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Anoxic monimolimnia: Nutrients devious feeders

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Abstract

This study focuses on the role of the meromictic anoxic basins' internal load: a) during storm events and b) under the environments' typical stratification conditions. Measurements of physicochemical parameters, nutrients, chlorophyll and hydrogen sulfide, four days after an anoxic crisis in Aitoliko basin as well as data obtained from a biennial basin's monitoring, were used. The relationships between temporal nutrient variations in the surface layer of an anoxic basin with the changes on its water column physicochemical characteristics, the changes on the bottom water phosphorus and nitrogen concentration and their effect on the basin's primary productivity, were studied.

In coastal environments, storm events could result in water column total mixing. This disturbance affects almost all the ecosystem's physical, chemical and biological parameters. The basin becomes anoxic, massive fish kills occur and H_2S, PO_4^{3-} and NH_4^+ release from bottom waters to the interface and surface waters promoting algal blooms. Bottom layer can supply the surface waters with nutrients, even during periods of high water column stratification. Small scale, usually subtle, changes in physicochemical and hydrological basin's characteristics promote this supply, affecting the ecosystem's primary production and shifting its quality character.

Keywords: anoxia, eutrophication, internal load, storm events, stratification, meromictic conditions

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1. Introduction

Coastal regions are under strong human influence which is reflected into their water quality. Oligotrophic estuaries and coastal systems have changed into mesotrophic and/or eutrophic, as shown by an increase in toxic algal blooms, hypoxic/anoxic events, and massive mortalities of many aquatic and benthic organisms. The relevance between the worldwide eutrophication increase and the rapid spread of the hypoxic/anoxic environments is confirmed during the last decades. The increase of organic and nutrient loading of the coastal zone, has caused the enhanced deterioration of oxygen conditions depleted in environments where anoxia/hypoxia is a natural consequence of their morphology and hydrodynamics (Vollenweider et al., 1992; Petricoli et al., 1996; Sorokin, et al., 1996a; 1996b; Druon et al., 2004; Diaz, 2008; Rouso et al., 2009; Kemp et al., 2009; Zhang et al., 2010; Rabalais et al., 2010).

In strongly stratified and productive water basins, bottom water dissolved oxygen is depleted due to the excessive organic matter decomposition in these depths. Distribution and recycling of nutrients in the overlying water column is inextricably dependent on oxygenation and redox conditions. Nitrates (NO_3^-) are the predominant nitrogen form in oxygenated epilimnia, while nitrites (NO_2^-) can be detectable throughout the water column with rather low concentrations. NO_2^- profiles usually presents peaks near the oxic/anoxic interface. Their levels can go through a maximum in the oxic waters as part of the ammonium (NH_4^+) oxidation and NO_2^- can be formed again because of the NO_3^- use as an oxidant in anoxic waters. Bottom water anoxia accelerates phosphates (PO_4^{3-}), ammonium (NH_4^+) and hydrogen sulphide (H_2S) generation and recycling with the accumulation from organic matter decomposition. PO_4^{3-} is also released into the pore water and diffused into the overlying bottom water when iron (oxy)hydroxides in the sediments are since to FeS because FeS minerals do not bind PO_4^{3-} (Rozan et al., 2002; Diaz and Rosenberg, 1995; Rabalais, 2002; Luther et al., 2004).

In permanently stratified water basins, the isolated bottom layers are not renewed under the typical environments' conditions. Storm events (e.g. prolonged and severe winds) could result in stratification destruction and water column total mixing. The water column turnover brings large amounts of H_2S to the surface resulting in low levels of oxygen throughout the water column and H_2S in the surface water, which are the conditions necessary for fish kills. Hydrogen sulfide release into shallower depths is accompanied by PO_4^{3-} and NH_4^+ release from bottom waters to the interface and surface waters promoting algal blooms. Thus more organic matter is produced fueling anoxia (Dassenakis et al., 1994; Fallesen et al., 2000; Kršinić et al., 2000; Astor et al., 2003; Luther et al., 2004; Brandi et al., 2008; Njiru et al., 2010). The decisive influence of the internal load (accumulated H_2S, PO_4^{3-} and NH_4^+) in the ecosystem's function has been reported by numerous researchers in different types of water environments, such as the salty lake Rogoznica in Croatia (Baric et al., 2003), the Mariager fjord in Denmark (Fallesen et al., 1999), the internal bays of Delaware in USA (Luther et al., 2004), the Aitoliko lagoon in Greece (Dassenakis et al., 1994; Leonardos and Sinis, 1997; Demetriou et al., 2010) and the Tapi estuary in India (Ram et al., 2014).

Water column balance is usually restored in a relatively short period of time, after a holomictic event, under the influence of local meteorological and hydrological processes. Geochemical processes equilibrium is initiated by dissolved oxygen concentrations being reset to the normal levels and this take longer, but not more than a few months. Environmental recolonisation, starts soon after the physicochemical conditions return to normal, but requires several years before the ecosystem be restored to the former conditions. An ultimate biological recovery is usually unattainable (Leonardos and Sinis, 1997; Fallesen et al., 2000; Baric et al., 2003).

The question now becomes, is whether the balance of an anoxic water ecosystem is under the threat of its hypolimnetic nutrient and sulfide load only in the case of storm events and water column total mixing.

In polymictic water basins it is clear that the accumulated nutrients in the bottom layer will supply surface waters after the pycnocline overturns. Besides this mechanism of basin water quality degradation, it is now recognized as a one of the largest obstacles in eutrophic environmental management and restoration efforts (Conley et al., 2009). The role of internal load, in permanently stratified water basins, is not so clear. In the Baltic Sea, one of the largest anoxic environments of the world, internal nutrient load, is implicated for the surface layers replenishment through vertical

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mixing processes, exacerbating the basin's eutrophic conditions caused by the high external nutrient and organic inflows in the Baltic Sea. Quantitative data cannot fully support this scenario and therefore further research is required (Reissmann et al., 2009).

In the present study, the impact of storm events on water column stability and bottom water hypoxia/anoxia of enclosed coastal basins is investigated. The importance of internal loading (H_2S, PO_4^{3-} and NH_4^+) has emerged. This leads to the disturbance on the main nutrients, dissolved oxygen, hydrogen sulfide and chlorophyll distribution, following total water column mixing. Additionally, the relationship between temporal nutrients variations in surface layers, of permanent anoxic coastal basins with; a) changes in the physicochemical characteristics of the water column, b) changes in the bottom water phosphorus and nitrogen concentrations, and c) their effect on the basin's primary productivity, are studied.

