Assessment of Ecological Importance and Anthropogenic Change of Subaquatic Springs in Ancient Lake Ohrid

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Abstract

Apart from their contribution in the water balance of Lake Ohrid, Republic of Macedonia, subaquatic springs are also expected to affect the water quality. A simple experiment was developed and applied to subaquatic spring in Kališta region (south-east of Kališta village, in the north-western part of the Lake) and at the spring at Veli Dab (eastern side of the Lake) based on physicochemical spring water properties. Different sampling methods were established with the aim of uncovering a more suitable way of sampling pure subaquatic spring water. The goal was to test these findings and adapt them for further analysis with higher temporal and measurement related resolution. Measurements were also aimed at gathering additional knowledge and methodology for characterizing the hydrogeology of the watersheds. Of interest was information on the general chemical composition of spring water, interactions between aquifer and groundwater and on the origins of groundwater. Integration of this knowledge adds to a better understanding on how and what kind of groundwater is delivered to Lake Ohrid. The obtained results show that the examined springs are rich in nutrients and, comparing to Lake water, exhibit temporal variations in temperature, pH, conductivity and dissolved oxygen. Investigated springs have a very constant flow, as well as water quality.

Keywords: Lake Ohrid, subaquatic springs, sampling, water quality

Introduction

Lake Ohrid is located in the central Balkan Peninsula (Southeast Europe) on the border between Albania and Macedonia. With a maximum length and width of 30.3 and 15.6 km, respectively, the lake covers a surface area of 358 km^2, reaches a maximum depth of 288.7 m and a volume of 55 km^3 (Table 1, Figure 1). With a limnological age of likely 4 to 10 million years, it is one of the oldest lakes in Europe (Wagner, 2008). Lake Ohrid is a steep-sided graben of rift formation (Albrecht, 2008a).

Different to most other inland waters, Lake Ohrid is mostly fed by subaquatic and surface springs which play a significant role in the formation of this unique system. From a water balnce poit of view, an inflow ratio of ~50% of total spring discharge into Lake Ohrid was estimated which equals to estimated ~25% of total inflow for subaquatic springs (Matzinger *et al.* 2006b). Upstream Lake Prespa (Figure 2) discharges lake water by underground flow which feeds certain springs in the Lake Ohrid region.

Spring lakes systems are very unique and special ecosystems. The specific ecology of Lake Ohrid reflects conditions of its catchment, its eco-climatological setting and is strongly dependent on subaquatic springs (Matter *et al.*, 2010).

The important role of subaquatic springs in the Lake's development has been mentioned in other lakes, such as Lake Kivu, Lake Cadagno (Tonolla *et al.*, 2005, Schmid *et al.*, 2010). Lake Ohrid is an oligotrophic lake; it is inhabited with more than 200 endemic and relic forms of organisms. Clearly the most spectacular quality of the Lake is its impressive endemism and subaquatic springs which contribute to and support its biodiversity, as several endemic organisms are found exclusively close to the springs (Stanković, 1960; Šapkarev *et al.*, 1988; Salemaa, 1994).

While considerable knowledge has been acquired on various aspects of the Lake Ohrid ecology over the past decades, the subaquatic springs were

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not considered, as their existence is only known from a few recent observations and from indirect water balancing (Wüest, 2005). Knowing a small catchment area of the Lake it can be presumed that subaquatic springs are numerous, but their distribution is unknown. In shallow zones, several subaquatic spring inflows have been described in the past (e.g., Gilbert and Hadzisce 1984). In recent years, subaquatic spring inflows were also detected at large depths (Matzinger and Wüest 2004). Input of pure spring waters (Matzinger *et al.*, 2006a; Jordanoska *et al.*, 2010, 2012) is important for the oligotrophic status of Lake Ohrid and its subsequent low algae productivity. All previous findings postulate that the subaquatic springs have a significant influence on the overall physicochemical properties of Lake Ohrid by supplying nutrients and dissolved oxygen at different depths and by creating distinctly different, but constant boundary conditions for the organisms near the springs.

Explanation of hydrogeological relations, correlation and circulation of subaquatic spring water is very difficult task, and sometimes includes a lot of assumptions. Although contribution of these springs to the water balance the Lake Ohrid has been calculated, there is still little knowledge on the physics and chemistry of water that seeps from the lake floor. Very little is known about the flux in the seepage of such water, the path and processes that it passes. Because of theirs complex flow paths, difficult collection and limited in depth analysis, little data exists that would identify springs of conservation concern, although rare species associated with springs have been detected.

