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Fish kill in Ismarida lake, Greece: identification of drivers contributing to the event

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Abstract

During the end of August 2013, a massive fish mortality occurred in Ismarida Lake, a small and shallow system of northern Greece, where approximately 10-18 tons of euryhaline fish died. This study attempts to describe the fish kill that occurred in Ismarida Lake during the night of 28 August 2013, and to identify the possible drivers that may have triggered this event. Combined hydrographic, ichthyological and phytoplankton surveys were carried out with a toxicological analysis. The study proposes both short-term and long-term measures for the management of both quality and quantity of the water (ground and surface) resources in the broader basin of Ismarida Lake.

Keywords: Bacteria, fish kill, hydrographic conditions, ichthyotoxic, Ismarida, phytoplankton, shallow lake**,** water quality.

Introduction

Fish kills (formerly reported in the international bibliography also as massive fish mortalities) are a common phenomenon and are generally defined as localized mass fish die-offs that can occur in marine, estuarine, or freshwaters (Meyer & Barclay, 1990). An event should be defined as a fish kill if the mortality event is (1) not part of the fishes' natural life cycle (e.g., mass mortality following spawning activity in semelparous fish); (2) if a minimum of 25 dead fish are found in one square kilometer (lentic) or river kilometer (lotic) and within a 48-hr period, and (3) if mortality was not caused by predation, including by humans (i.e., harvest) (La & Cooke, 2011). They occur as a result of a broad variety of natural and human-induced causes. Natural causes include phenomena such as extreme temperature fluctuations, starvation, diseases and dissolved oxygen depletion (Hoyer *et al*., 2009). Human-induced fish kills may be attributed to known and accidental additions of sewage/organics (Forsberg & Ryding, 1982), pesticides (Bálint *et al.,* 1995), acids (Beamish & Harvey, 2011), fertilizers (Reese *et al.*, 2008) and toxic compounds to the sediment (Hirata *et al*., 2011). Other factors include the occurrence of long-lasting summer droughts, common in the Mediterranean environment, placing stress on the freshwater hydrology of small and shallow lakes (Beklioglu *et al*., 2006). The resulting changes in the hydrological regimes may rapidly affect biotic growth rates, timing of reproduction, balance of photosynthesis and respiration, rates of mineralization, with local to large-scale regional effects (Coops *et al*., 2003). Fish kills are also linked to phenomena such as dissolved oxygen depletion, resulting from water column stratification during sustained periods of warm weather, coupled with low-flow conditions (Thronson & Quigg, 2008). High salinity fluctuations have also been suggested as a factor leading to fish kills, especially when they are linked to low basin run-offs and sustained periods of high temperatures (Whitfield *et al*., 2006). Fish kills in inland waters are known to occur with greater frequency, especially in eutrophic lakes (Oikonomou *et al.,* 2012). This phenomenon often accompanies harmful cyanobacteria and algal blooms (Abrantes *et al*., 2006). The haptophyte alga *Prymnesium parvum* has also been blamed for several fish kill incidents in inland waters of Texas, USA (Roelke *et al*., 2011). Blooms of *Pfiesteria* species have been associated with fish kills, particularly in the brackish waters of lakes and estuaries (Glasgow *et al*., 2001). Finally, *Prorocentrum minimum* has been associated with fish and shellfish mortalities in estuaries and coastal waters worldwide (Heil *et al*., 2005).

In Greece, fish kills have been observed many times both in fresh and transitional water systems; several reports also exist in marine waters. Unfortunately, in very few cases the causes of the fish kills are thoroughly examined. The most well-studied inland water event occurred in August and September 2004 in Lake Koronia, when thousands of different fish species such as *Alosa macedonica*, *Alburnus macedonicus*, *Aspius aspius*, *Rhodeus amarus*, as well as 30,000 birds of 41 different species perished (Moustaka-Gouni *et al*., 2004; Genitsaris *et al*., 2009). Other fish kills have been reported for the same lake in August 1995 and 2007, but with no other information on environmental conditions or potential causes of the fish kill (Gregoriadou *et al.,* 1997; Manakou *et al*., 2008). Fish kills have also been documented to occur in intermittent streams due to drought conditions during the summer (Skoulikidis *et al.,* 2011). The most recent fish kills were observed in the recently reconstructed Lake Karla (Oikonomou *et al*., 2012; Papadimitriou *et al*., 2013) where, during the initial period of lake's rehabilitation (2009-2011), several events occurred each year in spring when hundreds of fish, mainly *Cyprinus carpio*, were found dead in each case. A fish kill occurred in Etoliko lagoon, a transitional water system located in western Greece, part of the Messolonghi-Etolikon wetland complex in November 1990 (Leonardos & Sinis, 1997) when a large number of fish, mainly eels (approx. 140 tons) were found dead at the shore. While this event was the most catastrophic one, two minor events, in December 1998 and in December 2008, also occurred in Etoliko Lagoon. Instances such as these are probably much more frequent but the lack of reporting and assessment is a serious problem. As in many cases worldwide, reports of fish kills are rarely published as scientific investigations and they tend to re-occur in the same water bodies (La & Cooke, 2011).

During the end of August 2013, a massive fish kill occurred in the shallow Ismarida Lake, in northern Greece, also referred to as Mitrikou Lake. Ismarida has no history of similar events, even if small scale fish kills were randomly observed (pers. comm. Fisheries Department of Rhodope Prefecture, Region of East Macedonia and Thrace), and although the lake has endured multiple stresses over the years (Pavlikakis & Tsihrintzis, 2003). This study attempts to describe the event of a fish kill that occurred in the lake during the night of 28 August 2013, and to identify the possible drivers that may have triggered it. The study also proposes both short-term and long-term measures for the management and improvement of both quality and quantity of the water (ground and surface) resources in the broader basin of Ismarida Lake.