In order to achieve the objectives of this study, two different sets of Aitoliko basin's data were used. The first one includes measurements of physicochemical parameters, nutrients, chlorophyll and sulfides, four days after a storm event and the consequent anoxic crisis in Aitoliko basin on 4th of December 2008. The second one contains a similar data set obtained from a biennial (May 2006-May 2008) Aitoliko basin monitoring.

2. Materials and methods

2.1. Study area

Aitoliko is a semi-enclosed basin in western Greece. It covers an area of about 16 km^2 and its maximum depth is 27.5m. It is characterized as non-typical lagoon by its depth and the fact that its longitudinal axis is perpendicular to the shoreline. Aitoliko basin communicates southerly with a typical shallow lagoon (Messolonghi lagoon) with mean depth of about 0.5 m. The lagoonal system communicates southerly with Patraikos Gulf (maximum depth 100 m) (Fig. 1A). The two lagoons are connected through shallow and narrow openings under the bridges that connect the town of Aitoliko with the mainland.

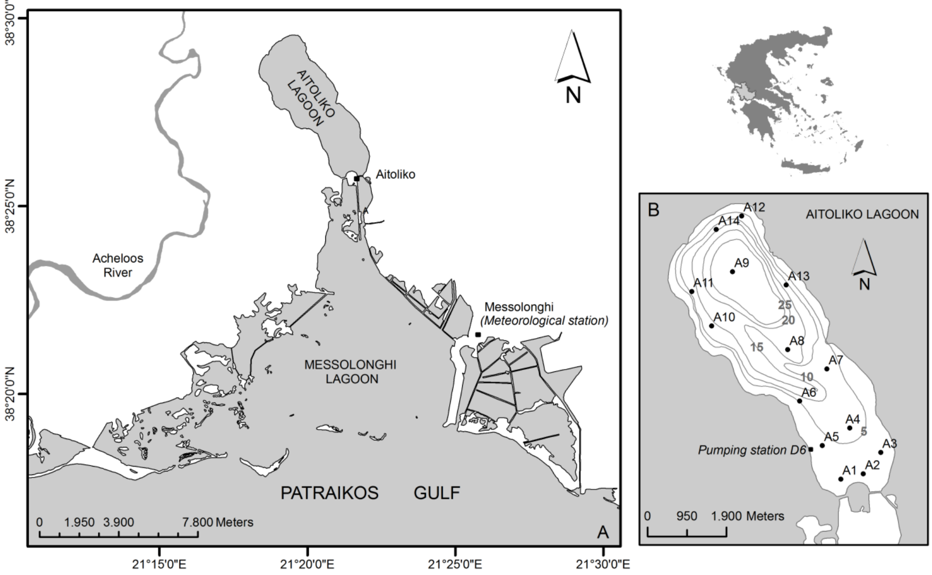

Fig. 1. (A) Map of the extended study area. (B) Sampling stations in Aitoliko basin.

Aitoliko is a permanently highly-stratified coastal basin with its isolated bottom water known to be anoxic and sulfidic. The large fresh water inflows are mainly arise through a pumping station (Fig. 1B) which is located near the basin's sill. The inflows are implicated both for the basin's permanent stratification and for the deterioration of water fluxes with its source basin (Messolonghi lagoon).

2.2. Data collection

Four days after the storm event of the 4th of December 2008, a sampling cruise was carried out in Aitoliko basin. During this cruise, continuous profiles of physicochemical parameters such as temperature, conductivity, dissolved oxygen, redox potential and pH were measured in situ using a Troll 9500 water quality multi-parameter instrument, at a network of fourteen (14) sampling sites (Fig. 1B). Water samples were collected from the deepest part (sampling site A_9) of the Aitoliko basin (Fig.1B), with 5 meters vertical intervals; using a 2.5 l Hydro Bios free flow sampler, during that cruise. Water samples for sulfides, nutrient and chlorophyll determination were brought to the laboratory in a portable refrigerator at 4ºC.

Identical data were colected, on a monthly base, during a biennial (May 2006-May 2008) monitoring of the Aitoliko basin.

There were no direct meteorological observations within the Aitoliko lagoon. Therefore, in order to assess the effect of the wind on the lagoon's hydrography, wind measurements from a station on the nearby Messolonghi town were used. This station is approximately 10 kilometres southeast of Aitoliko town (Fig. 1A). Wind speed and wind direction time series, with 10 minutes temporal resolution, were available. Daily means were calculated and studied for the analysis of the wind time series.

2.3. Data processing

Measured temperature and conductivity data were corrected by a low pas filter to minimize sharp spikes in salinity for the short-term mismatch of the sensor responses between temperature and conductivity, using Matlab. Any spikes remaining in the salinity data were removed by calculating a 1m running average. The despiked temperature and salinity data were used to construct density (sigma-t) profiles.

In practice, density was not measured; it was calculated from *in situ* measurements of pressure, temperature, and conductivity using the equation of state for sea water. For simplification, physical oceanographers often quote only the last 2 digits of the density, a quantity they call *density anomaly* or *Sigma* (*s, t, p*):

$$\sigma\,(s,t,p) = \rho\,(s,t,p) - 1\ \mathrm{g/cm^3} \qquad (1)$$

When ocean surface layers are studying, compressibility can be ignored, and the sigma-t quantity (written σ_t) can be used:

$$\sigma_t = \sigma\,(s,t,0) \qquad (2)$$

This is the density anomaly of a water sample when the total pressure on it has been reduced to atmospheric pressure (*i.e.* zero water pressure), but the temperature and salinity are *in situ* values.

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In this study the *Brünt–Väisälä frequency* or the *static stability frequency* was used, evaluating the strength of density stratification in Aitoliko lagoon water column. The frequency quantifies the importance of stability, and it is a fundamental variable in the dynamics of stratified flow. In simplest terms, the frequency can be interpreted as the vertical frequency excited by a vertical displacement of a fluid parcel.

In the ocean where salinity is important, the *Brünt–Väisälä frequency* (N^2) is expressed by the equation:

$$N^2 = -\frac{g}{\rho}\frac{d\rho}{dz} \qquad (3)$$

where ρ, the potential density, depends on both temperature and salinity, and g the acceleration of gravity.