The objective of this paper is to describe the experimental observation that uses physicochemical data to improve localization and characterization of subaquatic springs and its hydrology as well as best and uniform way of sampling the pure spring water. It was shown that these springs presented temporal variations in temperature, pH, conductivity and dissolved oxygen (DO) compared to lake water (Kunz, 2006), so these parameters were used as tracers by vertical profiling in the water column. The physical characterization of subaquatic springs involves mainly their influence on local turbulence and stratification, as well as the horizontal distribution of spring water, the sources and factors controlling ground water and surface water quality including a discriminating natural background and anthropogenic impact. Characterization of the subaquatic springs is an important contribution for protection and preservation of Lake Ohrid. Here, we present the basic physicochemical compositions of two different spring waters, and the potential implications on aquatic organisms living nearby.

Table 1: Characteristic of Lake Ohrid and its water balance (Matzinger *et al.*, 2006b)

Lake Ohrid	
Location*	40.900°-41.174° N; 20.628°-20.810°E
Surface area	358 m^2
Altitude	693.7 m.a.m.s.l.
Length (maximum)	30.3 km
Width (maximum)	15.6 km
Depth (mean)	155 m
Volume	55 km^3
Watershed	1.002 km^2
Hydraulic water residence time	70 years
Outputs	
Surface outflow	24.9 m^3/s
Evaporation	13.0 m^3/s
Total output	37.9 m^3/s
Inputs	
Precipitation of lake surface	8.8 m^3/s
River inflow:	
Albanian catchment	0.5 m^3/s
Macedonian catchment without river Sateska	1.9 m^3/s
River Sateska (diversion into Lake Ohrid in 1960)	5.5 m^3/s
Temporary inflows (estimation)	1.0 m^3/s
Surface spring inflow	10.3 m^3/s
Sublacustrine springs (from closing balance)	9.9 m^3/s
Total input	37.9 m^3/s

*(Albrecht, 2008)

Figure 1: Map of Lake Ohrid.Bathymetry with 25 m contour intervals and marked surface and subaquatic sources (Albrecht, 2008)

Figure 2: Ohrid and Prespa Lakes and their watershed boundaries (Gorsevski, 2008)

Materials and Methods

Study area

Lake Ohrid (40° 54' to 41°10' N and 20 38' to 20 48' E) (Figs. 1 and 2) is located on the central Balkan with approximately two-third of its surface area belonging to Macedonia and about one-third belonging of Albania. The hydrography reveals the karstic character of Lake Ohrid and it is almost exclusively fed by underground water from more or less abundant karstic sources (Stankovič, 1960). Even in the beginning of the century Cvijić pointed out that there is underground connection between Lake Ohrid and upstream Lake Prespa (higher for 158 m.a.m.s.l.). Two lakes are bounded with mountains massive water plunges trough the karts areas of Galičica and Suva Gora mountains. According to results (Anovski, 1980) with application of nuclear tracers and relevant techniques tracers was proven that the Prespa and Lake Ohrid are connected and the groundwater of the Thate-Galičica karst massive is fed by three spring groups: Biljana Springs-Bej Bunar; St. Naum springs, Thusemište and Bilihti valley. Together, both Lakes are unique regional hydro systems in the world. Many sublacustrine and surface springs, particularly on the south-eastern and southern side of Lake Ohrid, are charged by neighbouring Lake Prespa as well as by mountain range precipitation seeping through the karstic rocks and mixing with the waters originating in Lake Prespa (Anovski *et al.*, 1980; Eftimi & Zoto, 1997; Matzinger *et al.*, 2006a; Amataj *et al.*, 2007; Popovska & Bonacci, 2007).

The studied subaquatic springs are located in the north-western part of the Lake close to Kališta village (Figure 3) within a large area with many small and a few large springs at a depth of 8-10 m (LAT 41°09.671`N; LON 20°41.080′ E). At this location subaquatic springs are spread over a ~50 m-diameter area at a distance of 100 m from the shore, where there are many so-called littoral subaquatic springs. To draw the map (Figure 3), divers followed the outline of the active spring zone and some coordinates were recorded following their bubble-course (a dotted white line, with some inconsistencies in the coordinates). Water depths of the sampling positions, subaquatic spring 1 and 2 were recorded by echosounder and the help of the divers. The surface spring and littoral subaquatic springs' approximate locations are shown close to the shore.

