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ORIGINAL ARTICLE

Observing lake-sea tidally driven water exchange in Lake Zmajevo Oko (Rogoznica, Croatia)

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Abstract: Lake Zmajevo Oko (ZO), a marine lake near Rogoznica, Croatia, known for sudden anoxic overturns and progressive deoxygenation, has been studied extensively for its physical and biogeochemical properties over the past 30 years. However, the water exchange with the sea through the porous karst remains poorly understood. From 2020 to 2023, opportunistic temperature, salinity, dissolved oxygen, nutrients, and sea level measurements were conducted at various sites in and around ZO, including underwater caves, which are believed to facilitate the greatest exchange with the sea. Although tides in ZO are attenuated compared to the sea, tidal exchange influences the upper-layer lateral boundary temperatures. Moreover, the water that enters ZO has different effects in winter and summer and may alter hydrostatic stability depending on its salinity. Spectral analyses of water levels show that the karst between ZO and the sea acts as a natural filter, protecting against high-frequency water level fluctuations in the neighbouring sea. Fluctuations in dissolved oxygen and nutrients were also observed during the tidal cycle at locations with considerable subsurface exchange. However, more precise sampling strategies are needed to quantify the effects of ZO-sea exchange on ZO's biogeochemical properties. As anoxic overturn events become more frequent, further studies are essential to understand ZO's sensitivity to water exchange, particularly in the context of a changing climate that is expected to bring higher air temperatures, increased salinity, and more extreme precipitation in the Adriatic.
Keywords: Marine Lake Zmajevo Oko; anoxia; stratification; layer overturn; tidal cycles; karstic water exchange

Sažetak: OPAŽANJA IZMJENE VODE IZMEĐU ZMAJEVOG OKA (ROGOZNICA, HRVATSKA) I MORA UZROKOVANE PLIMOTVORNOM SILOM. Jezero Zmajevo oko (ZO), morsko jezero blizu Rogoznice u Hrvatskoj, poznato po naglim anoksičnim promjenama i progresivnoj deoksigenaciji, intenzivno je proučavano posljednjih 30 godina u kontekstu fizičkih i biogeokemijskih svojstava. Međutim, izmjena vode s morem kroz porozni krš i dalje je nedovoljno istražena. Od 2020. do 2023. godine provođena su povremena mjerenja temperature, saliniteta, otopljenog kisika, hranjivih tvari i razine mora na lokacijama unutar i oko ZO-a, uključujući podvodne špilje, za koje se vjeruje da se kroz njih odvija najveća izmjena vode s morem. Iako je plimni signal u ZO-u prigušen u usporedbi s morem, plimni tokovi vode utječu na lateralne temperature u gornjem sloju. Nadalje, voda koja ulazi u ZO različito djeluje zimi i ljeti, potencijalno mijenjajući stabilnost vodenog stupca ovisno o salinitetu. Spektralne analize razine vode u jezeru pokazuju da stijene između ZO-a i mora djeluju kao prirodni filter, gušeći visokofrekventne oscilacije prisutne u susjednom moru. Fluktuacije u koncentracijama otopljenog kisika i hranjivih tvari također su uočene tijekom plimnog ciklusa na mjestima s izraženom podzemnom izmjenom vode. Međutim, potrebne su preciznije strategije uzorkovanja kako bi se kvantificirali učinci izmjene između mora i ZO-a na biogeokemijska svojstva ZO-a. Kako se učestalost anoksičnih događaja povećava, daljnja istraživanja su nužna za razumijevanje osjetljivosti jezera na izmjenu vode, posebno u eri klimatskih promjena koje se manifestiraju kroz više temperature zraka, povišeni salinitet i ekstremnije oborine nad područjem Jadrana.
Ključne riječi: Morsko jezero Zmajevo oko; anoksija; raslojavanje; obrtanje slojeva; plimni ciklusi; izmjena vode kroz krš