2.4. Water samples storage and analysis

Water samples for sulfides (S^{-2}) measurements were always the first taken from the sampling bottle immediately after the sampler was back on board, taking special care to obtain samples with minimum aeration. To preserve samples for total sulfide determination, zinc acetate and sodium hydroxide solutions were put into the bottles before filling them with sample. Four drops of 2N zinc acetate solution per 100ml sample were used, while the final pH was always at least 9. The bottles were always filled completely and stoppered immediately. Water samples for sulfide analyses were measured unfiltered and freshly within 24h using the iodometric method (APHA, 1998).

The different nitrogen forms, ammonia, nitrite and nitrate, were determined using the indophenol blue, the colorimetric and the cadmium reduction method respectively. The ascorbic acid method was used in order to determine orthophosphates in the water samples (APHA, 1998).

Chlorophyll, acetone extraction was following as soon as the samples were transferred to the laboratory and about twenty hours later chlorophyll-a, b and c was determined using the trichromatic colorimetric method (APHA, 1998).

3. Results

3.1. Nutrients, chlorophyll and water quality characteristics of the Aitoliko basin

In this section, the data obtained during the biennial Aitoliko basin's monitoring are presented, focusing on the analyses of nutrients, chlorophylls and sulfides spatiotemporal variations.

Relatively low concentrations of ammonium were determined at the surface layer of the Aitoliko basin throughout the sampling period. More specifically, the average NH_4^+ concentrations ranged between 0.1mg/l and 0.5mg/l at the upper 10m of the basin's water column. From this depth to the basin's bottom a sharp increase of the ammonium concentrations was recorded, while the maximum value of about 12mg/l was determined at the deepest sampling depth of 25m (Fig. 2A).

Focusing on the basin's epilimnion, remarkable temporal changes in ammonium concentration were recorded. The relative high values, of about 0.25-0.5mg/l, identified in the surface layer of the Aitoliko basin during the summer and autumn months of 2006 was sharply reduced during the winter period 2006-2007 taking values in the range of 0.14-0.28mg/l. Comparable NH_4^+ concentrations (mean value of about 0.2mg/l), were measured in all the surface samples from the spring to the autumn of 2007. A further reduction of the determined ammonium values was observed during winter 2007-2008. In the March 2008 surface samples, 0.11mg/l NH_4^+ (mean value) were measured (Fig. 2B). During the sampling period, characteristic temporal changes in the NH_4^+ concentrations were recorded in the Aitoliko basin bottom layer as well. In particular, NH_4^+ concentration of up to 10mg/l were determined at the maximum sampling depth (25m) from May to October, while in the following winter months ammonium concentration decreased to below 8mg/l. From the first spring months of 2007, NH_4^+ concentrations in the basin's hypolimnion returned to the summer 2006 level, while a slight increase in its values (maximum concentration of 12mg/l) was observed during spring of 2008 (Fig. 2A).

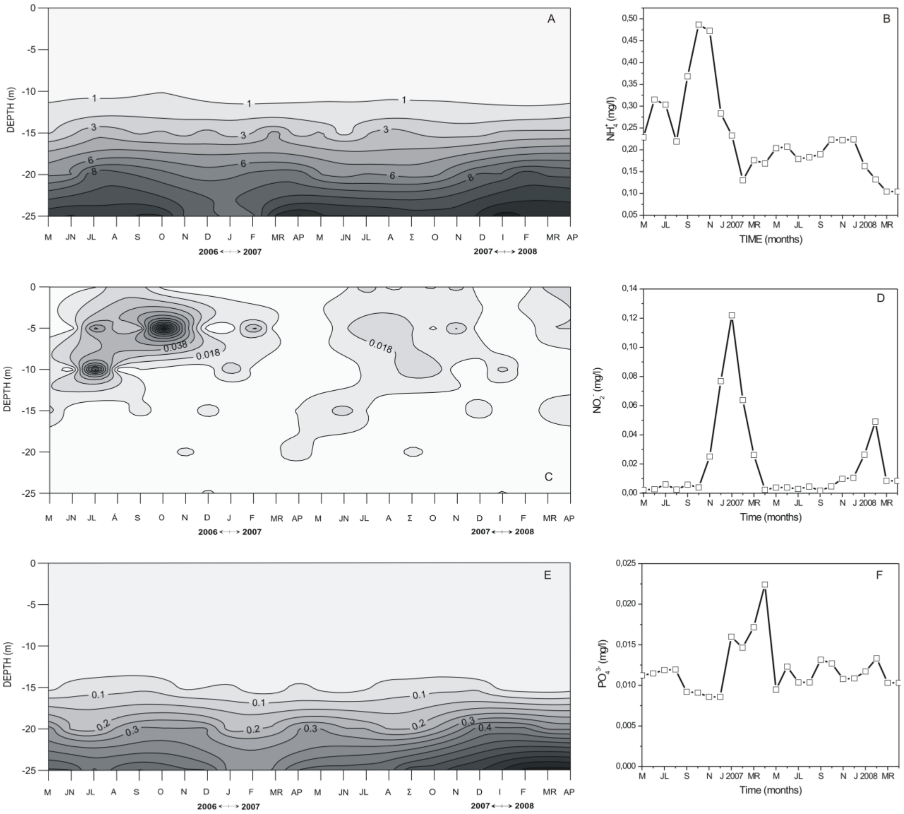

Fig. 2. (A) Depth – time diagram of ammonium concentration (NH_4^+) in mg/l, in the deepest sampling station A_9 during the period May 2006-May 2008. (B) Monthly variation of mean NH_4^+ epilimnetic values during the period May 2006-May 2008. (C) Depth – time diagram of nitrate concentration (NO_3^-) in mg/l, in the deepest sampling station A_9 during the period May 2006-May 2008. (D) Monthly variation of mean NO_2^- epilimnetic values during the period May 2006-May 2008. (E) Depth – time diagram of phosphate concentration (PO_4^{3-}) in mg/l, in the deepest sampling station A_9 during the period May 2006-May 2008. (F) Monthly variation of mean PO_4^{3-} epilimnetic values during the period May 2006-May 2008.