Another sampling spot is on the Eastern side of the Lake where two subaquatic springs were detected in the Veli Dab area (40 ˚59.341' N; 20 ˚47.971' E). Water is emerging from horizontal cracks in rocks from the shoreline. Both sampling sites have surface springs in the near vicinity which were used for water quality comparison.

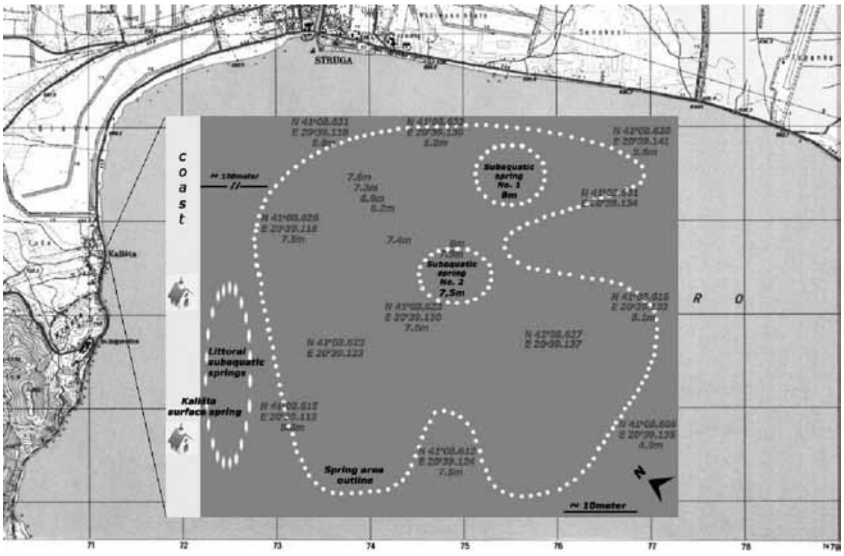

Figure 3: Schematic map of the Kališta subaquatic spring area and deep zones

Regular water samples were taken from Lake Ohrid springs from November 2006 until December 2008. Chemical analyses were carried out in laboratories at HBI, Ohrid and at the Faculty of Natural Sciences and Mathematics, Skopje.

Methods

In situ measurements were performed for the following parameters: T, Conductivity, pH, alkalinity and CO_2. For oxygen measurements, Winkler bottles were used. Samples for other analysis were drawn using a plastic bottle of 1500 ml. Samples were stored in new or acid-rinsed bottles and cooled for transport. At HBI's laboratory, water samples for measurements of major ions composition and nitrate concentration were filtered using nitrocellulose filters (pore diameter = 0.45 µm). Concentrations of nitrate were determined photometrically (Strickland and Parsons, 1968). Oxygen measurements were carried out following the Winkler method (Clesceri *et al.*, 1998). Phosphate concentration was performed photometrically (Strickland and Parsons, 1968). Cations and trace elements were analyzed by atomic absorption spectrometry (AAS) and electrothermal AAS (Varian, SpectrAA 55B and SpectrAA 640Z, respectively). In addition, profiles were taken in Lake Ohrid with the Seabird SBE 19 CTD profiler.

Evaluation of sampling strategies for subaquatic springs

Three sampling methods were evaluated within the spring mouth. Interaction of lake and groundwater which enters the Lake can cause serious misinterpretation of the physicochemical properties due to the difficulty of sampling the clear spring water.

The first method is based on one sampling instrument which was adapted from Lee (1977), built in Eawag, Kastanienbaum. The seepage meter (Lee, 1977) is a device that allows direct measurement of subaquatic spring water and the seepage flux. It consists of a plastic barrel, valve and plastic bag. Clarifying device utility was used to clarify sampling errors associated with its use. It should be noted that its use is limited to shallow spring inflows and where work with divers is possible. Springs inflow is very strong and has permanent sediment that rises above and is covered by the barrel flows constantly from the valve.

Another sampling method used was with Niskin flask placed directly to the spring mouth. Different sampling methods were established with the aim of finding a more suitable method of sampling the pure subaquatic spring water. Neither the flask nor the seepagemeter provide high reliability in their utilization. Table 2 shows a comparison between different sampling methods for few important parameters, showing differences that were observed, and wihich can be attributed to inappropriate sampling methods. Differences can also be observed in the Figure 4.