INTRODUCTION

Marine lakes (ML) are a group of coastal lakes connected to the sea, located in the karst areas near sea level (Blanchette *et al.*, 2020) and protected from wind by tree canopies and steep karst ridges (Hamner and Hamner, 1998). Such lakes form and disappear due to transgressions and regressions at the geological scale, while the connection between sea and lake can be permanent or sporadic (e.g. during extreme water levels in the sea; Fleury *et al.*, 2007). In addition to sea level, meteorological conditions, lake-sea and lake-groundwater exchanges, intra-lake currents can also determine the physical dynamics of MLs (Blanchette *et al.*, 2020). Depending on the type of lake-sea connection and its morphology, there is usually damping and phase shift of a marine signal, e.g. the tidal signal, within the lake (e.g. Peharda and Vilibić, 2008). Another consequence of the reduced lake-sea connectivity is the strong and persistent stratification, as observed in the meromictic Palau Lakes (Hamner and

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Hamner, 1998), which can, in exceptional cases, be interrupted during a short period of time when a complete lake overturn takes place, as in lake Zmajevo Oko (ZO) (Ciglenečki *et al.*, 2017; Dominović *et al.*, 2023; Marguš *et al.*, 2023). Often, MLs also have an inflow of groundwater through the porous karst area or through submarine karst springs (Wunsam *et al.*, 1999; Blanchette *et al.*, 2020), which can influence the salinity, temperature and nutrient balance as well as the stratification of a lake.

A unique and well-studied example among Palau's lakes is Jellyfish Lake (JL; Palau), which is 30 m deep and rectangular in shape (approx. 4000 m^2; Burnett *et al.*, 1989). The lake is well stratified, meromictic, with an anoxic bottom layer and an oxygen-rich upper layer that is both nutrient-poor and turbid. Water exchange at the surface occurs through small cracks in the rocks and is considered slow compared to mid-waters, where water exchange occurs via lake-sea tunnels in tidal cycles (Hamner and Hamner, 1998). As it is a tropical lake, there is no temperature inversion caused by the strong cooling at the surface, which could weaken the stratification and cause strong vertical mixing. Nevertheless, colder and saltier water occasionally enters the lake with the tides, which maintains the pycnocline by settling in the deeper layers (Blanchette *et al.*, 2020). Interestingly, the lake exhibits changes in stratification related to hemispheric processes that are known to influence the climate of the entire planet, such as ENSO (El Niño/Southern Oscillation, Martin *et al.*, 2006) and can therefore serve as an indicator of global and regional climate change.

Another well-documented example of a ML is ZO, a karstic sea lake on the eastern Adriatic coast, which can also serve as an archive for regional climate change. It is larger but shallower than the JL and has the shape of an irregular ellipse (area 9904 m^2; max. depth 13.5 m; Panđa, 2020). Deep circulation in ZO occurs once per year (autumn) causing the lake to mix either completely (to the bottom) or partially (to the chemocline), thus, ZO is considered as fluctuating between meromictic and holomictic mixing regimes (Ciglenečki *et al.*, 2017). In contrast to JL, there are no visible tunnels, and the lake is connected to the Adriatic Sea *via* small fissures in the surrounding rocks (Ciglenečki *et al.*, 2015). The water column of ZO is most of the time divided into two parts: the oxygen-rich upper layer and the anoxic deeper layer, while the pycnocline is well defined and inhabited by a diverse community of sulphur bacteria (Ciglenečki *et al.*, 2017; Čanković *et al.*, 2017, 2019). Despite the connection between the sea and the lake, the volume of anoxic water in the lake has increased over the last 30 years (Simonović *et al.*, 2023) and the connection itself seems to have weakened (Dominović *et al.*, 2023). Previous studies have pointed out that extreme meteorological conditions can cause mixing of the lake at the end of the warmer period of the year (Ciglenečki *et al.*, 2015), especially when there is weak vertical stratification due to a lack of precipitation in spring and summer (Zaninović *et al.*, 2008; Dominović *et al.*, 2023). The overturning causes mass mortality of aerobes in the lake (Kršinić *et al.*, 2000; Ciglenečki *et al.*, 2015). In addition, a pronounced halocline combined with long periods of high solar radiation, creates conditions for temperature maxima below the water surface (Legović *et al.*, 1991; Izhitskiy *et al.*, 2021; Marguš *et al.*, 2023). This further increases hydrostatic stability and prevents the renewal of nutrients and oxygen, which is why the deepest layers of many MLs are anoxic (Zadereev *et al*., 2017).