Nitrates were detectable only in the basin's surface layer. The measured concentrations varied from 0mg/l to 0.06mg/l, with the maximum values to characterize the depths of 5m and 10m in July and October of 2006. The measured NO_3^- concentrations characterized by seasonal variation, with the highest measured values to be recorded during the period July-November for both years of basin's monitoring. The second year maximum was significantly lower than the first year corresponding one (Fig. 2C).

Two seasonal maxima were observed in the vertical distribution of nitrites throughout the Aitoliko basin water column. The first one characterized the basin's surface layer during the period November 2006-March 2007. The maximum concentration of about 0.18mg/l was determined in the depth of 0m, in January 2007. The second one characterized the period December 2007-March 2008. During that time, nitrite concentrations in the Aitoliko basin's surface layer (maximum concentration ~0.05mg/l) were significantly lower than those determined during the corresponding period of the first monitoring year. In all the other samples, nitrite concentrations were lower than 0.01mg/l (Fig. 2D).

Relatively low values of orthophosphates characterized the surface layer of the Aitoliko basin throughout the sampling period. More specifically, the average PO_4^{3-} identified in the surface 10m of the Aitoliko basin water column varied from 0.005mg/l to 0.023mg/l. A remarkable increase on PO_4^{3-} concentration, in the 2006-2007 winter period, was recorded at the Aitoliko basin epilimnion. The maximum values (~0.023mg/l PO_4^{3-}) were identified in the first spring months of 2007; while in May onwards the mean epilimnetic PO_4^{3-} values were 0.012mg/l (Fig. 2E). A sharp increase of the phosphate concentrations with depth was recorded, and a maximum value of about 0.75mg/l was determined at the deepest sampling point of 25m. Just as in the case of the ammonium measured values, orthophosphate concentrations in Aitoliko basin bottom layer, varied temporally. In particular, PO_4^{3-} concentrations up to 0.5mg/l were determined at the maximum sampling depth (25m) during the summer and autumn months of 2006. In the winter period of the first sampling year, a slight decrease in the PO_4^{3-} concentration was recorded (maximum concentration of 0.45mg/l). From the first spring months of 2007, PO_4^{3-} concentrations in the basin's hypolimnion returned to the summer 2006 level, while a slight increase in its values (maximum concentration of 0.75mg/l) was observed during spring of 2008 (Fig. 2F).

As expected, higher chlorophyll-a concentrations characterized the surface layer of the Aitoliko basin throughout the sampling period. More specifically, during the summer months of

2006, chlorophyll-a concentrations higher than 12μg/l were determined at the depth of 10m, while the 5 surface meters were characterized values lower than 4μg/l. At the deep layer (below the depth of 15m) chlorophyll-a values varied from 1μg/l to 3μg/l. In October, November and December of 2006 at the entire basin's water column notably low chlorophyll-a concentrations were determined. Since January 2007, a gradual increase was recorded in the measured chl-a values. In the winter period, chl-a concentration increases were noticeable at the 5 surface meters, while during the spring months the higher values were determined at the depth of 10m. Concentrations higher than 22μg/l were measured at the depth of 10m in April 2007, when the chlorophyll-a values of the basin's shallower layer were about 10μg/l. During the summer period high chlorophyll-a concentrations up to 18μg/l were determined at the depth of 10m. In agreement with the first sampling year, chlorophyll-a distribution, low chl-a concentrations characterized the entire basin's water column in the late fall of 2007, while an increasing trend of the parameter's values at the surface of Aitoliko was evident during the first months of 2008. In the early spring of 2008 a chlorophyll-a concentration of 7.4μg/l was measured at the depth of 10m (Fig. 3A).

The seasonality of chlorophyll-a distribution in Aitoliko water column characterized chlorophyll-b variations as well. The surface 5m, layer of the Aitoliko basin was characterized by chlorophyll-b concentration lower than 5μg/l throughout the sampling period. From June to October 2006 high concentrations of chlorophyll-b were identified in the depth of 10m, with the maximum value of 43μg/l to be measured at this depth in August. Nearly zero chl-b concentrations were identified throughout the basin's water column during the winter period 2006-2007. In April 2007, 61μg/l chl-b was identified at the depth of 10m, whereas high concentrations were determined at this depth throughout the spring and summer period of 2007. From October 2007 to April 2008 notably low chlorophyll-b values characterized the entire Aitoliko basin water column (Fig. 3B).