Materials and Methods

Study Area Description

Lake Ismarida (40.9° N, 25.3° E) was thought to be the last natural freshwater lake of Thrace, northern Greece, covering an area of 3.4 km^2 with a mean depth of 1.5 m. Currently the lake is turned into a shallow, freshwater system during the rainy season (October-May), changing gradually into a mixed fresh-salt water system in the dry season (June-September), with complex interactions between surface, groundwater and sea water taking place (Gemitzi & Stefanopoulos, 2011). The lake is holomictic with a distinctive hydrological regime, which affects the variability of the water level and circulation (Moustaka *et al*., 2011). Thus, the water level fluctuates between 50 cm to 230 cm during the year, depending mainly on land-based inflows. The lake experiences strong watershed effects because of the high basin-to-lake area ratio of 245. The dominant land use types in the watershed (320.5 km^2) is agriculture, representing 48.4 % of the catchment, followed by woodlands and semi-natural vegetation, corresponding to 45.4 % of the drainage basin. Urban land cover accounts for 4.2 % of the lake's basin (Katsiapi *et al*., 2012). The climate in the broader basin is typical Mediterranean, with mean annual precipitation of 628 mm and monthly temperatures ranging from 1 to 35° C, with a mean value of 15.8° C.

At the northern part of the lake, the Vosvozis River enters gradually, forming a small deltaic confluence. Another river named Lissos flows nearby, approximately 4.5 km to the east of Ismarida Lake. The Vosvozis comprises the only surface water influx of Ismarida Lake (Fig. 1), having a mean annual discharge of 0.78 m^3/s, ranging between 0.18 m^3/s and 1.94 m^3/s. During the dry season (July-October), the river usually dries out as its limited discharge is filtered through the sandy sediments towards the shallow, local aquifer (Giannakopoulou, 1995). Part of the Vosvozis discharge derives from Komotini Waste Water Treatment Plant (WWTP), where the sewage of 79,500 inhabitants or 11,000 m^3/day ($\approx$ 0.13 m^3/s) is subject to a secondary treatment. During the dry season, the total discharge of the river originates almost entirely from these treated plant outflows. Another WWTP, smaller in capacity than the first, was designed and constructed to treat the waste water of Pagouria village, approximately 2 km northwards from the confluence of the river with the lake (Bousbouras *et al.,* 2010). This WWTP has never worked properly, resulting in Pagouria village's waste water being transferred, daily, by tanker trucks to Komotini's WWTP. This procedure possibly causes waste water leakages to the Vosvozis River.

At its southern end, the lake is connected to the Thracian Sea through a natural channel, 5.2 km long and 3-10 m wide, which was artificially widened and dredged in 1986 to allow the discharge of fluvial overflow (Fig. 1). This channel is partially occluded by a weir with cement base and side supports to mount wooden panels and block the entry of water. The weir is managed by the local fishermen (weir's panels are easily dismounted when needed). The weir is situated at approximately 900 m downstream from the lake, blocking the intrusion of seawater into the

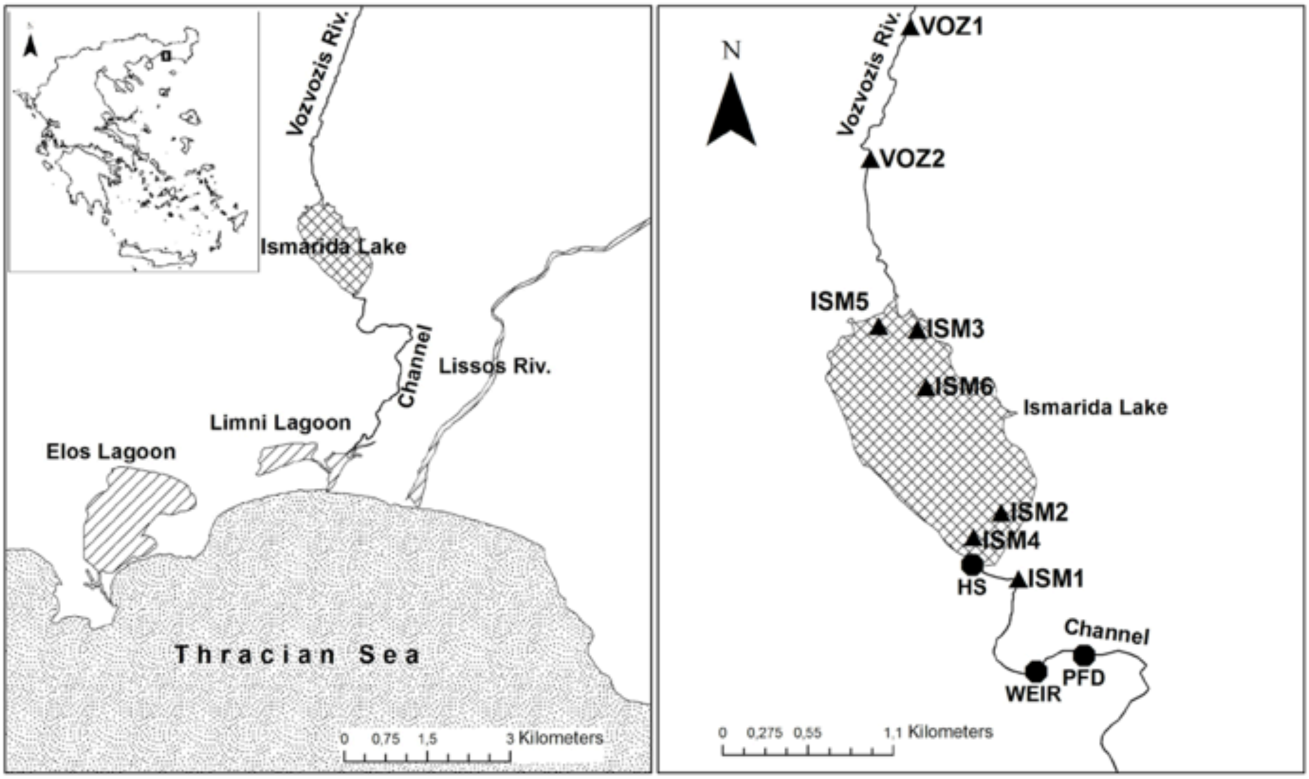

Fig. 1: Map of the study area. The fish fauna sampling stations are marked as triangles (see table 1 for a short description of each site), where the dot labeled as "WEIR" is its position, the one labeled "PFD" represents the position of the Permanent Fishing Devices, and the dot labeled "HS" shows the position of the hydrographic station.

lake, mainly during the dry season. Permanent fishing devices are located on a cross-section of the channel, 200 m downstream from the weir (Fig. 1).

