1   **Anoxic monimolimnia: Nutrients devious feeders**

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8   **Abstract**

9   This study focuses on the role of the meromictic anoxic basins' internal load: a) during

10   storm events and b) under the environments' typical stratification conditions. Measurements of

11   physicochemical parameters, nutrients, chlorophyll and hydrogen sulfide, four days after an

12   anoxic crisis in Aitoliko basin as well as data obtained from a biennial basin's monitoring, were

13   used. The relationships between temporal nutrient variations in the surface layer of an anoxic

14   basin with the changes on its water column physicochemical characteristics, the changes on the

15   bottom water phosphorus and nitrogen concentration and their effect on the basin's primary

16   productivity, were studied.

17   In coastal environments, storm events could result in water column total mixing. This

18   disturbance affects almost all the ecosystem's physical, chemical and biological parameters.

19   The basin becomes anoxic, massive fish kills occur and H_2S, PO_4^{3-} and NH_4^+ release from

20   bottom waters to the interface and surface waters promoting algal blooms. Bottom layer can

21   supply the surface waters with nutrients, even during periods of high water column

22   stratification. Small scale, usually subtle, changes in physicochemical and hydrological basin's

23   characteristics promote this supply, affecting the ecosystem's primary production and shifting

24   its quality character.

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

26   conditions

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

1   amounts of H_2S to the surface resulting in low levels of oxygen throughout the water column and

2   H_2S in the surface water, which are the conditions necessary for fish kills. Hydrogen sulfide release

3   into shallower depths is accompanied by PO_4^{3-} and NH_4^+ release from bottom waters to the interface

4   and surface waters promoting algal blooms. Thus more organic matter is produced fueling anoxia

5   (Dassenakis et al., 1994; Fallesen et al., 2000; Kršinić et al., 2000; Astor et al., 2003; Luther et al.,

6   2004; Brandi et al., 2008; Njiru et al., 2010). The decisive influence of the internal load (accumulated

7   H_2S, PO_4^{3-} and NH_4^+) in the ecosystem's function has been reported by numerous researchers in

8   different types of water environments, such as the salty lake Rogoznica in Croatia (Baric et al., 2003),

9   the Mariager fjord in Denmark (Fallesen et al., 1999), the internal bays of Delaware in USA (Luther

10 et al., 2004), the Aitoliko lagoon in Greece (Dassenakis et al., 1994; Leonardos and Sinis, 1997;

11 Demetriou et al., 2010) and the Tapi estuary in India (Ram et al., 2014).

12   Water column balance is usually restored in a relatively short period of time, after a

13 holomictic event, under the influence of local meteorological and hydrological processes.

14 Geochemical processes equilibrium is initiated by dissolved oxygen concentrations being reset to the

15 normal levels and this take longer, but not more than a few months. Environmental recolonisation,

16 starts soon after the physicochemical conditions return to normal, but requires several years before

17 the ecosystem be restored to the former conditions. An ultimate biological recovery is usually

18 unattainable (Leonardos and Sinis, 1997; Fallesen et al., 2000; Baric et al., 2003).

19   The question now becomes, is whether the balance of an anoxic water ecosystem is under

20 the threat of its hypolimnetic nutrient and sulfide load only in the case of storm events and water

21 column total mixing.

22   In polymictic water basins it is clear that the accumulated nutrients in the bottom layer will

23 supply surface waters after the pycnocline overturns. Besides this mechanism of basin water quality

24 degradation, it is now recognized as a one of the largest obstacles in eutrophic environmental

25 management and restoration efforts (Conley et al., 2009). The role of internal load, in permanently

26 stratified water basins, is not so clear. In the Baltic Sea, one of the largest anoxic environments of

27 the world, internal nutrient load, is implicated for the surface layers replenishment through vertical

1   mixing processes, exacerbating the basin's eutrophic conditions caused by the high external nutrient

2   and organic inflows in the Baltic Sea. Quantitative data cannot fully support this scenario and

3   therefore further research is required (Reissmann et al., 2009).

4   In the present study, the impact of storm events on water column stability and bottom water

5   hypoxia/anoxia of enclosed coastal basins is investigated. The importance of internal loading (H_2S,

6   PO_4^{3-} and NH_4^+) has emerged. This leads to the disturbance on the main nutrients, dissolved oxygen,

7   hydrogen sulfide and chlorophyll distribution, following total water column mixing. Additionally,

8   the relationship between temporal nutrients variations in surface layers, of permanent anoxic coastal

9   basins with; a) changes in the physicochemical characteristics of the water column, b) changes in the

10   bottom water phosphorus and nitrogen concentrations, and c) their effect on the basin's primary

11   productivity, are studied.

12   In order to achieve the objectives of this study, two different sets of Aitoliko basin's data

13   were used. The first one includes measurements of physicochemical parameters, nutrients,

14   chlorophyll and sulfides, four days after a storm event and the consequent anoxic crisis in Aitoliko

15   basin on 4th of December 2008. The second one contains a similar data set obtained from a biennial

16   (May 2006-May 2008) Aitoliko basin monitoring.