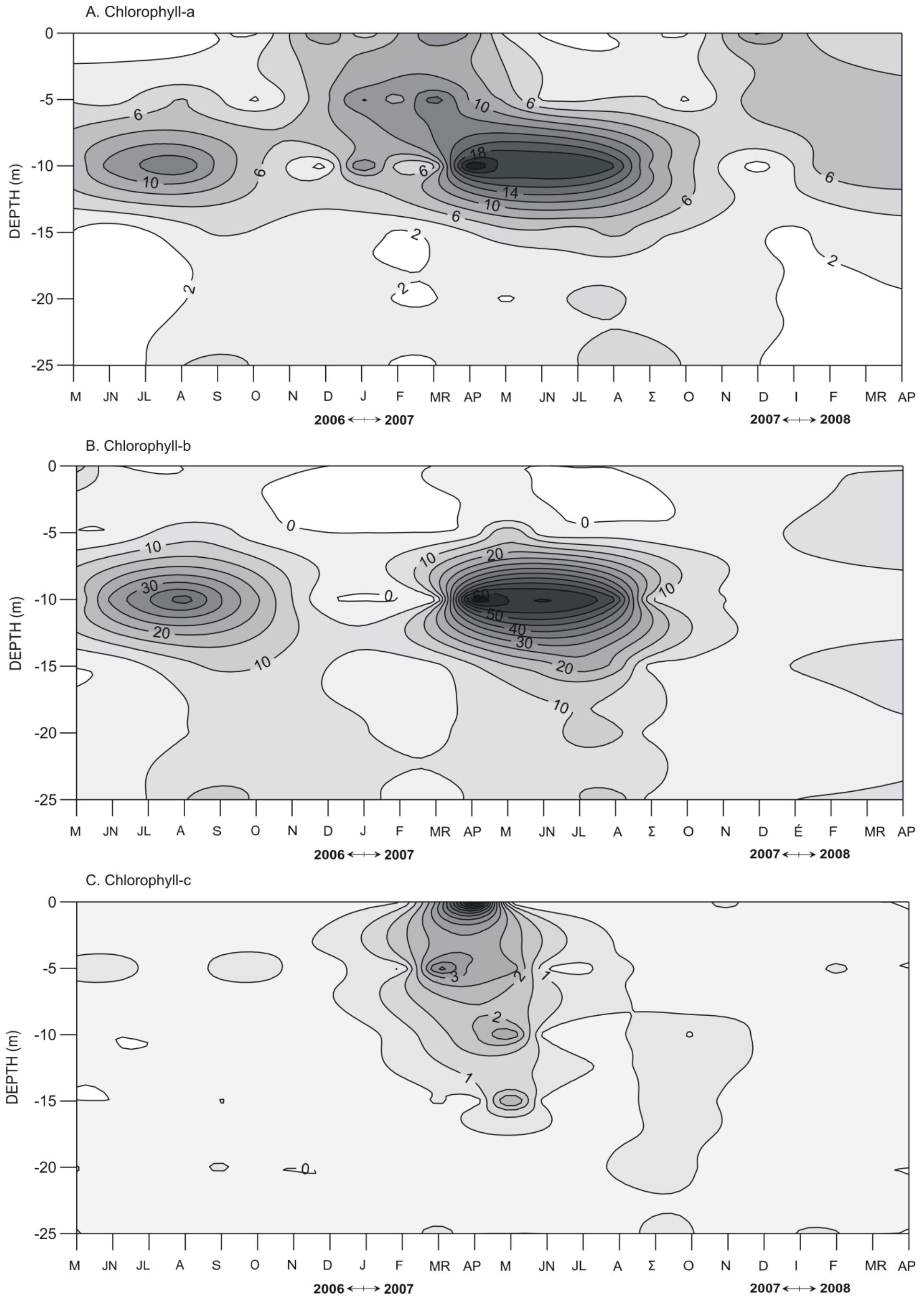

Fig. 3. Depth – time diagram of chl-a (A), chl-b (B) and chl-c (C) in μg/l, in the deepest sampling station A_9 during the period May 2006-May 2008.

Chlorophyll-c distribution was differentiated from those of chl-a and b. In general, low concentrations (0-0.5μg/l) of chlorophyll-c were determined in Aitoliko basin. Only exception was the spring of 2007 during which relative high values of chl-c determined up to the depth of 15m. The maximum concentration characterized April 2007, where about 9μg/l was determined on the basin's surface (0m) (Fig. 3C).

During the biennial monitoring period, accumulated sulfides reached concentrations up to 56.8mg/l near the bottom. In table 1 the determined sulfide concentrations at the maximum sampling depth (25m) throughout the sampling period (May 2006-May 2007), are presented. The higher sulfide values characterized the summer periods of both sampling years, while reduced concentrations were determined on February 2007 (31mg/l) and February 2008 (30mg/l).

Table 1. Sulfide concentrations at the maximum sampling depth (25m) throughout the sampling period (May 2006-May 2007). Mean monthly D_6 pumping station, discharge, during the period May 2006-May2008.

Year	**Month**	**Sulfide concentration A9 (25m) (mg/l)**	**D_6 Discharge (x10^6 m^3/month)**
2006	M	41,1	1,02
	JN	41	1,16
	JL	47,6	1,33
	AU	42,6	1,65
	S	47,8	1,23
	O	48,8	0,77
	N	50,8	0,71
	D	50,8	0,69
2007	J	49	0,63
	F	31	0,56
	MR	44,24	0,70
	AP	52,2	0,78
	M	52,92	0,72
	JN	46,12	0,69
	JL	55,16	0,64
	AU	51,8	0,64
	S	39,4	0,66
	O	46,1	0,78
	N	50,67	0,85
	D	55,6	0,80
2008	J	55,36	0,74
	F	30	0,71
	MR	44,6	0,87
	AP	55,48	0,98
	M	56,8	1,28

3.2. The storm event of December 2008

Description of the event: On the 4th of December, easterly to southerly winds, with speed up to 50km/h were blowing in the area of Aitoliko basin (Fig. 4). These winds caused, the forcible enter of water from Messolonghi lagoon into Aitoliko basin disturbing its water column balance and leading to a holomictic event.

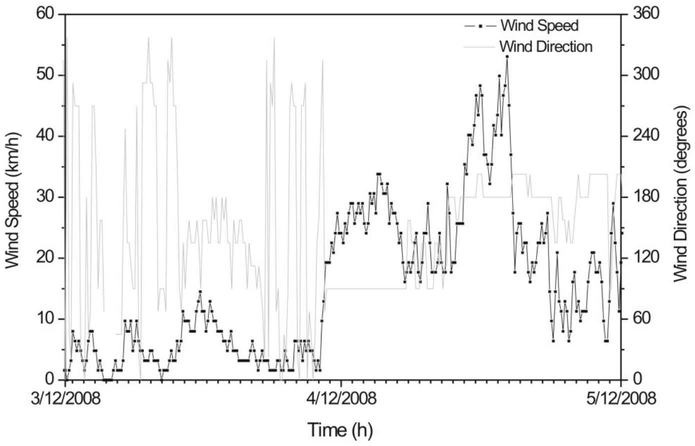

Fig. 4. Wind speed and wind direction, during 3-4/12/2008.

When the wind stopped, low dissolved oxygen concentrations were reported at the south part of the basin (Dimitriou et al., 2010) and zero oxygen was recorded in the northern part. This represents the spatial character in this anoxic crisis. During the following hours, birds were observed on the surface of the water feeding, while fishes which had reached the shore were gulping at the surface of the water in an attempt to obtain oxygen. The fish kills, that followed this storm event reflected the direct impact of the anoxic crisis in the ecosystem's biology.

Physical-chemical parameters: Just after the holomictic event, the basin's water column was nearly homogenous and quite unstable. After an intense storm capable for basin's mixing, water column static stability is reduced at all depths. Alternating of low negative and positive static stability values illustrated water column instabilities and indicated the mixing depth in the Aitoliko basin water column (Fig.5A).

Slight differences between surface and bottom temperature, salinity and density (sigma-t) values were recorded in Aitoliko basin water column four days after the anoxic crisis of the 4th of December 2008. The measured surface temperature, salinity and sigma-t were about 15.5ºC, 24‰ and 16.5 respectively, when the corresponding bottom values were just about 16.5ºC, 25‰ and 17.5. In the vertical distributions of these parameters, residual characteristics of the Aitoliko basin stratification were retained (Fig. 5B, C, D).

