a, and TP) do not show significant differences. Thus, it does not take into account the environments where there is phosphorus release from the sediment. The Amvrakikos Gulf is a system with important phosphorous release from the bottom as it is shown in Fig. 4, and as a result, the TSI produces a diminished value in comparison with the index's actual value in the gulf. So, Fig. 6(b) presents another distribution of TSI (TSI [entire water column]), that takes into account the TP measurements from the entire water column and according to that, the Amvrakikos Gulf could be characterized as a eutrophic gulf throughout the year.

#### 4. Discussion and conclusions

To conclude, the Amvrakikos Gulf is strongly stratified almost throughout the entire year, due to salinity or/and temperature fluctuations. The surface salinities increased from north to south as a result of the two rivers' impact in the northern part, while the temperature increased from west to east. From the data observation and in comparison to previous studies, it is apparent that the influence of the rivers is increased over the years, especially during the winter. This can also be confirmed, by the surface salinities which were less in 2009 (12–20‰) than they were in 1989 (26–33‰) [14].

Taking into account DO measurements from 1989 [21] and according to Kountoura and Zacharias [22], the DO conditions in the study area of the Amvrakikos Gulf have been gradually degraded over the last 20-30 years. This is also confirmed by the DO measurements, as the eastern seasonally hypoxic area in 1989 was converted to seasonally anoxic in 2009. On the other hand, in the western part of the gulf, major changes in the DO conditions were not observed. Due to the lack of information about the circulation patterns in the Amvrakikos Gulf, however, we can only assume that the maintenance of dissolved oxygen conditions in the western part of the gulf possibly resulted from the direct connection with the open sea (Ioanian Sea), whose waters are characterized by higher DO concentrations.

In 1989, the highest surface chl-a concentrations were detected in spring ranging from 21  $\mu$ g L<sup>-1</sup> in the northern part to 44.8  $\mu$ g L<sup>-1</sup> near the entrance of the gulf. This distribution may be attributed to the fact that the use of pesticides was particularly

increased to that of the past. As a result, the gulf received a larger amount of nutrients through the two rivers, which in combination with the relative low salinities in spring favored the phytoplankton growth and resulted in higher chl-a concentrations [8]. Nevertheless, in recent years, due to the reduction of the agricultural activities in the broader watershed, rivers discharged less amounts of nutrients than those of previous years and this resulted in less phytoplankton growth and smaller chl-a concentrations in spring 2009.

We can conclude that the study area is a mesotrophic and seasonally eutrophic gulf, where nonalgal particulates and color dominant light attenuation are present. On the one hand, the chl-a concentrations were less in 2009 than they were in 1989. On the other hand, the increasing use of fertilizers from agriculture, the pollution load from fish farming, the phosphate geological layers discharged through the Louros river into the Amvrakikos Gulf, and the wastes from the cities around the gulf increase the nutrient pollution of the Amvrakikos Gulf. In addition, the lack of similar measurements from previous years and the fact that the TSI produces a diminished value in comparison with the index's actual value in the gulf do not allow us to draw a conclusion about the future trend of the gulf's environmental conditions.

Finally, there is urgent need for greater accuracy in the determination of the gulf's eutrophication conditions in the future, using nitrogen measurements and another more appropriate index for intermediate waters. In addition, a more detailed study of the hydrodynamic circulation of the Amvrakikos Gulf is necessary; in order to better determine its contribution to the oxygenation of the water column.

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