18   **2. Materials and methods**

19   *2.1. Study area*

20   Aitoliko is a semi-enclosed basin in western Greece. It covers an area of about 16 km^2 and

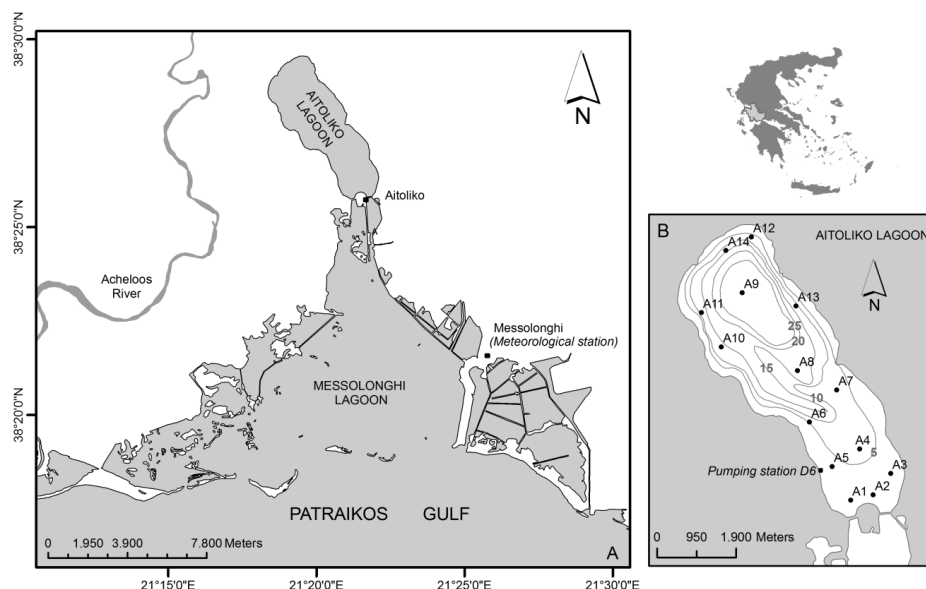
21   its maximum depth is 27.5m. It is characterized as non-typical lagoon by its depth and the fact that

22   its longitudinal axis is perpendicular to the shoreline. Aitoliko basin communicates southerly with a

23   typical shallow lagoon (Messolonghi lagoon) with mean depth of about 0.5 m. The lagoonal system

24   communicates southerly with Patraikos Gulf (maximum depth 100 m) (Fig. 1A). The two lagoons

25   are connected through shallow and narrow openings under the bridges that connect the town of

26   Aitoliko with the mainland.

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

4   Aitoliko is a permanently highly-stratified coastal basin with its isolated bottom water known

5   to be anoxic and sulfidic. The large fresh water inflows are mainly arise through a pumping station

6   (Fig. 1B) which is located near the basin's sill. The inflows are implicated both for the basin's

7   permanent stratification and for the deterioration of water fluxes with its source basin (Messolonghi

8   lagoon).

10   *2.2. Data collection*

11   Four days after the storm event of the 4th of December 2008, a sampling cruise was carried

12   out in Aitoliko basin. During this cruise, continuous profiles of physicochemical parameters such as

13   temperature, conductivity, dissolved oxygen, redox potential and pH were measured in situ using a

14   Troll 9500 water quality multi-parameter instrument, at a network of fourteen (14) sampling sites

15   (Fig. 1B). Water samples were collected from the deepest part (sampling site A_9) of the Aitoliko

16   basin (Fig.1B), with 5 meters vertical intervals; using a 2.5 l Hydro Bios free flow sampler, during

1 ~~that cruise~~. Water samples for sulfides, nutrient and chlorophyll determination were brought to the

2 laboratory in a portable refrigerator at 4°C.

3 Identical data were ~~colected~~ on a monthly base, during a biennial (May 2006-May 2008)

4 monitoring of the Aitoliko basin.

5 There were no direct meteorological observations within the Aitoliko lagoon. Therefore, in

6 order to assess the effect of the wind on the lagoon's hydrography, wind measurements from a station

7 on the nearby Messolonghi town were used. This station is approximately 10 kilometres southeast of

8 Aitoliko town (Fig. 1A). Wind speed and wind direction time series, with 10 minutes temporal

9 resolution, were available. Daily means were calculated and studied for the analysis of the wind time

10 series.

12 *2.3. Data processing*

13 Measured temperature and conductivity data were corrected by a low pas filter to minimize

14 sharp spikes in salinity for the short-term mismatch of the sensor responses between temperature and

15 conductivity, using Matlab. Any spikes remaining in the salinity data were removed by calculating a

16 1m running average. The despiked temperature and salinity data were used to construct density

17 (sigma-t) profiles.

18 ~~In practice, d~~ensity was not measured; ~~it was~~ calculated from *in situ* measurements of

19 pressure, temperature, and conductivity using the equation of state for sea water. ~~For simplification,~~

20 ~~physical oceanographers often quote only the last 2 digits of the density, a quantity they call~~ *density*

21 *anomaly* or *Sigma* (*s, t, p*):

22 $\sigma\,(s,t,p) = \rho\,(s,t,p) - 1\ \mathrm{g/cm^3}$ (1)

23 When ~~ocean surface layers are studying,~~ compressibility can be ignored, and the sigma-t

24 quantity (written σ_t) can be used:

25 $\sigma_t = \sigma\,(s,t,0)$ (2)

26 This is the density anomaly of a water sample when the total pressure on it has been reduced

27 to atmospheric pressure (*i.e.* zero water pressure), but the temperature and salinity are *in situ* values.

1   In this study the *Brünt–Väisälä frequency* or the *static stability frequency* was used,

2   evaluating the strength of density stratification in Aitoliko lagoon water column. The frequency

3   quantifies the ~~importance~~ of stability, and it is a fundamental variable in the dynamics of stratified

4   flow. In simplest terms, the frequency can be interpreted as the vertical frequency excited by a

5   vertical displacement of a fluid parcel.

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

7   the equation:

8    $$N^2 = -\frac{g}{\rho}\frac{d\rho}{dz} \tag{3}$$

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

10   gravity.

12   *2.4. Water samples storage and analysis*

13   Water samples for sulfides (S^{-2}) measurements were always the first taken from the

14   sampling bottle immediately after the sampler was back on board, taking special care to obtain

15   samples with minimum ~~aeration~~. To preserve samples for total sulfide determination, zinc acetate

16   and sodium hydroxide solutions were put into the bottles before filling them with sample. Four

17   drops of 2N zinc acetate solution per 100ml sample were used, while the final pH was always at

18   least 9. The bottles were always filled completely and stoppered immediately. Water samples for

19   sulfide analyses were measured unfiltered and freshly within 24h using the iodometric method

20   (APHA, 1998).

21   The different nitrogen forms, ammonia, nitrite and nitrate, were determined using the

22   indophenol blue, the colorimetric and the cadmium reduction method respectively. The ascorbic acid

23   method was used in order to determine orthophosphates in the water samples (APHA, 1998).