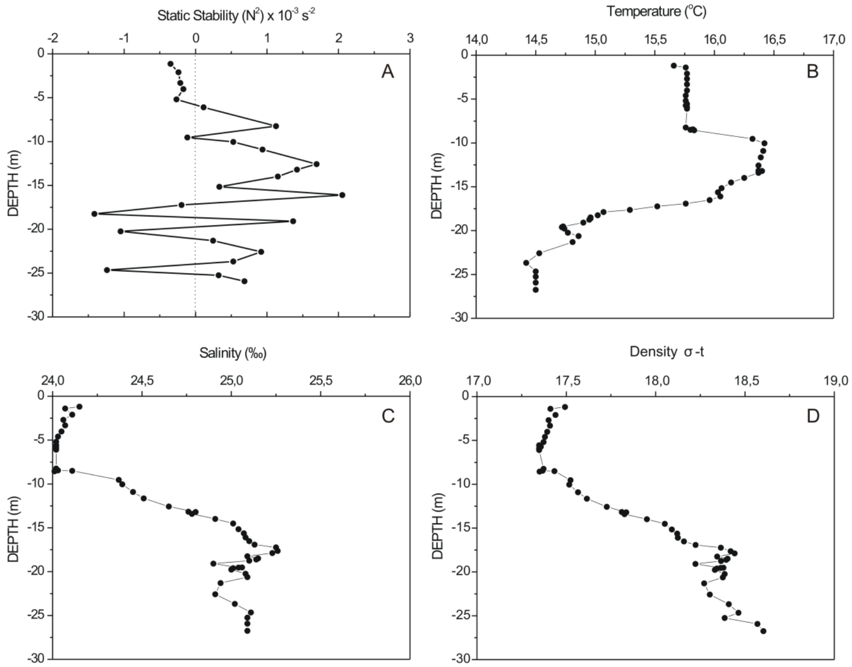

Fig. 5. Vertical profiles of static stability (A) temperature (B), salinity (C) and density (sigma-t) (D) in the deepest station A_9 during December 2008.

During the sampling of the 8th of December 2008, a well oxygenated surface layer down to about 5m deep was recorded at the southern and the central part of the Aitoliko basin. Below that depth dissolved oxygen concentrations decreased rapidly. The hypoxic and anoxic zones were spatial related and reached their maximum extent in the northern part of the lagoon (Fig. 6A). It is interesting to mention that the hypoxic zone extended below 14m, 8m and 4m at A_9, A_{12} and A_{14} stations respectively. Concurrently, zero oxygen concentrations were measured below 18m, 11m and 10m at A_9, A_{12} and A_{14} stations respectively.

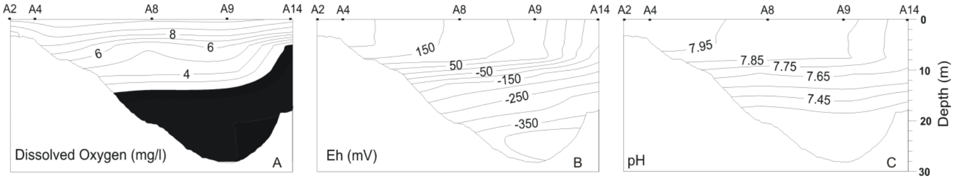

Fig. 6. Dissolved oxygen (A), Eh (B) and pH (C) vertical distribution across A_2-A_4-A_8-A_{14} cross section, during December 2008.

Redox potential and pH values were in accordance with the vertical and spatial distribution of dissolved oxygen (Fig. 6B, C). At the northern part of Aitoliko lagoon lower surface redox potential (~-80) and pH (~7.6) values existed, compared to the rest of the Aitoliko basin. Moreover, bottom layer redox potential decreased to about -400mV, reflecting pH (~ 7.3-7.4) and dissolved oxygen decreases.

Nutrients, sulfides and chlorophyll: During the holomictic period (December 2008), nitrates and nitrites were below the detection limits throughout the Aitoliko basin water column. The mean ammonium concentration determined at the 10 surface meters was about 0.25mg/l, while a maximum value of 3.67mg/l was characterized the 20m depth. A slight decrease of NH_4^+ (3.09mg/l) was recorded at greater depths (Fig. 7A).

Orthophosphates vertical distribution after the anoxic crisis is presented in Fig. 7B. Down to the depth of 15m the measured PO_4^{3-} concentration was lower than 0.06mg/l. A peak of 0.3mg/l was determined at the depth of 20m while a slight decrease (0.23mg/l) of orthophosphate concentrations characterized greater depths.

About 0.8mg/l sulfides were determined in the 10 meter surface waters, while a maximum value of 33mg/l was characterized the 20m depth. A slight decrease of sulfides concentration (31mg/l) was recorded at greater depths (Fig. 7C).

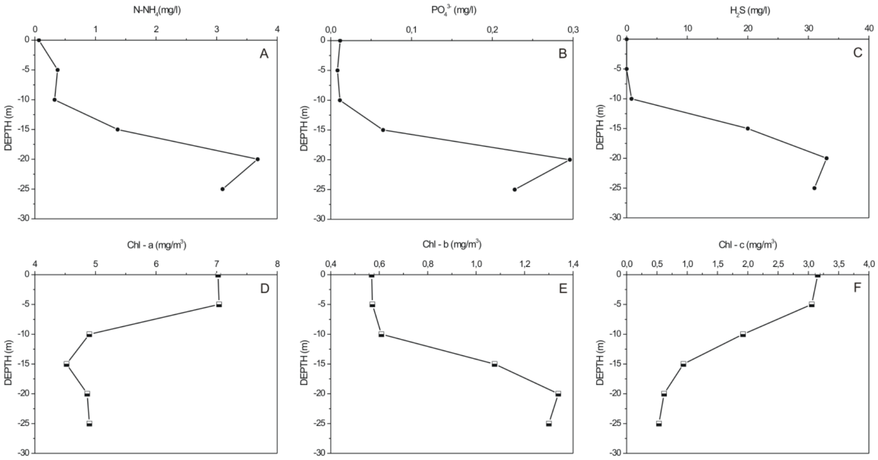

Fig. 7. Vertical profiles of ammonia (A), phosphate (B), sulfides (C) and chlorophyll-a, b, c (D, E, F) in Aitoliko lagoon during December 2008.