Table 2: Some parameters measured after different sampling techniques

Sampling method	Total phosphorous, TP [µg/l]	Free CO_2 [mg/l]
Lake water	69.9	0.28
Kališta - plast.bag	83.0	9.24
Kališta flask	57.6	29.3
Kališta barell	13.2	2.64
Kališta pump	2.42	0.28

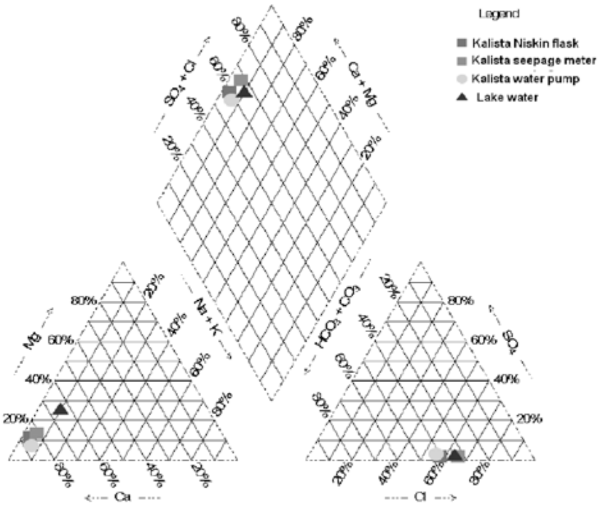

Figure 4: Piper diagram for different sampling techniques at Kališta spring area

The rocky formation around the Veli Dab spring area imposes another sampling method which, at the end was found to be most appropriate. This method utilizes a hose and vacuum pump. A vacuum pump was attached to the boat, and a hose to the spring mouth. Colouring was used in order to estimate the spring water, when colour appears at the surface there is assurance that it is spring water at both spring areas.

The chemical composition of surface water and ground water is controlled by many factors that include the composition of precipitation, mineralogy of the watershed and aquifers, and climate. Variations within the dataset are related to variations in calcium, magnesium, SO_4^{2-} which are derived from natural weathering reactions and pH, NO_3^-, chlorine and some trace elements which indicate anthropogenic impact. Seasonal changes in the water chemistry of individual sites were analyzed to better characterize the spatial variability of vertical hydraulic conductivity. The integrated result provides a method for characterizing hydrogeology of the watershed that fully utilizes traditional data.

The Kališta subaquatic spring contains a sandy sediment bottom where currents create ripples and drive pore water exchange. In the ripple troughs water penetrates into the sediment and flows along a curved path in 10-30 cm vertical plumes forming over the spring outlets. The sediment surface of the springs is characterized by numerous spring craters and algae (Figs. 5 and 6). Outflow rates were quite constant ~0.6 L s^{-1}. This ion-rich spring waters play a major role in this ecosystem. Depth allows vertical profiling which describes temporal variations and differences between Lake and spring water.

In the Veli Dab area, spring water with diffusive character is emerging from horizontal cracks in rocks forming different environments having similar characteristics to the Kališta spring area.

Figure 5: Calcite formation of subaquatic springs at Kališta region

Figure 6: Small pores and ripples of spring water emerging in the lake water

Results and Discussion

Vertical profiles were done by CTD profiler. Spring water temperature differences in the Lake can be seen in the Figs. 7 and 8a. During the autumn and winter periods show temperature stratification due to the depth of the water column of only ~8 m.

Veli Dab spring had an average temperature of 10.4 °C which is approximately equal to the Kališta spring average temperature of 10.5 °C. Comparing to the data from both surface springs that are located at both subaquatic spring areas, Kališta surface spring had a temperature of 11.5 °C and Veli Dab surface spring at both measurements before it dried had 8.8 °C.