The eastern riparian shore of Ismarida Lake used to be an extensive floodplain, where wet meadows existed, but in the 1980's an embankment was constructed to allow the use of floodplains for farming. Measurements on the plains of Ismarida Lake exhibited a hydraulic connection of the system with an underlying semi-confined aquifer of 50-100 m thickness (Pisinaras *et al*., 2007).

Prior to the widening and dredging of the seaward channel, the lake's fishery production was approximately 4 tons, comprised mainly of Common carp (*Cyprinus carpio*) and European eel (*Anguilla anguilla*). The widening and dredging of this channel shifted fishery production into euryhaline species, mainly of the Mugilidae family. This shift was associated with the frequent influx of seawater through the channel, causing periodical salinity raises that favor euryhaline species. In the first years after dredging and widening of the channel, the total fishery production increased to approximately 5.5 tons (during 1990-1996), dropping rapidly after 1996 and halved by 2009.

Measurements prior and during the event

These measurements include: a) continuous hydrographic data collected by an auto-recording monitoring station in Ismarida Lake, b) the dead fish biomass estimation, and c) the toxicological analysis on tissues from the dead fish.

Hydrographic data

Water level, conductivity, salinity and water temperature of Ismarida Lake were measured at a permanently-installed hydrographic station, equipped with a series of sensors for water temperature, conductivity, salinity, TDS and pressure. The station was installed on January 1, 2013, thus covering a significant period before the event and the whole period of interest. The hydrographic station is operated by the Management Body of Nestos Delta-Vistonida and Ismarida Lakes and it is located at the southern part of the system, close to its connection with the channel (Fig. 1). Parameters were continuously recorded at fifteen-minute intervals, and data were daily transmitted to the server of the Management Body, via the standard GSM network. Dissolved oxygen was measured ad hoc, when the event of mass mortality was firstly observed, using a portable DO meter.

Harmonic analysis was performed on the hourly values of water level and water conductivity, to determine the relative contribution of the tidal harmonics of the diurnal and semi-diurnal constituents. These time-series were subsequently reconstructed, to reveal the tidal and non-tidal signals in the above time-series. The World

Tides Matlab software (Boon, 2006) was used for the harmonic analyses of the hydrographic station data.

Dead fish biomass estimation

The total biomass of the dead fish was estimated based on approximate ad hoc measurements of the density (fish biomass in kg per m²) of the dead fish layer in three distinctive parts of the lake and the channel (samplings were carried out on PFD, HS, ISM4 sites of the fish-fauna survey on Figure 1). The fish were collected using a round scoop. The dead fish layer was roughly reconstructed on a GIS, for the extent of the layer to be assessed, based on photos of the layer taken from many different spots around the lake and personal observations. The biomass of dead fish was estimated three times by multiplying the extent of the layer each time with one of the three different biomass values per m², resulting in a range of values. This procedure was followed because it was unclear where the layer had different densities.

Toxicological analysis on dead fish tissues

On the day of the event, samples of dead fish were collected in order to question the possibility that the event was the result of a toxicological infection. After being packaged in a frozen container, the fish were sent to the Veterinary Research Institute of Thessaloniki (VRIT Department of Infectious and Parasitic Diseases), where fish were diagnosed for parasitic, bacterial, fungal, environmental non-communicable and histological diseases, according to the OIE (2003). Specifically, 12 *Mugilidae* spp. specimens were sent for diagnosis at the V.R.I.T. All of them were at least two years old. Cells and tissues were obtained from all individuals.

Post-event measurements

Post-event measurements include: a) hydrographic data collected at the fishing devices of Ismarida Lake; b) fish fauna survey, taken place concurrently to the hydrographic measurements; and c) an intense phytoplankton survey.

Hydrographic survey

The hydrographic conditions in Ismarida Lake were surveyed during a typical spring tidal cycle on 12 November 2013. A tidal gauge (Valeport 740), a CTD probe (Valeport MIDAS), a dissolved oxygen and pH probe (WTW) and a current-meter (Valeport 105) were deployed at the southern part of the lake, at the permanent fishing devices (Fig. 1), while water samples were collected, stored properly and analyzed for nitrogen and phosphorus compounds (ammonia, organic nitrogen and total phosphorus), organic content (BOD and COD), total suspended solids and chlorophyll-a concentrations. Similar analysis was also performed at the ichthyofauna sampling stations of the lake and the lower segment of Vosvozis River.

Fish fauna survey

The fish fauna of Ismarida Lake and Vosvozis River was sampled on 12 November 2013. Six sampling site were selected in Ismarida Lake, and two for the lowland part of Vosvozis River (Fig. 1). Sites were located aiming to cover all different habitats of the lake and river, and concurrently to catch most of the species reported by previous studies (Economidis *et al*., 1991; Economou *et al*., 2007). Special attention was placed on covering the parts of the lake near Vosvozis River outflow and the region of the channel. The description of the sampling sites (Fig. 1), along with the sampling tools that were used in each of them, is presented in Table 1.