In the first days of December 2008, just after the total mixing event in Aitoliko basin, relative high chlorophyll-a concentrations were determined throughout the water column. The five surface meters were characterized by 7μg/l chl-a, while from this depth and deeper, 5μg/l chl-a were determined (Fig. 7D). In the surface layer 0.67μg/l chl-b were measured while at greater depths parameter's increase was recorded (Fig. 7E). In contrast, the maximum chl-c values (3μg/l) characterized the basin's surface and they decreased with depth's increase (Fig. 7F).

4. Discussion

Permanent stratified coastal basins, like Aitoliko, are characterized by anoxic and sulfidic hypolimnia. In enclosed or silled basins, anoxia occurs naturally, through their morphology and high water residence times and its origin is rarely linked with eutrophication phenomena.

Anoxia in Aitoliko lagoon is known since 18th century, as historical records are referred in total mixing events, massive fish kills and H_2S sulfide release. Measurements of physicochemical parameters of Aitoliko go back to 1951. During the period 1951-2004, bottom layer temperatures show relatively constant values while salinity values ranged about 5‰. The oxic/anoxic interface progressively decreased from a depth of 18m in 1951 to 4m in 2003-2004. During the summer period of the years 2006 and 2007, the oxic/anoxic interface developed in a depth of 17-18m. In the winter periods of the same years low dissolved oxygen concentrations were determined in Aitoliko basin's monimolimnio. This fact was ascribed to the anthropogenic deepening of the sill that connects Aitoliko and its source basin (Messolonghi lagoon). Greater amounts of dense-salty water flowed from Messolonghi lagoon, causing limited vertical mixing of the Aitoliko basin. The hydrodynamic processes controlled the small scale mixing of the basin's water column, introduced oxygen into the halocline and the bottom waters, without destroying stratification (Gianni et al., 2011; Gianni and Zacharias, 2012).

Quite a few times in the past, anoxic conditions reached the water surface and the whole water column of Aitoliko basin was anoxic for several days. Usually, during autumn or winter months after intense southern or south-eastern winds, the balance that keeps the anoxic water under the lighter well oxygenated water is disturbed. Wind enforces large quantities of salty water to enter from Messolonghi into Aitoliko basin. This dense water mass is passing under the relative lighter anoxic water of the Aitoliko basin, helping it to emerge. During these events, hydrogen sulfide, which is accumulated in the lagoon's bottom layer, is released in the upper layers killing all the aquatic organisms, while the atmosphere becomes stuffy for the local residents. The first reported mass mortality event in the Aitoliko lagoon is referred to in 1881. Since then a numerous of such events have been recorded in 1963, 1990 (Dassenakis, 1994; Leonardos and Sinis, 1997), 1992, 1995 and 2001. Such events have a decisive influence into the basin's physical, chemical and biological characteristics and processes. The most recent holomictic event in Aitoliko basin is the one described in this study and is occurred on the 4th of December 2008.

Regarding the Aitoliko basin's nutrient budget and productivity the most comprehensive overview is presented in Daneilides (1991) study. Orthophosphate, total phosphorus, ammonium, nitrite, nitrate and chlorophyll-a (chl-a) profiles were identified in the deepest part of Aitoliko basin during the period April 1984-April 1985. Nutrient concentrations in the Aitoliko basin's surface layer were not significantly high, indicating their consumption of the planktonic organisms and their removal to greater depths through dead cells. The year average concentrations of TP, PO_4^{3-}, NH_4^+, NO_3^- and NO_2^- in this layer were approximately 0.03mg/l, 0.02mg/l, 0.2mg/l, 0.1mg/l and 0.012mg/l, respectively (Daneilidis, 1991). After the spring plankton bloom, nitrogen concentrations were significantly reduced in the epilimnion, resulting in the inorganic N to inorganic P ratio reduction. Increase of all the inorganic nitrogen forms in the winter period, rendered phosphorus the growth limiting factor. A general reduction of all nutrient concentrations and increases in chl-a values occurred, with the spring bloom onset. During the 1984-1985 period the reported spring chl-a maximum was about 10μg/l. The basin's deep layer was characterized by high ammonium, orthophosphate and total phosphorus concentrations with constant values throughout the sampling period. Specifically, about 15mg/l, 2mg/l and 2.5mg/l NH_4^+, PO_4^{3-} and TP were determined in Aitoliko basin bottom layer during the 1984-1985 period.

Under the typical meromictic conditions prevailed in Aitoliko basin, throughout the two years of monitoring (2006-2008), low ammonium, nitrate, nitrite and orthophosphate concentration were determined in the surface 10m. At greater depths where, anoxic conditions prevailed, a sharp increase in ammonium and orthophosphate concentrations were recorded. Just after the Aitoliko basin total mixing (December 2008), nitrate and nitrite were below the detection limits throughout the water column, while the measured ammonia concentrations below 15m were approximately 4 times lower than that measured under the typical meromictic conditions that commonly prevail during the winter time. This reduction is probably due to ammonium oxidation during water column mixing, as large amounts of oxygenated water flows into basin's bottom. Changes in orthophosphate concentrations after the storm event were observed as well. The orthophosphate deficiency during this time was probably caused by its removal from the water column by absorption of orthophosphate on re-oxidized mineral phases of Mn- and Fe- oxides (Sundy et al., 1992) and by precipitation of high insoluble calcium phosphate (Bjerum et al., 1958; Drever, 1982).

Bottom overturn increases the nutrient content of surface layers, enhancing algal blooms. Chlorophyll-a and algal cell abundances in surface layers are correlated with changes of the oxic/anoxic interface depth. Chlorophyll-a in surface waters decreases sharply as the oxic/anoxic interface moves deeper, while the depth of oxic/anoxic interface affects the prevalence of phytoplankton species (Ma et al., 2006). Chlorophyll-a is the most appropriate parameter to follow the growth of both algae and *Cyanobacteria*, in a coastal environment. Chlorophyll-a is a good indicator of the biomass of algae present in waters (Räike et al., 2003). The increase of nutrients in surface layers can also amplify the growth and prevalence of harmful algal species in the water column which grow quickly with division rates of 1 to 5d^{-1} (Kreiberg, 1999). Higher harmful algal cell density occurred when more H_2S developed in the bottom waters and the interface moves to shallower depths. Large blooms of dinoflagellates were recorded in the surface waters of Torquay Canal when the interface migrates upward, while diatoms prevail when anoxic conditions limited in great depths (Ma et al., 2006).