However, no studies for ZO have yet dealt with the exact nature of the connection between the lake and the sea and the physico-chemical properties of the exchanged water. This is because it can be assumed that seawater flows into the lake when water level in the sea is higher than in the lake. However, Blanchette *et al.* (2020) have shown for JL that this need not be the case and that the tidal inflow through the karst can have different physico-chemical properties than lake and seawater, which also leads to greater mixing during storms. As ZO is a small, mostly isolated and fragile ecosystem that can be easily affected by extremes and fluctuating mixing regimes (also referred to as a natural "experiment" or "laboratory", together with JL; e.g. Ciglenečki *et al.*, 2017; Blanchette *et al.*, 2020), we decided to continue exploring its connection to the sea by first locating the contact points between ZO, karst and the sea and investigating the characteristics of the inflowing water and the corresponding water level trends. Following several opportunistic campaigns organised between 2020 and 2023, this study aims to fill some of the knowledge gaps related to the exchange between ZO and the sea. The results will bring several benefits. Firstly, they will reveal the footprint and volume of inflowing water, answering the question of whether seawater enters the lake at some point during the inflow. The detected connection points between the lake and the karst will allow for more accurate monitoring of the inflowing water, as the researchers will know the best places to deploy the instruments and take water samples. Secondly, the results of this study will provide information needed for more precise tidal predictions for future ZO studies. Finally, the results of this study will enable the continuation of ZO research in the context of ongoing climate change, as climate scenarios for the Croatian coast predict a general rise in both mean sea level and sea level extremes due to changes in the winds that drive storm surges (Denamiel and Vilibić, 2023). Furthermore, these processes are expected in combination with strong warming and less precipitation in summer (Branković *et al.*, 2013) as well as salinization of the sea (e.g. Verri *et al.*, 2024), which calls into question the quantity and quality of water that will be entering ZO.

MATERIALS AND METHODS

ZO (Fig. 1) is located on a karstic peninsula near the village of Rogoznica (43°32' N and 15°58' E; Croatia) surrounded by steep cliffs (4-23 m height; Ciglenečki *et al.*, 2015). To investigate its connection with the sea

Fig. 1. Zmajevo Oko Lake (ZO) and the nearby Soline (noted as "*Bay 1*") and Koprišće (noted as "*Bay 2*") bays. Data loggers were positioned at six locations in the lake (marked as *LB E*, *LB W*, *Cave 1*, *Cave 2* (mouth), *Cave 2* (back), *Centre*), three locations in the sea (marked as *Bay 1a*, *Bay 1b*, *Bay 2*), and one land location (marked as *Land*). Water was sampled at two location inside the lake (*Cave 1* and *Cave 2* (back)) and two reference points (*Centre* and *Bay 2*). Sources for the background maps: https://www.bing.com/maps/aerial (Croatian coast) and http://preglednik.arkod.hr/ARKOD-Web (ZO and its surroundings).

and the consequent water exchange, opportunistic *in-situ* measurements were carried out with multi-monthly installed sensors and water samples in the vicinity of ZO and in the nearby Adriatic Sea. The main objective was to determine whether there are differences between the physico-chemical properties of the lake water, the seawater and the water that enters the lake as its water level rises. The next step was to investigate the differences, if any, and describe them using the known causal relationships associated with ZO and other similar systems.