A beach seine, used in the particular sampling session had a length of 12 m and a height of 1.7 m and the mesh size was 1.2 mm. A portable electrofishing device was used (Hans Grassl Direct Pulse Current Electrofishing Device IG200/2), operating at 80 Hz. Gill nets of different mesh sizes (7 to 70 mm) were used for sampling the deepest part of Ismarida lake (ISM6). The main objective of the particular sampling session was to determine all species composing the ichthyofauna of Ismarida Lake and the lowland part of Vosvozis River hydrological basin. For this purpose, samples were not conducted following a standardized protocol for quantitative analysis.

Collected samples from each sampling site were placed separately in formaldehyde solution (10%) and transferred to the laboratory, where taxonomic classification of species was carried out according to Kottelat & Freyhof (2007). The common names used are from Economidis *et al*. (2009).

Table 1. Sampling sites, description and tools used for the ichthyological survey.

Code Name	Sampling site Description	Sampling tool
ISM1	Permanent fishing devices	Beach seine
ISM2	Lake’s southern part	Beach seine
ISM3	Vozvozis’ outfall – east	Electro-fishing device
ISM4	Hydrographic Station of Ismarida Lake	Beach seine
ISM5	Vozvozis’ outfall – west	Electro-fishing device
ISM6	Center of the Lake	Nordic Nets
VOZ1	Vozvozis’s bridge at Pagouria village	Electro-fishing device
VOZ2	Vozvozis’s Irish bridge passage	Electro-fishing device

Phytoplankton survey and analysis

Surface water samples were collected from a site at the center of Ismarida Lake, covering the period from 5 September 2013 until the end of October 2013, with a bi-weekly frequency. Due to the fish kill event, water samples were additionally collected from Vosvozis River on 5 September 2013. Fresh and preserved samples were examined using an inverted microscope (Nikon SE 2000) and species were identified using taxonomic keys. Phytoplankton counting was performed using Utermöhl's sedimentation method. At least 400 phytoplankton individuals were counted in each sample.

Results

Description of the event

In the morning of 29 August 2013, a thick layer of dead fish was observed at the littoral part of the lake (Gemitzi, pers. comm.) (Fig. 2). Air odor due to dead fish decay was inducing discomfort. At that time, dissolved oxygen levels in the lake water were approximately 4 mg/L (Gemitzi, pers. comm.) and conductivity ranged between 26 and 30 mS/cm. During the second half of August 2013 the water level of Ismarida Lake dropped to its lowest annual point (near 45 - 50 cm depth), probably due to the combined action of evaporation and the limited Vosvozis inflow (annual minimum was reached). This inflow comprised mostly of processed waste water from Komotini WWTP. Mean air temperature during the day of the event was measured as 26.6 °C at the closest weather station, Imeros, Rhodopi, 4.7 km southeast of the lake, and the most common wind direction during 25 – 29 August was SSW, SW and S. On 28 August, daily-averaged water temperature reached its dry season maximum. During this day, the daily-averaged water conductivity increased sharply, probably due to the partial opening of the weir resulting in the inflow of seawater into the lake. The major part of the dead fish layer was collected at the southern part of the lake, close to ISM4 sampling site (Fig. 1), yet dead fish were observed at all shores of the lake, except the northern part.

Hydrographic station

Time-series analysis on the dataset collected by the hydrographic station (1/1/2013 – 9/9/2013) reveals that water temperature varied from 2 ˚C to 31 ˚C, with a mean value of 22.8 ˚C. Water conductivity varied from near zero values to 37.3 mS/cm, depicting a time-mean of 7.25 mS/cm. Similarly, water salinity ranged between 2 to 31, with a period-mean value of 7.4. Water level from sensors gauge ranged from 0.3 m to 1.6 m, having an average at 0.62 m.

Harmonic analysis

The results from the harmonic analysis on the water level series are shown in Table 2, while for the water conductivity they are presented in Table 3. The contribution of the diurnal constituents S_1, P_1 and K_1 on the water level variability is apparent. A similar behavior is also shown in the harmonic analysis of the conductivity time-series, where S_1, K_1 and P_1 dominate over the remaining harmonics. Correlation coefficients between the observed and modeled through harmonic analysis series reached 0.936 and 0.891 for water level and conductivity, respectively. Reconstruction of these series and comparison to the observed ones shows that during August 2013 tidal

Fig. 2: Dead fish along the seaward entry channel and inside the permanent fishing devices.

Table 2. Harmonic analysis performed on the water level time-series recorded by the Ismarida Lake hydrographic station.

Tidal Constituent	Period	Amplitude (m)	Phase (deg)
J_1	23.0984	0.0011	310.0084
K_1	23.9344	0.0355	143.9525
S_1	24.0000	0.0723	249.3199
P_1	24.0658	0.0383	352.9877
M_1	24.8412	0.0010	50.6220
O_1	25.8193	0.0010	242.0826
Q_1	26.8683	0.0006	271.2146
$2Q_1$	28.0062	0.0007	105.7767
M_2	12.4206	0.0019	58.0431
S_2	12.0000	0.0023	87.9983
N_2	12.6583	0.0005	115.4631
v_2	12.6260	0.0005	58.4853
MU_2	12.8717	0.0003	37.4363
λ_2	12.2217	0.0044	125.2909
L_2	12.1916	0.0048	81.5335
K_2	11.9672	0.0034	54.8606

Table 3. Harmonic analysis performed on the water conductivity time-series recorded by the Ismarida Lake hydrographic station.