24   Chlorophyll, acetone extraction was following as soon as the samples were transferred to the

25   laboratory and about twenty hours later chlorophyll-a, b and c was determined using the trichromatic

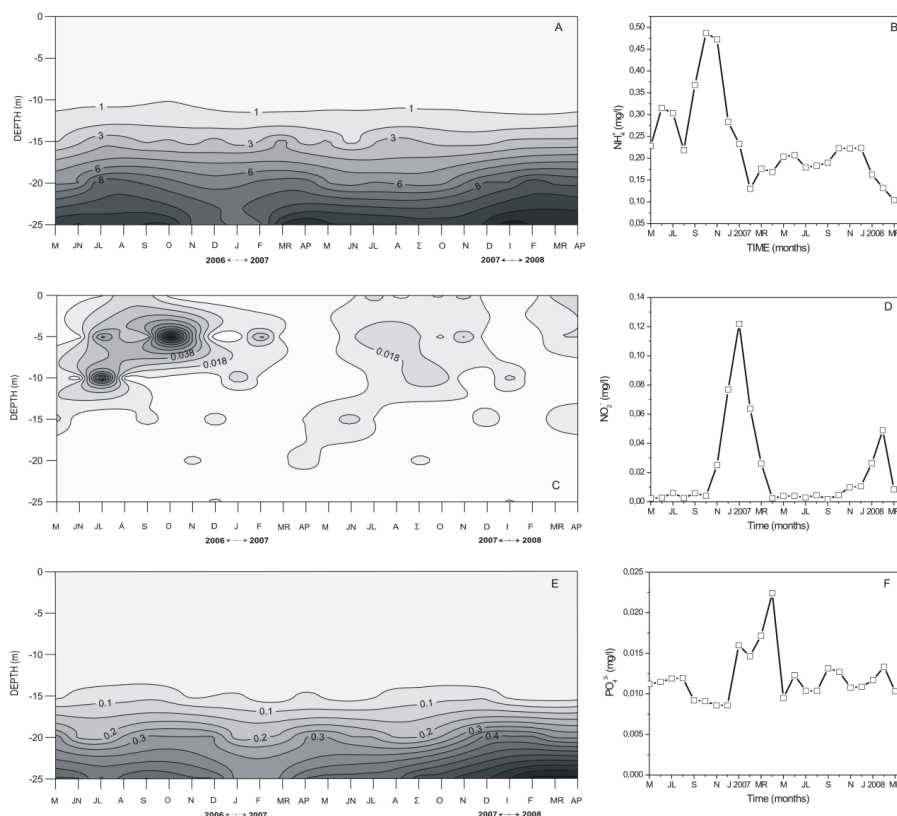
26   colorimetric method (APHA, 1998).

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).

2 Fig. 2. (A) Depth – time diagram of ammonium concentration (NH_4^+) in mg/l, in the deepest sampling station

3 A_9 during the period May 2006-May 2008. (B) Monthly variation of mean NH_4^+ epilimnetic values during the

4 period May 2006-May 2008. (C) Depth – time diagram of nitrate concentration (NO_3^-) in mg/l, in the deepest

5 sampling station A_9 during the period May 2006-May 2008. (D) Monthly variation of mean NO_2^- epilimnetic

6 values during the period May 2006-May 2008. (E) Depth – time diagram of phosphate concentration (PO_4^{3-})

7 in mg/l, in the deepest sampling station A_9 during the period May 2006-May 2008. (F) Monthly variation of

8 mean PO_4^{3-} epilimnetic values during the period May 2006-May 2008.

10   Nitrates were detectable only in the basin's surface layer. The measured concentrations

11 varied from 0mg/l to 0.06mg/l, with the maximum values to characterize the depths of 5m and 10m

12 in July and October of 2006. The measured NO_3^- concentrations characterized by seasonal variation,

13 with the highest measured values to be recorded during the period July-November for both years of

1   basin's monitoring. The second year maximum was significantly lower than the first year

2   corresponding one (Fig. 2C).

3   Two seasonal maxima were observed in the vertical distribution of nitrites throughout the

4   Aitoliko basin water column. The first one characterized the basin's surface layer during the period

5   November 2006-March 2007. The maximum concentration of about 0.18mg/l was determined in the

6   depth of 0m, in January 2007. The second one characterized the period December 2007-March 2008.

7   During that time, nitrite concentrations in the Aitoliko basin's surface layer (maximum concentration

8   ~0.05mg/l) were significantly lower than those determined during the corresponding period of the

9   first monitoring year. In all the other samples, nitrite concentrations were lower than 0.01mg/l (Fig.

10   2D).

11   Relatively low values of orthophosphates characterized the surface layer of the Aitoliko

12   basin throughout the sampling period. More specifically, the average PO_4^{3-} identified in the surface

13   10m of the Aitoliko basin water column varied from 0.005mg/l to 0.023mg/l. A remarkable increase

14   on PO_4^{3-} concentration, in the 2006-2007 winter period, was recorded at the Aitoliko basin

15   epilimnion. The maximum values (~0.023mg/l PO_4^{3-}) were identified in the first spring months of

16   2007; while in May onwards the mean epilimnetic PO_4^{3-} values were 0.012mg/l (Fig. 2E). A sharp

17   increase of the phosphate concentrations with depth was recorded, and a maximum value of about

18   0.75mg/l was determined at the deepest sampling point of 25m. Just as in the case of the ammonium

19   measured values, orthophosphate concentrations in Aitoliko basin bottom layer, varied temporally.

20   In particular, PO_4^{3-} concentrations up to 0.5mg/l were determined at the maximum sampling depth

21   (25m) during the summer and autumn months of 2006. In the winter period of the first sampling year,

22   a slight decrease in the PO_4^{3-} concentration was recorded (maximum concentration of 0.45mg/l).

23   From the first spring months of 2007, PO_4^{3-} concentrations in the basin's hypolimnion returned to the

24   summer 2006 level, while a slight increase in its values (maximum concentration of 0.75mg/l) was

25   observed during spring of 2008 (Fig. 2F).

26   As expected, higher chlorophyll-a concentrations characterized the surface layer of the

27   Aitoliko basin throughout the sampling period. More specifically, during the summer months of

1   2006, chlorophyll-a concentrations higher than 12μg/l were determined at the depth of 10m, while

2   the 5 surface meters were characterized values lower than 4μg/l. At the deep layer (below the depth

3   of 15m) chlorophyll-a values varied from 1μg/l to 3μg/l. In October, November and December of

4   2006 at the entire basin's water column notably low chlorophyll-a concentrations were determined.