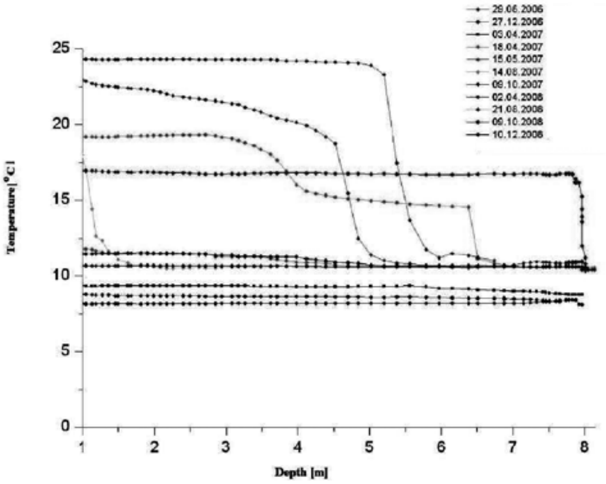

Figure 7: Temperature of all measured CTD profiles at Kališta spring area

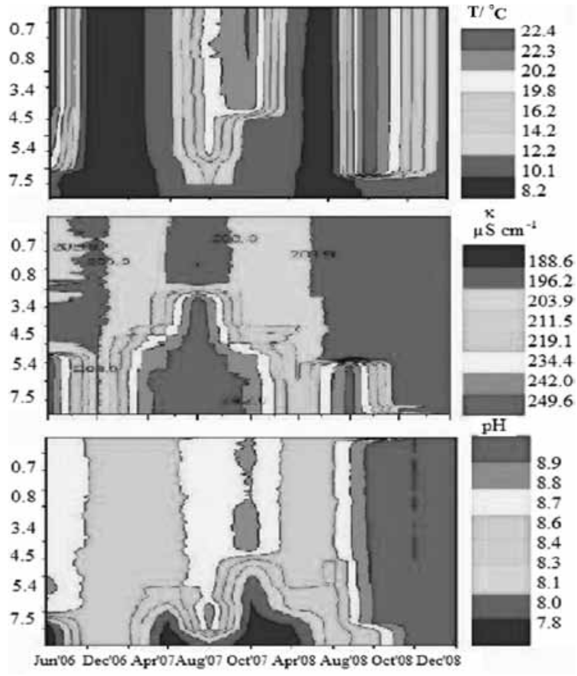

Figure 8: Vertical depth profiles at Kališta spring area over the period from 2006 to end of 2008
a) Temperature measured with CTD profile
b) CTD conductivity transformed into conductivity at 20°C base on ionic composition (Wüest *et al.*, 1996)
c) CTD pH depth profile

κ_T values were transformed to conductivity at 20°C base on ionic composition (Wüest at al., 1996). The signals found in these profiles originate from spring water intrusion. Depending on the different seasons of the presented measurements (Figure 8a), spring water is constant in comparison with Lake water at around 10.6°C. Additionally, conductivity of spring water is higher than that of Lake water (Figure 8b). Hence, when spring water mixes with Lake water, temperature is decreased, and conductivity is increased. Seasonal profiles describe mixing of lake and spring water taken in spring and winter periods and even autumn (Figure 9). Temperature has an effect on conductivity, but near the spring area inducted stability. During the two and a half year study, there was a strong pycnocline in the northern region of the study area. During the winter period lower conductivity at Kališta subaquatic spings water was mixing with lower conductivity Lake water, with both water types having close temperatures. pH remained fairly constant in both subaquatic spring sampling areas, with average values of 7.6 pH.

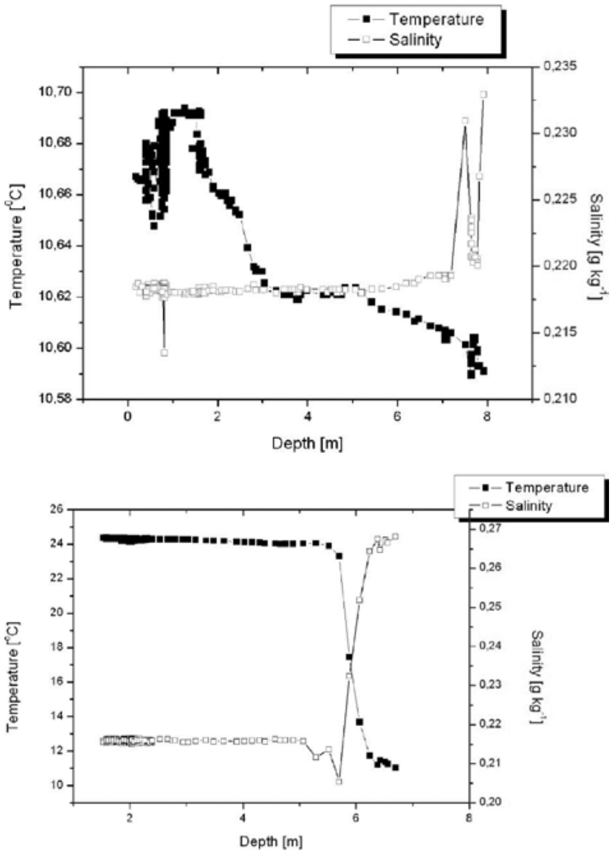

Figure 9: Stratification-based on CTD profiles taken at Kališta spring area taken on 10 December 2008 (a) and 23 August 2008 (b)

Lake Ohrid is a carbonate lake which results in great buffer capacity of the lake water (Wetzel & Likens, 1991). The accumulation rates of $CaCO_3$ within the spring area are high and suggest that authigenic carbonates are formed throughout the entire water column (Matter, 2008). That could also be observed from the difference between spring and Lake water. Both subaquatic springs, sampled at the bottom of the Lake showed higher values of ~145 mg/l of $CaCO_3$ than 125 mg/l of $CaCO_3$ for lake water.