Time series data

Since ZO is surrounded by karst rocks through which there is no direct connection to the Adriatic Sea (e.g. tunnel), but there are tidal fluctuations, our first goal was to identify potential contact points, i.e. cracks in the rocks through which water enters and leaves the lake. To this end, we first placed five temperature data loggers at several locations along the lateral boundaries of the lake. The locations are shown in Fig. 1: *LB E, LB W, Cave 1, Cave 2 (mouth),* and *Cave 2 (back)*, at depths of 2.8, 2.8, 0.7, 2.8 and 1.5 m, respectively. The sites in the caves, both submerged, were chosen because a diver had previously reported water with different optical/visual properties at these locations. We used MX2202 HOBO Pendant MX Temperature Data Loggers (Onset) with resolution of ±0.04 °C and accuracy of ±0.5 °C that were set to record at 15-minute intervals during the period from 29 June 2020 to 13 January 2021. Some loggers stopped recording earlier than planned.

After the preliminary review of the data, we decided to repeat the temperature measurements, but this time at a higher frequency (1 minute sampling rate), simultaneously in the lake and in the nearby sea (*Bay 1a, Bay 2*; Fig. 1, both at 0.5 m depth), and additionally record the water level data. For this set, we used HOBO U20-001-01-Ti Titanium water level data loggers (Onset) with a resolution of ±0.2 cm and an accuracy of ±0.5 cm. To distinguish tidal effects from daily atmospheric influences on water temperature (WT), we also recorded air temperature and pressure at the surface (*Land*; Fig. 1). Since the previous measurements were recorded at the lateral boundaries, we also used the temperature recorded in the middle of the lake for comparison (*Centre*; Fig. 1, at 0.5 m depth). This data set was part of another, longer experiment and was therefore recorded at 1-hour intervals using MX2202 HOBO Pendant Temp/Light (Onset). The measurements in this opportunistic experiment were recorded during the field campaign from 24

June 2021 to 27 June 2021 and all series mentioned in this paragraph refer to this period.

In the next campaign we were interested in comparing the water level of ZO and the sea. For this purpose, we deployed the instruments at the *Cave 1* and *Bay 1b* sites from 2 March 2023 to 12 July 2023. The loggers were placed at a depth of 1.3 m and set to take samples at 15-minute intervals. As these loggers also record temperature, this data was analysed in parallel with the water level data and noted as the "2023 temperature series".

Water sampling

When examining the 2023 temperature time series recorded at several locations in the lake (explained in the previous paragraph), we identified Cave *1* and *Cave 2* (Fig. 1) as probable lake-sea contact points due to having peculiar daily changes. Namely, in September and October, when surface heating may still be substantial, the near-surface points are expected to have a daily change that resembles the air temperature change. However, these two locations showed a contrasting temperature signal, which indicated the presence of another process influencing WT. This is why we selected the two caves at the lateral boundary as the most likely entry points of tidal water (*Cave 1* and *Cave 2 back*; Fig. 1) and took samples at these locations. Since we expected an inflow of water between the beginning of the lake water level rise and its peak, we took the samples shortly after the beginning (marked: *Inflow start*) and just before the end (marked: *Inflow end)* of the water inflow period in the lake. Sampling was conducted on 29 May 2023, based on the tidal prediction generated using the Python package *t_tide* and data from Dominović *et al.* (2023) (Fig. S1 in Supplementary Material). We also selected two reference points: one in the centre of the lake (*Centre*; a lake water reference; Fig. 1) and another in Koprišće Bay (*Bay 2*; a seawater reference; Fig. 1). As access to the described sites required a dive, samples were collected by a professional diver. Temperature and depth were recorded *in situ*, while salinity (S) and dissolved oxygen (DO [mg L^{-1}]) were measured *ex situ* using an HQ40d probe (Hach) with a resolution of ±0.01 and ±0.1 mg L^{-1}, respectively.

Analysis of water level and temperature

We used two approaches to analyse the results: the qualitative approach and the quantitative approach. The qualitative approach did not involve any data preparation other than trimming the data. Namely, the 2020 – 2021 temperature series were trimmed during visual inspection so that each data set corresponds to the shortest period (that of data logger deployed at site LB W): 29 June 2020 – 3 December 2020. As part of this approach, we examined the entire time series data and its shorter segments, looked for both recurring and unusual features, examined minima and maxima, and then drew our conclusions. In the Results section, we show the untrimmed data, i.e., the entire timeseries.