5   Since January 2007, a gradual increase was recorded in the measured chl-a values. In the winter

6   period, chl-a concentration increases were noticeable at the 5 surface meters, while during the spring

7   months the higher values were determined at the depth of 10m. Concentrations higher than 22μg/l

8   were measured at the depth of 10m in April 2007, when the chlorophyll-a values of the basin's

9   shallower layer were about 10μg/l. During the summer period high chlorophyll-a concentrations up

10   to 18μg/l were determined at the depth of 10m. In agreement with the first sampling year,

11   chlorophyll-a distribution, low chl-a concentrations characterized the entire basin's water column in

12   the late fall of 2007, while an increasing trend of the parameter's values at the surface of Aitoliko

13   was evident during the first months of 2008. In the early spring of 2008 a chlorophyll-a concentration

14   of 7.4μg/l was measured at the depth of 10m (Fig. 3A).

15   The seasonality of chlorophyll-a distribution in Aitoliko water column characterized

16   chlorophyll-b variations as well. The surface 5m, layer of the Aitoliko basin was characterized by

17   chlorophyll-b concentration lower than 5μg/l throughout the sampling period. From June to October

18   2006 high concentrations of chlorophyll-b were identified in the depth of 10m, with the maximum

19   value of 43μg/l to be measured at this depth in August. Nearly zero chl-b concentrations were

20   identified throughout the basin's water column during the winter period 2006-2007. In April 2007,

21   61μg/l chl-b was identified at the depth of 10m, whereas high concentrations were determined at this

22   depth throughout the spring and summer period of 2007. From October 2007 to April 2008 notably

23   low chlorophyll-b values characterized the entire Aitoliko basin water column (Fig. 3B).

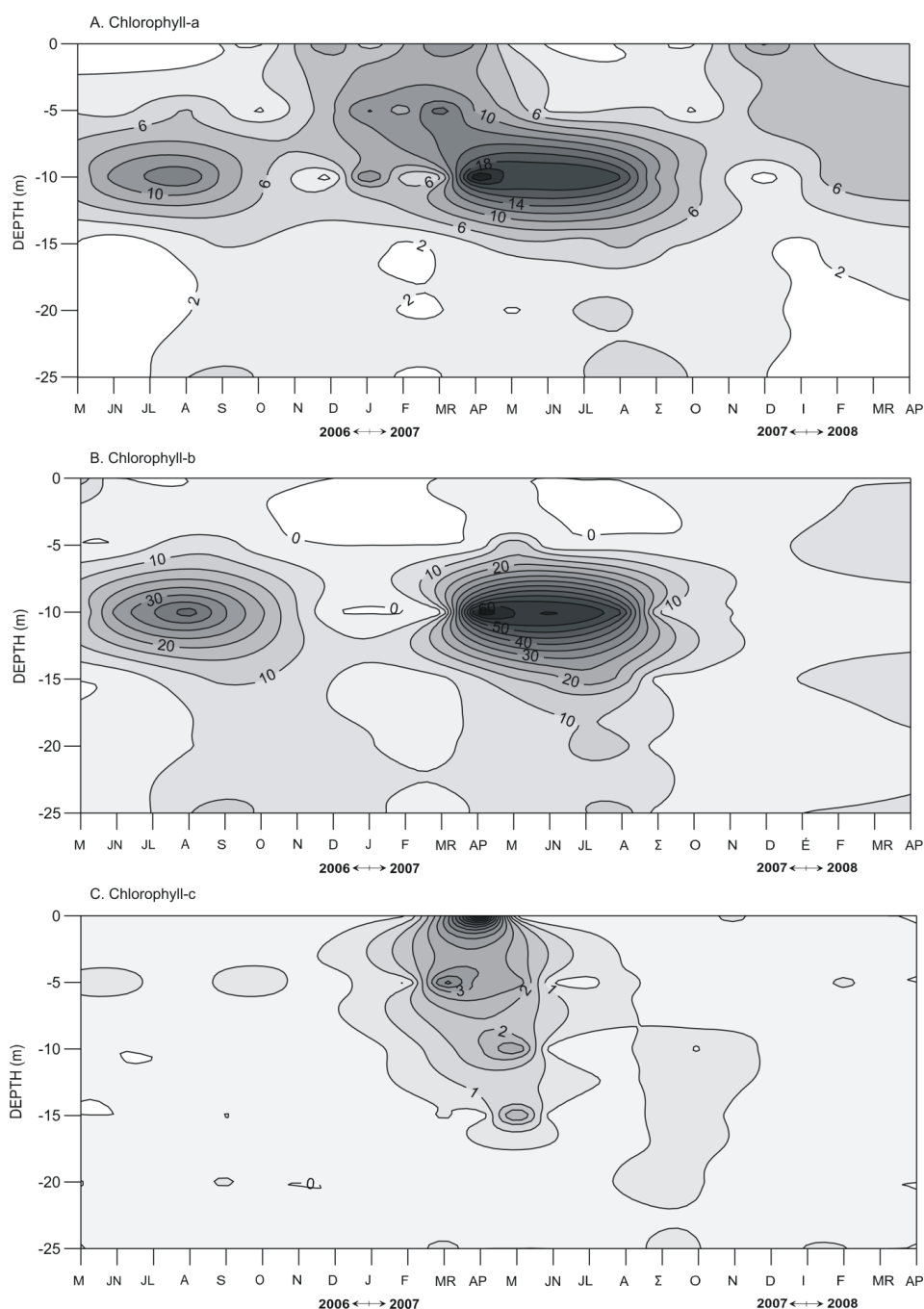



Fig. 3. Depth – time diagram of chl-a (A), chl-b (B) and chl-c (C) in µg/l, in the deepest sampling station A_9

during the period May 2006-May 2008.

1   Chlorophyll-c distribution was differentiated from those of chl-a and b. In general, low

2   concentrations (0-0.5μg/l) of chlorophyll-c were determined in Aitoliko basin. Only exception was

3   the spring of 2007 during which relative high values of chl-c determined up to the depth of 15m. The

4   maximum concentration characterized April 2007, where about 9μg/l was determined on the basin's

5   surface (0m) (Fig. 3C).