Tidal Constituent	Period	Amplitude (mS/cm)	Phase (deg)
J_1	23.0984	0.4726	225.6573
K_1	23.9344	14.0523	83.5130
S_1	24.0000	25.2522	182.7384
P_1	24.0658	12.5737	283.4796
M_1	24.8412	0.4547	8.3748
O_1	25.8193	0.4980	304.2586
Q_1	26.8683	0.2588	11.8520
$2Q_1$	28.0062	0.1106	151.6163
M_2	12.4206	2.2333	351.5049
S_2	12.0000	2.1390	301.1520
N_2	12.6583	0.5254	337.9537
v_2	12.6260	0.3643	269.3124
MU_2	12.8717	0.7803	35.9869
λ_2	12.2217	2.0837	66.7079
L_2	12.1916	2.5650	18.3078
K_2	11.9672	3.1358	317.9505

influence is limited in amplitude, as the length of the artificial entry canal dissipates the incoming tidal energy (Fig. 3). However, during the week prior to the event, the water level was reduced to a minimum value, probably due to summer evaporation and limited fresh and sea water inflow. From 23 August 2013 sea level displayed an increasing trend, associated with the sudden inflow of water in the system. Figure 4 illustrates the modeled and the recorded conductivity curves. Modeled conductivity appears in phase and closely follows the recorded series, with slight overestimation, especially at the beginning of the month. During the event, the model exhibits strong underestimation and fails to follow the recorded curve, which increases suddenly, explaining that the water level into the system increased due to an unexpected and violent sea water intrusion under normal conditions.

Hydrographic survey

Results from the survey implemented during a spring tidal cycle depicted the limited exchange of the system with the open sea, under normal conditions. Water flow speeds at the fishing devices (southern end) exhibited very low magnitudes, ranging between 0.01 and 0.1 m/s, with mostly a southerly direction, due to a recent rainfall event. Water level in the system exhibited an average of 0.12 m, water temperature was almost stable at 17 ˚C, pH at 8.1, while dissolved oxygen varied from 9.4 to 15.5 mg/L. Water conductivity at all sites of the lake was also found to be stable at 26.7 mS/cm, representing brackish water salinity of 20.4. Total suspended solids (TSS) were found higher at the riverine sites (38.52 mg/L) than those at the lake (29.92 mg/L). At the central part of the lake,

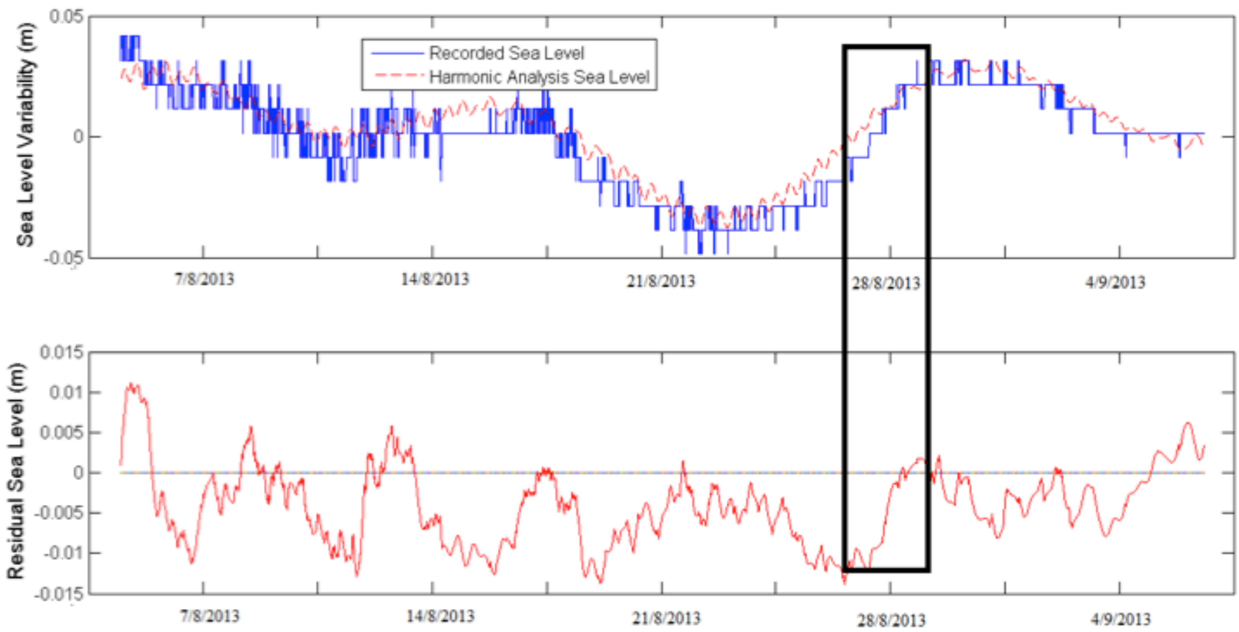

***Fig. 3**:* a) Upper panel: Water level variability in Ismarida Lake, as recorded by the auto-recording hydrographic station (blue line) and water level reconstruction model, based on harmonic analysis results (red line); b) Lower panel: Residual (non-tidal) fluctuation of sea level variability in Ismarida Lake. Rectangle represents the period of the fish mortality event.

surface TSS values were slightly lower (33.04 mg/L) than the corresponding bottom value (37.96 mg/L).

Increased values were also reported in terms of organic pollution of the system. BOD_5-values were at 23.3 mg/L at the south end (fishing devices), increasing gradually towards the center (25.6 mg/L) and the riverine part (28.6 mg/L). COD levels showed similar behavior with values along the south-to-north gradient of 153.40 mg/L, 158.35 mg/L and 178.14 mg/L, respectively. Total phosphorus at the area of the fishing devices varied between 552.64 μg/L and 1,089.98 μg/L. Lower values were observed at the center of the lake (465.76 μg/L at the surface and 650.22 μg/L at the bottom), while higher contents were recorded at Vosvozis River (949.71 μg/L). Ammonia was rather stable in time and between sites, ranging from 4.20 mg/L to 5.88 mg/L. TKN ranged from 10.98 mg/L at the southern part to 12.60 mg/L at the riverine sites. Higher chlorophyll-a levels were measured at the northern parts of the lake, near the confluence with Vosvozis River (75.94 μg/L), having moderate to low concentrations at the center of the system (52.82 μg/L) and the southern end (31.92 μg/L). On 20 August 2013, a high concentration of chlorophyll-a at the level of 206.6 μg/l was recorded at the surface waters of the lake (Charalambous pers. comm.).