All processes can be traced through the changes in concentration of Ca^{2+} and Mg^{2+} can which affect water hardness. Higher values in Ca^{2+} are recorded at the Veli Dab surface and subaquatic spring, which is expected due to the rocky surrounding of the spring. Average magnesium values were from 3 to 6 mg/l. Sodium is very soluble in water and can reach high concentrations, but here the average values showed high variations from 2.4 to 3.5 mg/l. Potassium found in the Lake and spring waters had values from 0.6 to 2.3 mg/l for lake water at the Veli Dab subaquatic spring (Figure 10).

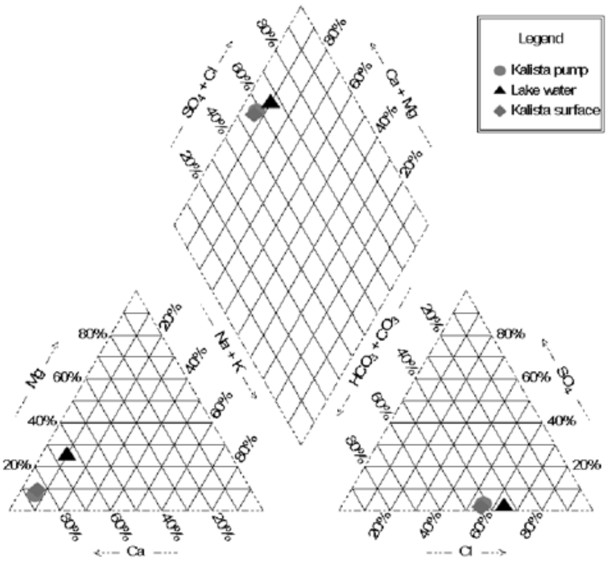

Figure 10: Piper diagram with difference between surface, subaquatic and Lake water

NO_2-N concentrations were almost always undetectable. Average values for all nitrogen forms are given in the Table 3. Values for NO_2-N at the Kališta surface spring were mentioned previously. Lake water average value is of 1.2 µg/L NO_3-N, due to a single measurement in June, 2006 when a high concentration was observed. No regularity was shown in any of the large fluctuations in concentrations except that rainfall considerably contributes to NO_3-N content. Values were in a large range from 0.06 to 1 mg/l. In comparison with subaquatic spring water, lake water has very low concentrations of NO_3-N, with average values of 0.06 mg/l at Kališta and 0.09 mg/l at the Veli Dab spring area. Generally NH_4-N concentrations were almost always undetectable.

Average values for the subaquatic spring at Kališta are from two measurements in April, 2008 and December, 2008 with values of less than 0.013 mg/l. Lake water concentrations of NH_4-N were detected in August, 2008 at both subaquatic springs area. Kališta lake water exceeded the value of 0.039 mg/l in summer 2008 which clearly associates the anthropogenic influence during the summer period.

Table 3: Average values for total phosphorus and nitrogen forms (in µg/L), DO (in mg/l) and O_{sat} (%)

Sampling site	TP	NO_3-N	NO_2-N	NH_4-N	TN- Kjeldahl	TN	DO	$O_{2,sat}$
Kališta surface spring	22.0	887	0.21	0.00	380	1267	8.43	78.0
Kališta lake water	14.1	68.4	0.09	3.23	448	516	9.66	109
Kališta subaquatic spring	9.74	402	<0.01	3.00	399	801	10.16	103
Veli Dab subaquatic	4.40	341	<0.01	0.00	326	667	10.42	105
Veli Dab lake water	3.42	97.6	<0.01	1.19	433	530	9.42	109
Veli Dab surface	18.6	237	<0.01	<0.01	354	590	10.88	105

The subaquatic springs area and lake water in the vicinity exhibited natural sources of phosphorus which is mainly due to decomposition of organic matter. Phosphorus associated with organic and mineral constituents of sediments in water bodies can also be mobilized by bacteria and released into the water column. That was the case with the Kališta lake water area, where higher variability was shown during the whole monitoring period. Concentrations varied from 0.003 to 0.070 mg/l.