6   During the biennial monitoring period, ~~accumulated~~ sulfides ~~reached~~ concentrations up to

7   56.8mg/l near the bottom. ~~In table 1 the determined sulfide concentrations at the maximum sampling~~

8   ~~depth (25m) throughout the sampling period (May 2006-May 2007), are presented.~~ The higher

9   sulfide ~~values~~ characterized the summer periods of both sampling years, while reduced

10   concentrations were determined on February 2007 (31mg/l) and February 2008 (30mg/l).

12   Table 1. Sulfide concentrations at the maximum sampling depth (25m) throughout the sampling period (May

13   2006-May 2007). Mean monthly D_6 pumping station, discharge, during the period May 2006-May2008.

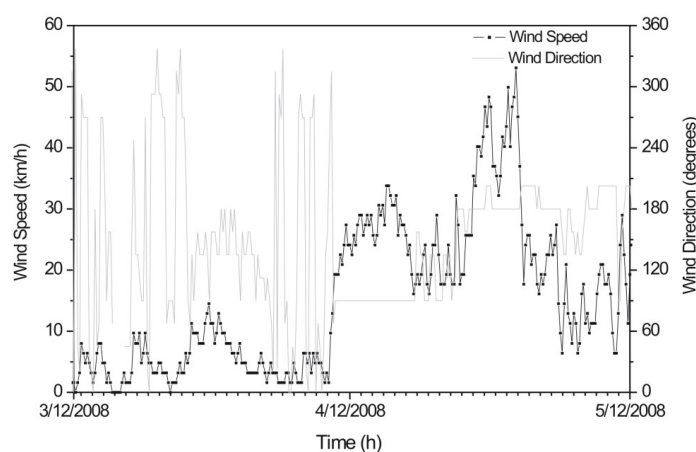
Year	Month	Sulfide concentration A9 (25m) (mg/l)	D_6 Discharge (x10^6 m^3/month)
2006	M	41,1	1,02
	JN	41	1,16
	JL	47,6	1,33
	AU	42,6	1,65
	S	47,8	1,23
	O	48,8	0,77
	N	50,8	0,71
	D	50,8	0,69
2007	J	49	0,63
	F	31	0,56
	MR	44,24	0,70
	AP	52,2	0,78
	M	52,92	0,72
	JN	46,12	0,69
	JL	55,16	0,64
	AU	51,8	0,64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

1   *3.2. The storm event of December 2008*

2   *Description of the event:* On the 4th of December, easterly to southerly winds, with speed up

3   to 50km/h were blowing in the area of Aitoliko basin (Fig. 4). These winds caused, the forcible enter

4   of water from Messolonghi lagoon into Aitoliko basin disturbing its water column balance and

5   leading to a holomictic event.

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

9   When the wind stopped, low dissolved oxygen concentrations were reported at the south part

10   of the basin (Dimitriou et al., 2010) and zero oxygen was recorded in the northern part. This

11   represents the spatial character in this anoxic crisis. During the following hours, birds were observed

12   on the surface of the water feeding, while fishes which had reached the shore were gulping at the

13   surface of the water in an attempt to obtain oxygen. The fish kills, that followed this storm event

14   reflected the direct impact of the anoxic crisis in the ecosystem's biology.

15   *Physical-chemical parameters:* Just after the holomictic event, the basin's water column was

16   nearly homogenous and quite unstable. After an intense storm capable for basin's mixing, water

17   column static stability is reduced at all depths. Alternating of low negative and positive static stability

1   values illustrated water column instabilities and indicated the mixing depth in the Aitoliko basin

2   water column (Fig.5A).

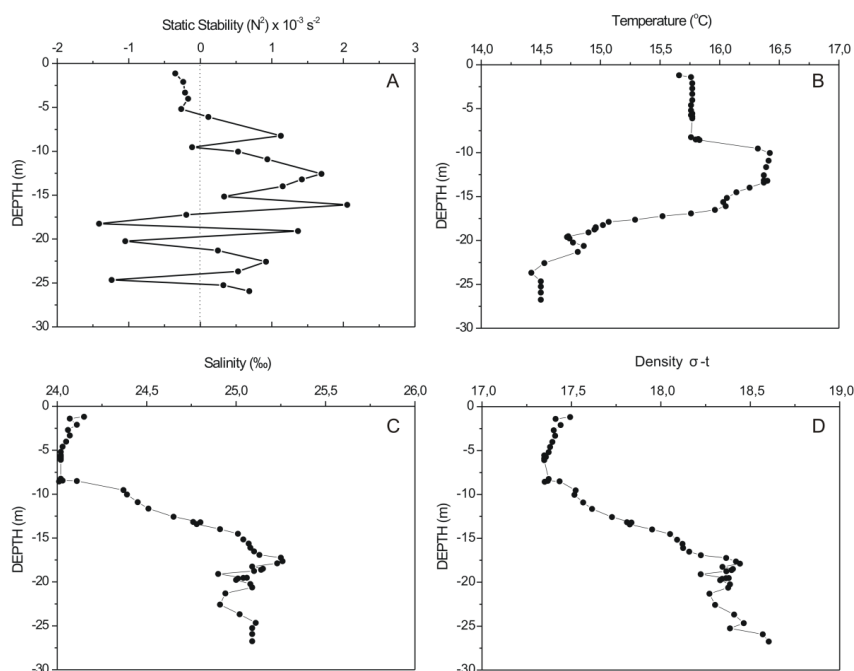
3   Slight differences between surface and bottom temperature, salinity and density (sigma-t)

4   values were recorded in Aitoliko basin water column four days after the anoxic crisis of the 4th of

5   December 2008. The measured surface temperature, salinity and sigma-t were about 15.5ºC, 24‰

6   and 16.5 respectively, when the corresponding bottom values were just about 16.5ºC, 25‰ and 17.5.

7   In the vertical distributions of these parameters, residual characteristics of the Aitoliko basin

8   stratification were retained (Fig. 5B, C, D).