Dead fish biomass estimation and fish fauna survey

Biomass of the dead fish was estimated to be 10-18 tons of fish in an area of 0.36 km². The dead fish layer comprised mainly by specimens of *Mugilidae* family (~ 90%) (*Liza ramada*, *Liza aurata*, *Liza saliens*, *Mugil cephalus*). However, specimens of *Anguilla anguilla*, *Carrasius gibelio*, *Cyprinus carpio* and *Squalius orpheus* were also collected. Toxicological tests indicated that the fish kill was not related to fish infections or toxic factors.

The fish species collected at five sampling sites in Ismarida Lake and in the two sampling sites at the lower part of Vosvozis River during the survey of 12 November 2013 are presented in Table 4. The total number of fish species captured was nine, separated in four families. More specifically, five species belonging to two families were found in Ismarida Lake, while at Vosvozis River four species belonging to two families were captured. The species found in the lake are euryhaline and are usually found in brackish water bodies such as lagoons. The species of Vosvozis are typical freshwater species. *Cobitis strumicae* inhabits mainly rivers, preferring slow to moderate flowing waters, while all other species captured at Vosvozis River prefer mainly the standing waters of lakes and rivers.

Phytoplankton survey

Microscopic analysis of water samples collected some days later from the Lake Ismarida and Vosvozis River, revealed a high number of phytoplankton species that are typical for the brackish waters of the lake. Among them, the ichthyotoxic dinoflagellate *Pfiesteria piscicida* was identified, although it was not the dominant one. A high abundance and dominance of the harmful bloom forming dinoflagellate *Prorocentrum minimum* was recorded. Finally, extremely high number of large-

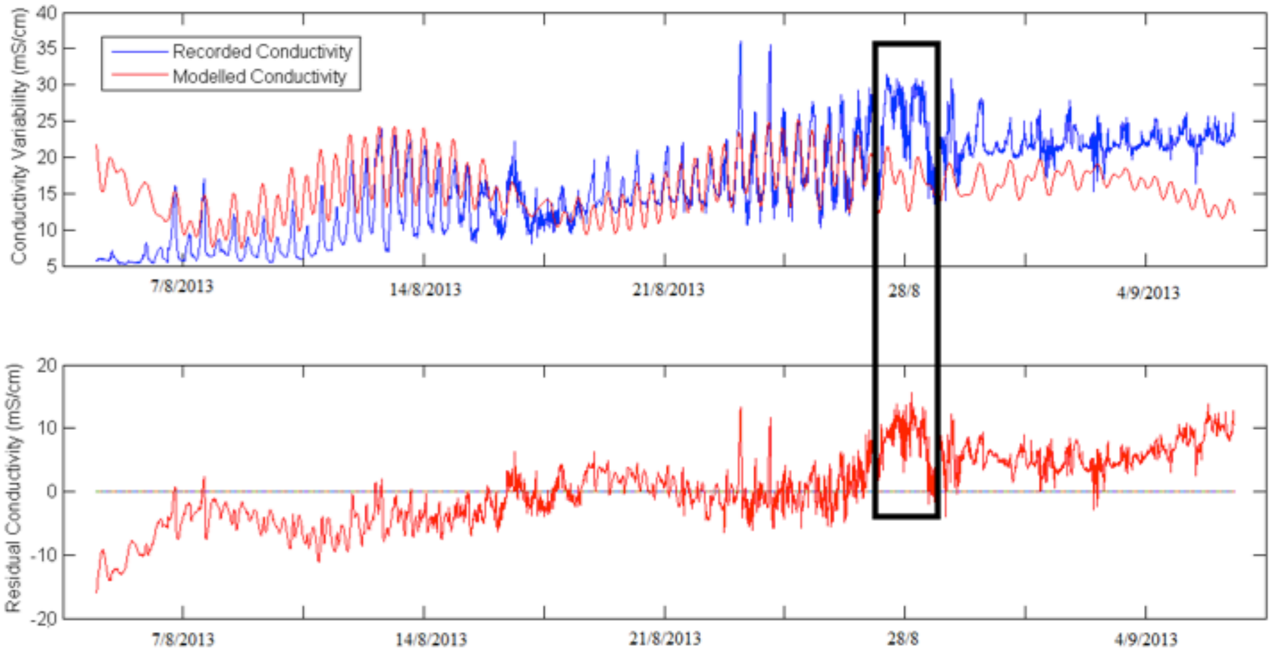

Fig. 4: a) Upper panel: Water conductivity in Ismarida Lake, as recorded by the auto-recording hydrographic station (blue line) and water conductivity reconstruction model, based on harmonic analysis results (red line); b) Lower panel: Residual (no-tidal) fluctuation of water conductivity variability in Ismarida Lake. Rectangle represents the period of the fish mortality event.

Table 4. List of fish species of Lake Ismarida and River Vozvozis that were captured on 12 November 2013 and the sampling stations (locations of the sampling stations are exhibited on the map of Figure 1).

Family	Species	Common Name	Sampling stations
Gobiidae	*Knipowitschia caucasica*	Caucasian dwarf goby	ISM1, ISM2, ISM3, ISM4
Mugilidae	*Mugil cephalus*	Flathead mullet	ISM5
Mugilidae	*Chelon labrosus*	Thicklip grey mullet	ISM5
Mugilidae	*Liza aurata*	Golden grey mullet	ISM5
Mugilidae	*Liza saliens*	Leaping mullet	ISM4
Cyprinidae	*Cobitis strumicae*	Thracian spined loach	VOZ1, VOZ2
Cyprinidae	*Squalius orpheus*	Orpheus chub	VOZ1, VOZ2
Cyprinidae	*Carrasius gibelio*	Prussian carp	VOZ1
Poeciliidae	*Gambusia holbrooki*	Eastern mosquito fish	VOZ1, VOZ2

sized heterotrophic bacteria in the water samples from Vosvozis River and lake water samples were reported.