DO and $O_{2,sat}$ show geographical trends. Springs from the East coast of Lake Ohrid are more undersaturated with DO than the Northern part. Simple comparison is made at both subaquatic sampling places with Figure 11 that describes Kališta surface, subaquatic and Lake water DO in comparison with free CO_2. Figure 12 shows the same trends but at the Veli Dab spring area. The oxygen status of subaqutaic springs is presented with average values which are around 2.4 mg/l O_2 for spring water, and slightly higher values that reached 3.34 mg/l O_2 for lake water at Kališta. The subaquatic spring in this area had the highest value of 3.66 mg/l O_2 in September. The lowest values exceeded 0.31 mg/l O_2 at both surface springs measured in October, 2008.

These subaquatic springs had almost standard fluctuation during the summer period with values that never exceeded a value of 1.7 mg/l for the Kališta spring area. The Veli Dab subaquatic spring BOD_5 ranged from 0.2 to 1.4 mg/l Kališta had higher values for BOD_5 in comparison with the Veli Dab area. Even lake water had obvious seasonal trends during the summer period at both subaquatic springs sampling locations, which is not that significant for the Kališta subaquatic spring. This sampling spot also showed rising trends during the winter period.

Robustness of the sampling and localization is showed at the depth sites. Littoral site identification is easier, however at deeper bottom sites of the lake assistance can only provided by certain ground structures such as fine sediment or gravelled ground. Where sediments are saturated, as expected in submerged materials, groundwater is synonymous with pore water.

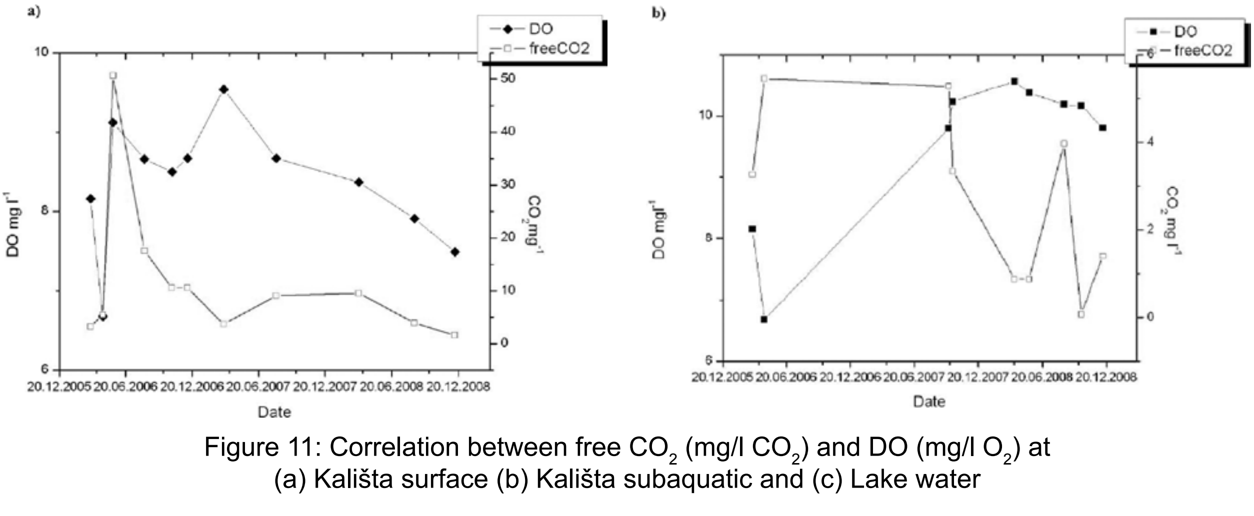

Figure 11: Correlation between free CO_2 (mg/l CO_2) and DO (mg/l O_2) at
(a) Kališta surface (b) Kališta subaquatic and (c) Lake water