The quantitative approach included the removal of the mean from each of the data sets and the additional removal of atmospheric pressure for 24 June 2021 – 27 June 2021. The water level (*h*) was calculated using the formula for hydrostatic pressure ($h = p / (\rho * g)$), where the gravitational acceleration (*g*) was assumed to be 9.81 ms^{-2}. The density (ρ) for ZO and the sea was calculated from salinity and temperature data measured in the period February 2023 – July 2023 using the python Seawater package and then used to obtain the water level data from the hydrostatic pressure. After data preparation, we proceeded with the spectral analysis of the 2023 temperature and water level datasets. First, we calculated the power density spectra - P_{xx} - using the MATLAB function *pwelch* with a 10-day Hamming window and a window overlap of 80 %. We did this separately for the lake and the sea signals. We then calculated the magnitude-squared coherence (using the function *mscohere)* and the cross-spectrum phase (function *cpsd*) between the lake and sea signals. Finally, we determined the square root of the power density for each of the two signals and then calculated the gain spectrum from the ZO-to-sea ratio (sqrt(P_{lake}/P_{sea})). For the 2023 temperature series, we did not calculate the phase and gain spectrum since the lake-sea temperature relationship is not a causal one, as opposed to the water level one.

To analyse the ZO volume, the 2023 water level dataset was filtered using a low-pass filter (moving average) with a cut-off frequency of 2 h. It was then multiplied by the surface area of the lake (A = 9904 m^2; Panđa *et al.*, 2020) and divided by the ZO volume (90691.738 m^3; Panđa *et al.*, 2020) to obtain the relative volume change. Finally, the data set prepared in the described manner was divided into smaller intervals, each 24 hours long and separated by the 15-minute sampling period (e.g., 2 March 00:00 – 2 March 23:59; 2 March 00:15 – 3 March 00:14, etc.). For each of these intervals, we calculated the maximum and minimum values and then subtracted the second from the first. This resulted in a new set of 24-hour volume changes for which we determined the maximum, minimum and mean 24-hour relative volume of exchanged water in the period February 2023 – July 2023.

Concentration of nutrients

Part of the physico-chemical properties of the exchanged water is its nutrient load, so we analysed the concentration of nutrients. Nutrients were measured in fresh, unfiltered samples within 48 hours of sampling. UV-Vis spectrophotometric analysis was performed using a Specord 200 plus spectrophotometer (AnalytikaJena) in a 1 cm quartz cuvette. We determined the concentrations of ammonium (NH_4^+), nitrite (NO_2^-), orthophosphate (PO_4^{3-}) and silicate (SiO_4^{4-}) in the samples according to Hipwell Strickland and Parsons (1972), with the exception of nitrate. Nitrate (NO_3^-) concentrations in the samples were determined according to Pai *et al.* (2021). In brief, nitrate was reduced to nitrite using a vanadium (III) chloride and then its concentration was determined using the Griess reagent. To obtain the nitrate concentration, the nitrite concentration determined in the previous step (i.e. before reduction) was subtracted. The timing of sampling was determined based on a tidal forecast, so that both samplings are carried out while the direction of water exchange is from the sea into the lake; the first at the start of the lake water level rise period and the second just before its end.

RESULTS

Fig. 2 shows a time series of temperature data measured at five locations in the lake from June 2020 to December 2020/January 2021. The daily fluctuations in WT are most pronounced at *Cave 2 back*. Namely, in the period from July to October, the *Cave 2 back* WT showed much stronger tidal cycles than the WT at the other two sites around the lake perimeter (western and eastern lateral boundary; *LB W* and *LB E*, respectively). The inflowing water was 4-5 °C colder during most of this period (July-August), then its WT amplitude gradually decreased until early October, when WTs at all locations became similar. The WT measured in *Cave 1* showed the same trend as in *Cave 2 back* in terms of daily fluctuations, only with 20-50% smaller amplitudes. The maximum daily WT range outside the caves went up to 1.3 °C at 2.8 m depth (6 July 2020; *LB E*), while it was more pronounced inside the caves - up to 6.4 °C (4 July 2020) in *Cave 1* and 6.6 °C in the back part of the submerged cave, i.e. *Cave 2 back*. The following period, 13 October 2020 – 12 November 2020 (also shown enlarged in Fig. 2), followed the holomixing of the water column (Dominović *et al.*, 2023). At the beginning of this period, WTs at the four sites were almost the same, but in *Cave 1* and *Cave 2*, WTs started to decrease in late October and early November and were 2-5 °C and 1-3 °C lower than WTs at the other loggers deployed from November to January, respectively. Such a difference may be the result of different sensor depths, or of colder sea waters residing deeply in caves, or substantial influx of freshwater through the karst that strongly stratifies ZO, as October and December 2020 were characterized by extreme precipitation in the area (CMHS, 2020a; 2020b).