The total mixing event in Aitoliko basin in the first days of December 2008 led to chlorophyll-a concentration increases throughout the water column, comparing with the measured values under the typical meromictic conditions prevail commonly during the winter time. Changes observed in the vertical distribution of chlorophyll-b as well. Unlike the typical winter profiles, chl-b maximum concentrations were recorded at the basin's surface while a sharp decrease was observed with increasing depth. Additionally, the vertical profile of chlorophyll-c four days after the storm event was remarkable. Chl-c mainly occurs in algae which are absent from the surface layer under the typical winter conditions in Aitoliko basin. Values equal to 3μg/l were measured on 8th of December 2008. The abrupt increase in chlorophyll-c, immediately after the total mixing event, could be associated with a bloom, for example, of dinoflagellates which encouraged under these conditions. Certainly the observed changes indicate change in the composition of the lagoon's phytoplankton community, can only confirmed by analytical determination of the prevailing algal types. In this study algal community structure was not determined.

The changes induced in the physical, chemical and biological characteristics of an anoxic meromictic basin as Aitoliko, following total mixing, highlight the impact of accumulated nutrients and sulfides in the bottom layer.

However, questions arising are related to the importance of hypolimnetic nutrient and sulfide load under meromictic conditions, and its relation with the surface water quality. Trying to address out this concern, the focus was on the temporal variations of specific nutrients in the Aitoliko surface layer and their relation with; a) changes on the physicochemical characteristics of the basin's water column, b) changes on the bottom water phosphorus and nitrogen concentration, and c) their effect on the basin's primary productivity.

The relative high concentrations of ammonium characterized the surface layer of the Aitoliko basin during the summer and autumn months of 2006 and that was sharply reduced during the winter. At the same period, increase of epilimnetic nitrites concentration, (ammonium oxidation intermediate product) and decrease of the bottom layer ammonium and orthophosphate values were recorded. These changes coincide with the Aitoliko basin's hypolimnion oxygenation due to weak/small scale mixing of the water column caused by the denser water inflow from the Messolonghi lagoon in the summer months (Gianni et al., 2011). Dissolved oxygen increase in Aitoliko basin's water column may be the answer to the observed nutrient variations during that period.

In addition, during the 2006-2007 winter period, a PO_4^{3-} concentration increase in the surface layer of the Aitoliko basin, was recorded. This increase could be attributed to external sourcing with inorganic phosphorus. This scenario could be strengthened, considering that the basin's main freshwater source is the D_6 pumping station. D_6 is located near the basin's entrance and supplies Aitoliko with water from an extensive drainage network which extends to the adjacent cultivated land. The seasonal PO_4^{3-} maximum surface concentrations coincide with the minimum flow of the D_6 pumping station (Table 1), and is lagging 3-4 months behind the main fertilization period, weakening the external loading scenario. In a second scenario, the observed epilimnetic PO_4^{3-}

increase could be attributed to the surface layers enrichment from the deeper waters. The hydrodynamic processes that control the small scale mixing of Aitoliko basin water column, introducing oxygen into the halocline and the bottom waters, without destroying the stratification (Gianni et al., 2011; Gianni and Zacharias, 2012), probably also governs the nutrients transport from the deep pool.

The phosphorus concentration increases in the Aitoliko epilimnion during winter and spring months of 2007 had an immediate impact on the basin's primary productivity. A particularly high chlorophyll-a concentration was determined the spring of 2007 and that was significantly higher than those typically characterize Aitoliko basin (see spring 2006 values; Daneilides, 1991). Moreover during this period, a maximum of chlorophyll-c in the surface of the Aitoliko basin was observed providing evidence that the spring-summer phytoplankton bloom accompanied changes in the dominant planktonic types.

During the 2007-2008 winter period an additional reduction on the epilimnetic NH_4^+ concentration was recorded and accompanied increased NO_2^- concentrations. The intensities of both changes were milder than that reported for the previous sampling year. No changes were observed in the surface PO_4^{3-} concentrations while hypolimnetic NH_4^+ and PO_4^{3-} values increased during that period. The spring 2007 algal bloom increased the organic load and was enhanced in the anoxic layers of the Aitoliko basin. The decomposition of this organic matter can be linked the bottom layer nitrogen and phosphorus content rise. It becomes obvious that the Aitoliko bottom water oxygenation episode in the winter months of 2008 had different impact on the basin's water column and this is reflected in nutrient and chlorophyll profiles.

5. Conclusions

Storm events in coastal environments can result in stratification destruction and water total column mixing. In systems where anoxic/sulfidic bottom waters exist, vertical mixing leads to a basin's anoxic crisis. The massive fish kills is the result of an intense disturbance of a natural system which led to the dissolved oxygen consumption in the surface water by hydrogen sulfide the anoxic water layer and direct H_2S toxicity. Hydrogen sulfide release is accompanied by PO_4^{3-} and NH_4^+ release from bottom waters to the interface and surface waters promoting algal blooms. This disturbance affects almost all the physical, chemical and biological parameters in a water basin.

A few days after the total mixing the physical rehabilitation of the basin's water column starts. The surface layer is gradually oxygenated; indicating water column balance restoration, under the influence of the local meteorological and hydrological processes. Geochemical processes equilibrium starts by dissolved oxygen concentrations reseting to the normal levels and this takes longer but not more than a few months. Environmental recolonization starts soon after the physicochemical conditions return to normal, but requires several years before the ecosystem be restored.

The induced changes in the physical, chemical and biological characteristics of an anoxic meromictic basin, after its total mixing, highlight the impact of the accumulated nutrients and sulfides in the bottom layer.

Just as significant is the role of hypolimnetic nutrient and sulfide load in meromictic periods. The H_2S, NH_4^+, PO_4^{3-} rich bottom layer can supply nutrients to the surface waters when the physicochemical and hydrological basin's water column characteristics permit it. Nutrient and organic matter flux water column turn over affects both quantitative nature of and qualitative the basin's primary productivity and shifts the ecosystems quality character.

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