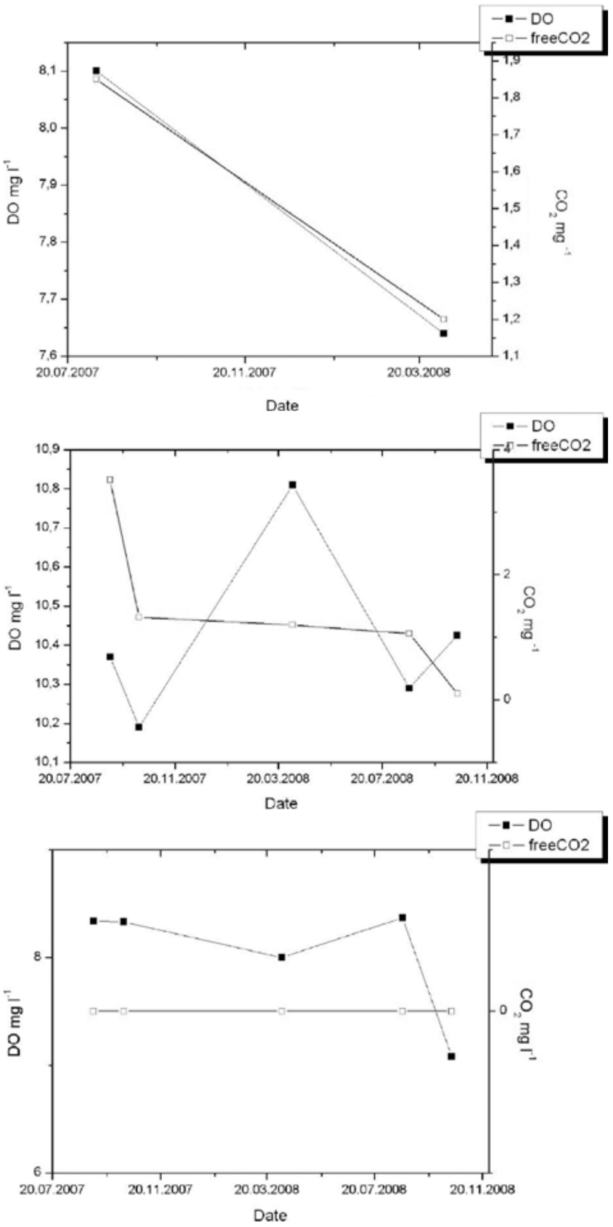

Figure 12: Correlation between free CO_2 (mg/l CO_2) and DO (mg/l O_2) at Veli Dab surface (a), subaquatic (b) and Lake water (c)

Data of echo-sound surveys (Wüest, 2005) indicates the presence of underwater "stalagmites" close to areas where underwater springs are expected. According to the low-resolution echo-sound images, the height of such formations in some cases seems to exceed 10 m from the lake bottom. The formation of such, possibly calcareous structures, might be explained by the higher concentration in calcium carbonate in the spring water (Calcium ratio "springs/Lake Ohrid" ~ 3:2) in combination with microbiological activity (Wüest, 2005).

Using a dense grid of CTD profiles, deep spring areas can be located using the spring signals in temperature and salinity. Based on the oxygen saturation of springs at the lakeshore, the detection of a spring-signals was also expected from a sensitive oxygen probe.

While sampling with the seepage meter divers caused turbidity, and neither the Niskin flask, nor the seepage meter gave satisfactory results. Finally the best results are presented using the vacuum water pump. All sampling strategies are impossible without the help of divers.

The aim of this paper was to evaluate the sampling strategies and identify subaquatic sources of physicochemical parameters. This was difficult due to the mixing and biochemical reactions in the lake water that modifies the chemistry of the subaquatic spring.

Spring water in the Lake Ohrid region was characterized by a remarkable stability over time and by significant variability when comparing individual springs. Occasionally, small fluctuations occurred for all parameters (i.e. T, k_{20}, pH, DO, $O_{2,sat}$, concentrations of major ions composition), but temporal stability could be illustrated in which variations of less than ±0.1 °C occured.

This study found that subaquatic springs provide a source of nutrients in the lake water and its estimation can provide data for the lake water cycle.

The precious conditions around the springs are however under increasing pressure: The main human impact in the catchment - water pollution, water consumption and climate change - are also expected to have an effect on spring properties. Regretfully, results from a sanitary aspect of the microbiological investigations suggest an anthropogenic influence, in other words, faecal waste waters (sewage) pollution (Lokoska, 2009).

The Kališta region was characterized with temporal constancy at the beginning, which so far was a general characteristic for all spring water, but occasionally parameter values were measured in large ranges. The main conclusions based on these differences must take into account seasonal variations, anthropogenic influence, and areal sampling depth.

At the Veli Dab spring area knowing the complex structures of the rocky area variations in chemical composition might be due to geochemical proceses.

Facing the differences between two different types of subaqutic springs there is temporal variation among their physical and chemical properties, although there were certain variabilities measured in different parameters including establishing appropriate and suitable sampling methods.

Clearly so far, based on all parameters there is a considerable difference between the Lake and spring water.

Acknowledgments

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