11   Fig. 5. Vertical profiles of static stability (A) temperature (B), salinity (C) and density (sigma-t) (D) in the

12   deepest station A$_9$ during December 2008.

14   During the sampling of the 8th of December 2008, a well oxygenated surface layer down to

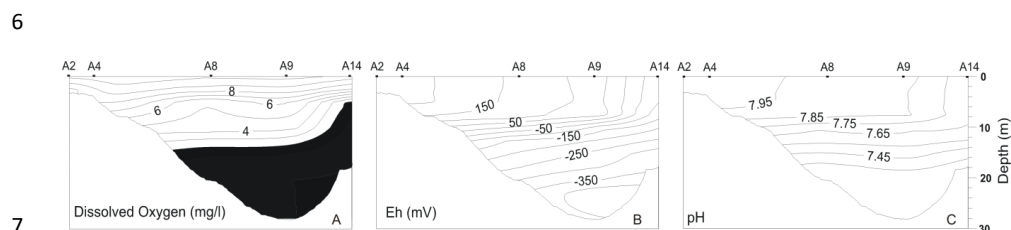
15   about 5m deep was recorded at the southern and the central part of the Aitoliko basin. Below that

1 depth dissolved oxygen concentrations decreased rapidly. The hypoxic and anoxic zones were spatial

2 related and reached their maximum extent in the northern part of the lagoon (Fig. 6A). It is interesting

3 to mention that the hypoxic zone extended below 14m, 8m and 4m at A_9, A_{12} and A_{14} stations

4 respectively. Concurrently, zero oxygen concentrations were measured below 18m, 11m and 10m at

5 A_9, A_{12} and A_{14} stations respectively.

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

9 during December 2008.

11 Redox potential and pH values were in accordance with the vertical and spatial distribution

12 of dissolved oxygen (Fig. 6B, C). At the northern part of Aitoliko lagoon lower surface redox

13 potential (~-80) and pH (~7.6) values existed, compared to the rest of the Aitoliko basin. Moreover,

14 bottom layer redox potential decreased to about -400mV, reflecting pH (~ 7.3-7.4) and dissolved

15 oxygen decreases.

16 *Nutrients, sulfides and chlorophyll:* During the holomictic period (December 2008), nitrates

17 and nitrites were below the detection limits throughout the Aitoliko basin water column. The mean

18 ammonium concentration determined at the 10 surface meters was about 0.25mg/l, while a maximum

19 value of 3.67mg/l was characterized the 20m depth. A slight decrease of NH_4^+ (3.09mg/l) was

20 recorded at greater depths (Fig. 7A).

21 Orthophosphates vertical distribution after the anoxic crisis is presented in Fig. 7B. Down to

22 the depth of 15m the measured PO_4^{3-} concentration was lower than 0.06mg/l. A peak of 0.3mg/l was

23 determined at the depth of 20m while a slight decrease (0.23mg/l) of orthophosphate concentrations

24 characterized greater depths.

1   About 0.8mg/l sulfides were determined in the 10 meter surface waters, while a maximum

2   value of 33mg/l was characterized the 20m depth. A slight decrease of sulfides concentration

3   (31mg/l) was recorded at greater depths (Fig. 7C).

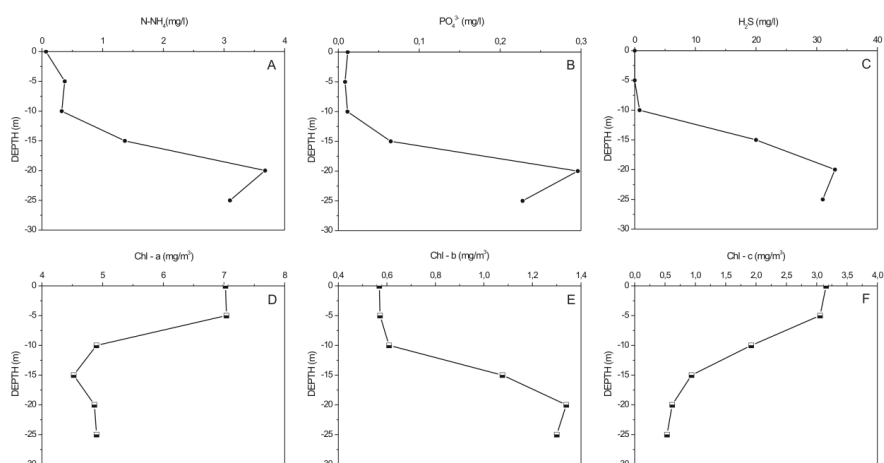



Fig. 7. Vertical profiles of ammonia (A), phosphate (B), sulfides (C) and chlorophyll-a, b, c (D, E, F) in

7   Aitoliko lagoon during December 2008.

9   In the first days of December 2008, just after the total mixing event in Aitoliko basin, relative

10   high chlorophyll-a concentrations were determined throughout the water column. The five surface

11   meters were characterized by 7μg/l chl-a, while from this depth and deeper, 5μg/l chl-a were

12   determined (Fig. 7D). In the surface layer 0.67μg/l chl-b were measured while at greater depths

13   parameter's increase was recorded (Fig. 7E). In contrast, the maximum chl-c values (3μg/l)

14   characterized the basin's surface and they decreased with depth's increase (Fig. 7F).

16   **4. Discussion**

17   Permanent stratified coastal basins, like Aitoliko, are characterized by anoxic and sulfidic

18   hypolimnia. In enclosed or silled basins, anoxia occurs naturally, through their morphology and high

19   water residence times and its origin is rarely linked with eutrophication phenomena.