In Fig. 3 there are two magnified examples of the temperature series from 2020, the first one after the anoxic ZO overturning event (Fig. 3A) and the second one showing strong temperature gradients across the lake and no WT change over time (Fig. 3B). During the overturning situation (7 October 2020; Fig. 3A), WTs had similar values throughout the lake, except in *Cave 2*, showing that lake water inflow was mainly limited in the cave (*Cave 2 back*; the tidal WT fluctuation was much larger in respect to *Cave 2 mouth* and compared to the rest of this period). During a stable winter situation (Fig. 3B), no tidal WT fluctuations were recorded in the caves, suggesting that seawater and lake water had similar temperatures. Nevertheless, there is a visible temperature inversion, with *Cave 1* being both the shallowest (0.7 m) and the coldest of the three locations. *Cave 2 back* at 1.3 m depth is warmer by 1.5-2.0 °C, and *LB E* is the warmest at 2.8 m depth and ~18.8 °C. This indicates that salinity has a major role in sustaining the ZO stratification, as such temperature gradients in a shallow lake could not persist without stable stratification being supported by a water property other than temperature (e.g. Boehrer *et al.*, 2008).

In June 2021, we further investigated the relationship between the inflowing water and the tides in the lake. Several temperature and water level loggers were placed at the edge of the lake, but also in the nearby sea (Soline and Koprišće bays; locations *Bay 1a* and *Bay 2* in Fig. 1, respectively), while the last logger was left on land for air temperature measurements (see Fig. 4A).

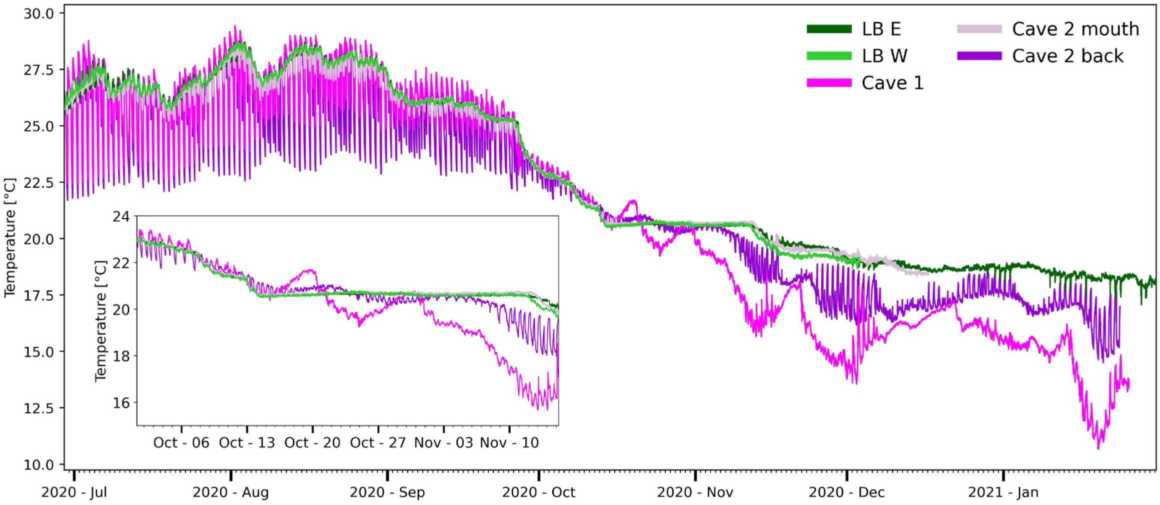

Fig. 2. Temperature series measured at five locations in the lake from July 2020 to February 2021. The period 1 October – 17 November 2020 is shown enlarged in the lower left part of the Figure. Locations and colours as shown in Fig. 1.