Fifteen phytoplankton species were identified during the sampling of September and October, belonging to the group of diatoms (5), dinoflagellate (3), cryptophytes (2), cyanobacteria (2), euglenophytes (1) and chlorophytes (2) (Table 5). During both months, total phytoplankton biomass was in eutrophic/hypertrophic levels ranging from 26 to 63 mg/L. The lake's phytoplankton was dominated by mainly brackish/coastal phytoplankton species, such as *Tetraselmis* sp. (abundance$_{max}$ = 315 × 10^6 individuals/L, biomass$_{max}$ = 19 mg/L), the cryptophyte *Plagioselmis* sp. (abundance$_{max}$ = 104 × 10^6 individuals/L, biomass$_{max}$ = 7 mg/L individuals/ml) and *Cryptomonas* sp. (abundance$_{max}$ = 43 × 10^6 individuals/L, biomass$_{max}$ = 11 mg/L), the known toxic dinoflagellate *Prorocentrum minimum* (abundance$_{max}$ = 33 × 10^6 individuals/L, biomass$_{max}$ = 48 mg/L), and a small centric diatom (abundance$_{max}$ = 2 × 10^6 individuals/L, biomass$_{max}$ = 36 mg/L. *Pfiesteria piscida* was found in lower abundances (~0.15 × 10^6 individuals/L). Large heterotrophic bacteria were

Table 5. Phytoplankton species that were identified during the sampling of September and October 2013.

Chlorophytes	**Euglenophytes**
Tetraselmis sp.	*Euglena* sp.
Monoraphidium contortum	**Cryptophytes**
Cyanobacteria	*Plagioselmis* sp.
Pseudanabaena limnetica	*Cryptomonas* sp.
Limnothrix redekei	**Dinophyceae**
Diatoms	*Pfiesteria piscicida*
Amphiprora sp.	*Prorocentrum minimum*
Nitzschia acicularis	*Amphidinium* sp.
Nitzschia closterioides	
Rhizosolenia cf. *setigera*	
Unknown centric diatom	

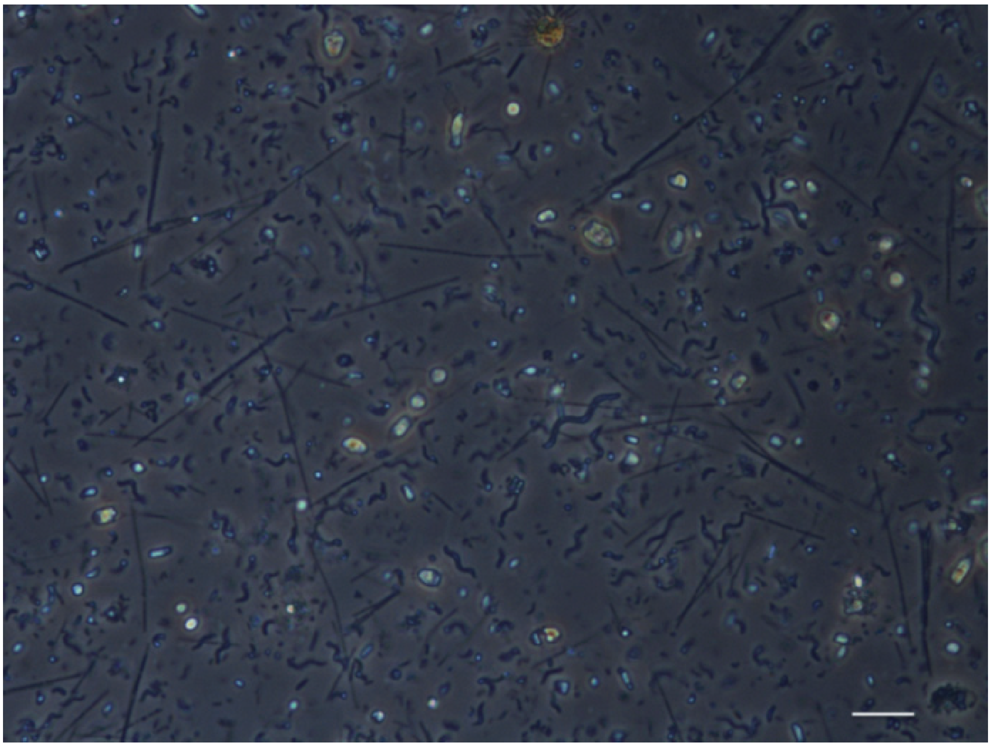

Fig. 5: Light micrograph (phase contrast) of a water sample containing predominantly large comma to spiral-shaped heterotrophic bacteria collected from Vosvozis River on 5 September 2013. Scale bar 10 µm.

conspicuous (~110 × 10^6 individuals/L) in phytoplankton samples collected from the Lake on 5 September 2013, exceeding 1,000 × 10^6 individuals/L in the water samples collected from Vosvozis River (VOZ1) (Fig. 5).

Discussion

Fish kills in natural aquatic ecosystems are indicative of poor water quality and downgraded ecosystem health (Klemm *et al*., 1993). Furthermore, the identification of the stress factors contributing to such events and the assessment of the relative contribution of each has been a tedious task. Concerning the past fish kills that were recorded in lacustrine systems in Greece, in each case a decisive factor leading to the fish kill was identified. In Lake Karla the decisive factor was the bloom of the ichthyotoxic phytoplankton species *Prymnesium parvum* and *Pfiesteria piscicida* and of several cyanobacterial species (Oikonomou *et al*., 2012; Papadimitriou *et al*., 2013), whereas in Lake Koronia during the 2004 event the dominant factor was the bloom of ichthyotoxic *Prymnesium* cf.*parvum* co- occurring with known toxic cyanobacteria (Moustaka-Gouni *et al*., 2004; Genitsaris *et al*., 2009). During the event of 1995 in Lake Koronia, the lake's water-level dropped (in combination with high temperature), along with suspension of fractionated phosphorus particles from the lake's sediments, possibly triggered by strong winds (Kaiserli *et al*., 2002), were the most decisive factors. A similar weather phenomenon also caused resuspension of sediment in Etoliko lagoon, leading to H_2S release and consequently to an intense growth of bacteria that rapidly consumed the dissolved oxygen of the water and led to the fish kill (Leonardos & Sinis, 1997).