1   Anoxia in Aitoliko lagoon is known since 18th century, as historical records are referred in

2   total mixing events, massive fish kills and H_2S sulfide release. Measurements of physicochemical

3   parameters of Aitoliko go back to 1951. During the period 1951-2004, bottom layer temperatures

4   show relatively constant values while salinity values ranged about 5‰. The oxic/anoxic interface

5   progressively decreased from a depth of 18m in 1951 to 4m in 2003-2004. During the summer period

6   of the years 2006 and 2007, the oxic/anoxic interface developed in a depth of 17-18m. In the winter

7   periods of the same years low dissolved oxygen concentrations were determined in Aitoliko basin's

8   monimolimnio. This fact was ascribed to the anthropogenic deepening of the sill that connects

9   Aitoliko and its source basin (Messolonghi lagoon). Greater amounts of dense-salty water flowed

10   from Messolonghi lagoon, causing limited vertical mixing of the Aitoliko basin. The hydrodynamic

11   processes controlled the small scale mixing of the basin's water column, introduced oxygen into the

12   halocline and the bottom waters, without destroying stratification (Gianni et al., 2011; Gianni and

13   Zacharias, 2012).

14   Quite a few times in the past, anoxic conditions reached the water surface and the whole

15   water column of Aitoliko basin was anoxic for several days. Usually, during autumn or winter months

16   after intense southern or south-eastern winds, the balance that keeps the anoxic water under the

17   lighter well oxygenated water is disturbed. Wind enforces large quantities of salty water to enter from

18   Messolonghi into Aitoliko basin. This dense water mass is passing under the relative lighter anoxic

19   water of the Aitoliko basin, helping it to emerge. During these events, hydrogen sulfide, which is

20   accumulated in the lagoon's bottom layer, is released in the upper layers killing all the aquatic

21   organisms, while the atmosphere becomes stuffy for the local residents. The first reported mass

22   mortality event in the Aitoliko lagoon is referred to in 1881. Since then a numerous of such events

23   have been recorded in 1963, 1990 (Dassenakis, 1994; Leonardos and Sinis, 1997), 1992, 1995 and

24   2001. Such events have a decisive influence into the basin's physical, chemical and biological

25   characteristics and processes. The most recent holomictic event in Aitoliko basin is the one described

26   in this study and is occurred on the 4th of December 2008.

1   Regarding the Aitoliko basin's nutrient budget and productivity the most comprehensive

2   overview is presented in Daneilides (1991) ~~study.~~ Orthophosphate, total phosphorus, ammonium,

3   nitrite, nitrate and chlorophyll-a (chl-a) profiles were identified in the deepest part of Aitoliko basin

4   during the period April 1984-April 1985. Nutrient concentrations in the Aitoliko basin's surface layer

5   were not significantly high, indicating their consumption of the planktonic organisms and their

6   removal to greater depths through dead cells. The ~~year~~ average concentrations of TP, PO_4^{3-}, NH_4^+,

7   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,

8   respectively (Daneilidis, 1991). After the spring plankton bloom, nitrogen concentrations were

9   significantly reduced in the epilimnion, resulting in the inorganic N to inorganic P ratio reduction.

10   Increase of all the inorganic nitrogen forms in the winter period, rendered phosphorus the growth

11   limiting factor. A general reduction of ~~all~~ nutrient concentrations and increases in chl-a values

12   occurred, with the spring bloom onset. During the 1984-1985 period the reported spring chl-a

13   maximum was about 10μg/l. The basin's deep layer was characterized by high ammonium,

14   orthophosphate and total phosphorus concentrations with constant values throughout the sampling

15   period. Specifically, about 15mg/l, 2mg/l and 2.5mg/l NH_4^+, PO_4^{3-} and TP were determined in

16   Aitoliko basin bottom layer during the 1984-1985 period.

17   Under the typical meromictic conditions prevailed in Aitoliko basin, throughout the two

18   years of monitoring (2006-2008), low ammonium, nitrate, nitrite and orthophosphate concentration

19   were determined in the surface 10m. At greater depths where, anoxic conditions prevailed, a sharp

20   increase in ammonium and orthophosphate concentrations ~~were~~ recorded. Just after the Aitoliko

21   basin total mixing (December 2008), nitrate and nitrite were below the detection limits throughout

22   the water column, while the measured ammonia concentrations below 15m were approximately 4

23   times lower than that measured under the typical meromictic conditions that commonly prevail

24   during the winter time. This reduction is probably due to ammonium oxidation during water column

25   mixing, as large amounts of oxygenated water flows into basin's bottom. Changes in orthophosphate

26   concentrations after the storm event were observed as well. The orthophosphate deficiency during

27   this time was probably caused by its removal from the water column by absorption of orthophosphate

1   on re-oxidized mineral phases of Mn- and Fe- oxides (Sundy et al., 1992) and by precipitation of

2   high insoluble calcium phosphate (Bjerum et al., 1958; Drever, 1982).

3   Bottom overturn increases the nutrient content of surface layers, enhancing algal blooms.

4   Chlorophyll-a and algal cell abundances in surface layers are correlated with changes of the

5   oxic/anoxic interface depth. Chlorophyll-a in surface waters decreases sharply as the oxic/anoxic

6   interface moves deeper, while the depth of oxic/anoxic interface affects the prevalence of

7   phytoplankton species (Ma et al., 2006). Chlorophyll-a is the most appropriate parameter to follow

8   the growth of both algae and *Cyanobacteria*, in a coastal environment. Chlorophyll-a is a good

9   indicator of the biomass of algae present in waters (Räike et al., 2003). The increase of nutrients in

10   surface layers can also amplify the growth and prevalence of harmful algal species in the water

11   column which grow quickly with division rates of 1 to $5d^{-1}$ (Kreiberg, 1999). Higher harmful algal

12   cell density occurred when more H_2S developed in the bottom waters and the interface moves to

13   shallower depths. Large blooms of dinoflagellates were recorded in the surface waters of Torquay

14   Canal when the interface migrates upward, while diatoms prevail when anoxic conditions limited in

15   great depths (Ma et al., 2006).

16   The total mixing event in Aitoliko basin in the first days of December 2008 led to

17   chlorophyll-a concentration increases throughout the water column, comparing with the measured

18   values under the typical meromictic conditions prevail commonly during the winter time. Changes

19   observed in the vertical distribution of chlorophyll-b as well. Unlike the typical winter profiles, chl-

20   b maximum concentrations were recorded at the basin's surface while a sharp decrease was observed

21   with increasing depth. Additionally, the vertical profile of chlorophyll-c four days after the storm

22   event was remarkable. Chl-c mainly occurs in algae which are absent from the surface layer under

23   the typical winter conditions in Aitoliko basin. Values equal to 3μg/l were measured on 8th of

24   December 2008. The abrupt increase in chlorophyll-c, immediately after the total mixing event, could

25   be associated with a bloom, for example, of dinoflagellates which encouraged under these conditions.