Ismarida Lake is a very shallow, eutrophic/hypertrophic ecosystem (Moustaka *et al*., 2011), experiencing severe stress during the last few decades. The most important stress factors identified are:

a) the dramatic decrease of water level during the last decades, due to the over-exploitation of groundwater (Pisinaras *et al*., 2007),
b) the increased sediment inflow, due to higher soil erosion at the upstream parts of Vosvozis River tributaries,
c) the large water level fluctuations during the year,
d) the high watershed-to-lake area ratio,
e) the high coverage of agricultural land over the lake's watershed (Katsiapi *et al*., 2012),
f) the intrusion of sea water (Moustaka *et al*., 2011),
g) the inflow of the effluents of the waste water treatment plants from the city of Komotini and Pagouria village, and
h) the extensive reed-beds around the lake (Bousbouras *et al*., 2010), at the expense of the lake's surface.

From this point of view, Ismarida Lake is a highly variable ecosystem where changes in water levels are vital. Specifically, the fluctuations of water level (increase due to the inflow of freshwater in winter and spring or drop due to evaporation in summer or drainage to the sea) and the intrusion of seawater have resulted in marked changes in the salinity of the lake, with direct consequences for the organisms living in it (phytoplankton, macrophytes, zooplankton, and hence fish). The high biomass values and frequent, ecologically destructive blooms of marine and freshwater phytoplankton species, including harmful cyanobacteria and dinoflagellates, indicate poor water quality (Moustaka-Gouni *et al.,* 2012).

Although, cyanobacterial blooms have been documented in Ismarida Lake previously (Moustaka *et al*., 2011), during the study period these species were not among the dominant phytoplankton. Nevertheless, a few days after the fish kill, a harmful brackish waters phytoplankton species was detected, the ichthyotoxic dinoflagellate *Pfiesteria piscicida* that coincided with fish kills in Lake Karla (Oikonomou *et al*. 2012). Although its abundance was lower than that reported in the literature to cause fish kills (> 600/ml), it is unclear what happened some days earlier and during the fish kill incident in such a highly fluctuating ecosystem. In addition, during our study after the fish kill, a bloom of another harmful brackish/coastal water dinoflagellate species, *Prorocentrum minimum,* occurred. Fish kills have been associated with this potentially toxic dinoflagellate under conditions of high temperatures and incident light irradiances in response to increasing coastal eutrophication (Heil *et al.,* 2005).

Concerning the ichthyofauna, large-sized specimens of both euryhaline and freshwater species were found in the dead fish layer during the day of the event. This could be attributed either to the fact that smaller-sized fish (e.g. *Knipowitchia caucasica*) are more resistant to harsh conditions or that smaller sized-fish were not collected by the scoop used. A reason explaining the small proportion of freshwater species in the species richness of the dead fish layer could be that the increased salinity in the lake two days prior to the event may induce the movement of freshwater species towards the confluence with Vosvozis River or even upstream of the river. Of the fish species that were collected from the dead fish layer, specimens of *A. anguilla* and *C. carpio* were not found during the post-event sampling (November 2013). This could be explained by the small number of specimens of the two latter species left in the lake because of its high salinity during the last decade; specimens of these species remaining in the lake died during the fish kill; and finally, the sampling tools used (electrofishing device) was inappropriate to collect specimens of these species especially eels.

Our thoughts on the causes of this incident are that the sea water entry triggering the event may have been more sudden than usual. This could be related to an inward horizontal pressure gradient applied between the two sides of the weir, resulting from the seasonal fall of lake's water level (recorded by the hydrographic site) and the raised coastal Thracian Sea level, due to the strong dominant north-eastern Etesian winds (recorded by synoptic meteorological data). Indeed, north-eastern winds blowing over the North Aegean Sea tend to accumulate the water towards the Thracian Sea coastline (Sylaios *et al*., 2013), thus pushing sea-water through the long and narrow entry channel into Ismarida Lake. This intense and continuous entry of seawater abruptly increased the salinity of the system during these two days of the fish kill. This violent entry may have caused a stirring of the lake's sediments, triggering the resuspension of inorganic and organic matter, providing an introduction of harmful coastal water phytoplankton species, resulting in a harmful bloom and an extremely high number of large heterotrophic bacteria that finally rapidly consumed the dissolved oxygen: all this may have led to the fish kill.

The fish kill could also be attributed to other factors: a) the system's limnological conditions reached limit levels for the large-sized fish living in the lake; b) adverse water quality conditions shown by the microscopic water analysis (phytoplankton blooms and a conspicuous bacterial bloom), which could have also stressed the fish in the lake; c) intense and continuous entry of seawater causing introduction of harmful marine algal species and stirring of the lake's sediment, triggering the fish kill, and d) the high organic load transferred from Vosvozis River into the lake. A combination of these factors could have led to the fish kill.

Based on the knowledge gained concerning Ismarida Lake, some management measures towards the improvement of the lake's ecosystem could be proposed:

- Permanent engineering construction that will prevent the entry of seawater to the lake, either at the site of the existing weir, or at a new location closer to the sea. This construction should have the ability to allow freshwater to flow towards the sea during floods.
- Construction of an artificial wetland as a filtration buffer downstream of Pagouria, to achieve the highest possible biological quality of the Vosvozis River before its outfall to Ismarida Lake.
- More efficient operation of the waste water treatment plants discharging their effluents into Vosvozis River.
- Regular monitoring of Ismarida Lake, including salinity, amounts of phosphorus and nitrogen compounds, harmful phytoplankton and cyanobacterial blooms.

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