26   Certainly the observed changes indicate change in the composition of the lagoon's phytoplankton

1   community, can only confirmed by analytical determination of the prevailing algal types. In this

2   study algal community structure was not determined.

3   The changes induced in the physical, chemical and biological characteristics of an anoxic

4   meromictic basin as Aitoliko, following total mixing, highlight the impact of accumulated nutrients

5   and sulfides in the bottom layer.

6   However, questions arising are related to the importance of hypolimnetic nutrient and sulfide

7   load under meromictic conditions, and its relation with the surface water quality. Trying to address

8   out this concern, the focus was on the temporal variations of specific nutrients in the Aitoliko surface

9   layer and their relation with; a) changes on the physicochemical characteristics of the basin's water

10 column, b) changes on the bottom water phosphorus and nitrogen concentration, and c) their effect

11 on the basin's primary productivity.

12   The relative high concentrations of ammonium characterized the surface layer of the Aitoliko

13 basin during the summer and autumn months of 2006 and that was sharply reduced during the winter.

14 At the same period, increase of epilimnetic nitrites concentration, (ammonium oxidation intermediate

15 product) and decrease of the bottom layer ammonium and orthophosphate values were recorded.

16 These changes coincide with the Aitoliko basin's hypolimnion oxygenation due to weak/small scale

17 mixing of the water column caused by the denser water inflow from the Messolonghi lagoon in the

18 summer months (Gianni et al., 2011). Dissolved oxygen increase in Aitoliko basin's water column

19 may be the answer to the observed nutrient variations during that period.

20   In addition, during the 2006-2007 winter period, a PO_4^{3-} concentration increase in the surface

21 layer of the Aitoliko basin, was recorded. This increase could be attributed to external sourcing with

22 inorganic phosphorus. This scenario could be strengthened, considering that the basin's main

23 freshwater source is the D_6 pumping station. D_6 is located near the basin's entrance and supplies

24 Aitoliko with water from an extensive drainage network which extends to the adjacent cultivated

25 land. The seasonal PO_4^{3-} maximum surface concentrations coincide with the minimum flow of the

26 D_6 pumping station (Table 1), and is lagging 3-4 months behind the main fertilization period,

27 weakening the external loading scenario. In a second scenario, the observed epilimnetic PO_4^{3-}

1   increase could be attributed to ~~the surface layers enrichment~~ from the deeper waters. The

2   hydrodynamic processes that control the small scale mixing of Aitoliko basin water column,

3   introducing oxygen into the halocline and the bottom waters, without destroying the stratification

4   (Gianni et al., 2011; Gianni and Zacharias, 2012), probably also governs the nutrients transport from

5   the deep pool.

6   The phosphorus concentration increases in the Aitoliko epilimnion during winter and spring

7   months of 2007 had an immediate impact on the basin's primary productivity. A particularly high

8   chlorophyll-a concentration was determined the spring of 2007 and that was significantly higher than

9   those typically characterize Aitoliko basin (see spring 2006 values; Daneilides, 1991). Moreover

10   during this period, a maximum of chlorophyll-c in the surface of the Aitoliko basin was observed

11   providing evidence that the spring-summer phytoplankton bloom accompanied changes in the

12   dominant planktonic types.

13   During the 2007-2008 winter period an additional reduction on the epilimnetic NH_4^+

14   concentration was recorded and accompanied increased NO_2^- concentrations. The intensities of both

15   changes were milder than that reported for the previous sampling year. No changes were observed in

16   the surface PO_4^{3-} concentrations while hypolimnetic NH_4^+ and PO_4^{3-} values increased during that

17   period. The spring 2007 algal bloom increased the organic load and was enhanced in the anoxic

18   layers of the Aitoliko basin. The decomposition of this organic matter can be linked the bottom layer

19   nitrogen and phosphorus content rise. It becomes obvious that the Aitoliko bottom water oxygenation

20   episode in the winter months of 2008 had different impact on the basin's water column and this is

21   reflected in nutrient and chlorophyll profiles.

24   **5. Conclusions**

25   Storm events in coastal environments can result in stratification destruction and water total

26   column mixing. In systems where anoxic/sulfidic bottom waters exist, vertical mixing leads to a

27   basin's anoxic crisis. The massive fish kills is the result of an intense disturbance of a natural system

1   which led to the dissolved oxygen consumption in the surface water by hydrogen sulfide the anoxic

2   water layer and direct H_2S toxicity. Hydrogen sulfide release is accompanied by PO_4^{3-} and NH_4^+

3   release from bottom waters to the interface and surface waters promoting algal blooms. This

4   disturbance affects almost all the physical, chemical and biological parameters in a water basin.

5   A few days after the total mixing the physical rehabilitation of the basin's water column

6   starts. The surface layer is gradually oxygenated; indicating water column balance restoration, under

7   the influence of the local meteorological and hydrological processes. Geochemical processes

8   equilibrium starts by dissolved oxygen concentrations reseting to the normal levels and this takes

9   longer but not more than a few months. Environmental recolonization starts soon after the

10   physicochemical conditions return to normal, but requires several years before the ecosystem be

11   restored.

12   The induced changes in the physical, chemical and biological characteristics of an anoxic

13   meromictic basin, after its total mixing, highlight the impact of the accumulated nutrients and sulfides

14   in the bottom layer.

15   Just as significant is the role of hypolimnetic nutrient and sulfide load in meromictic periods.

16   The H_2S, NH_4^+, PO_4^{3-} rich bottom layer can supply nutrients to the surface waters when the

17   physicochemical and hydrological basin's water column characteristics permit it. Nutrient and

18   organic matter flux water column turn over affects both quantitative nature of and qualitative the

19   basin's primary productivity and shifts the ecosystems quality character.

20   **Author contribution**: Areti Gianni and Ierotheos Zacharias designed and carried out the samplings

21   while the experiments were designed and carried out by Areti Gianni. Areti Gianni prepared the

22   manuscript with contributions from Zacharias.

23   **Competing interests**: The authors declare that they have no conflict of interest.

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