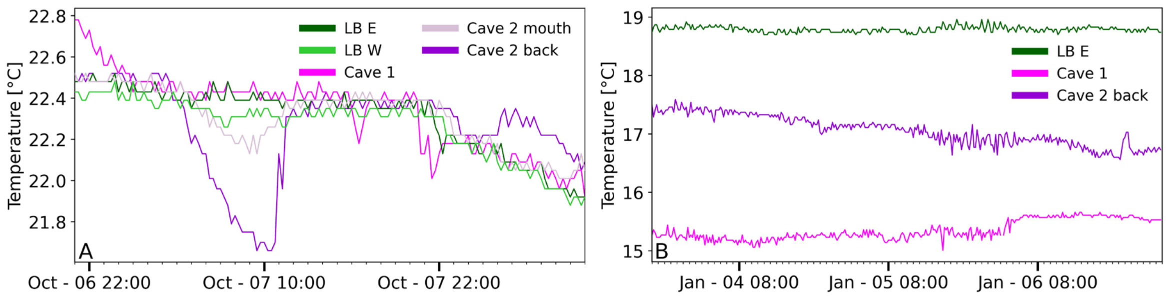

Fig. 3. Clips of temperature series measured at five locations in the lake from July 2020 to February 2021: 6 – 8 October 2020 (**A**) and 4 – 6 January 2021 (**B**). Locations and colours as shown in Fig. 1.

A daily WT trend is evident in *Bay 1a* and *Bay 2*, with a maximum temperature range of 1.4 °C and 1.7 °C, respectively (all depths and WT ranges shown in Table S1). Similar daily oscillations are observed in the surface layer in the middle of the lake (location: *Centre*, Fig. 1), which was measured at the same depth as in the sea (0.5 m) and has a maximum daily range of 1.2 °C. These ranges follow the diurnal cycle of temperature during the pronounced summer warming (Notarstefano *et al.*, 2006). In contrast, the loggers in the caves show a sudden drop in temperature that coincides with the onset of high tide, i.e. when the rising tide at sea creates the water level difference that directs the current towards the lake (marked as '*Inflow start*' in Fig. 4). This tidally driven temperature drop is the greatest when the difference between the measured sea level in the sea and in the lake is the largest, equalling to 2.0°C and 3.9°C on 25 June 2021 at *Cave 1* and *Cave 2 back*, respectively. After the tide in the sea reaches its maximum, the temperatures quickly start to increase. When the tide in ZO becomes higher than the tide in the sea (i.e. when the tidal inflow reverses and the water starts to flow out through the connecting pores towards the sea), *Cave 2 back* WT quickly relaxes to the lake ambient value. A very similar behaviour, but slightly less pronounced, can be seen for *Cave 1*, too.

To gain insight into the ZO-sea damping across different frequencies, spectral and cross-spectral analyses of the temperature and sea level data during the joint measurements in 2023 were performed (Figs. 5 and 6), where *Cave 1* and *Bay 1b* represent ZO and the sea, respectively. The power density and magnitude-squared coherence of the temperature data (Fig. 5) indicate dominant peaks at 24 h and 12 h, presumably reflecting both diurnal warming and tidal effects on the sea and lake. The energy at both peaks is higher in the lake than in the sea, especially in the 12-hour period. Moreover, additional peaks are visible in the lake at 8, 6 and 4.8 h, which are the result of an irregular temperature change caused by the tidally advected waters (trimmed cosine function), resulting in higher harmonics of the 24-hour periodic base oscillation. In fact, the higher harmonics derived from the time series measured in the caves are

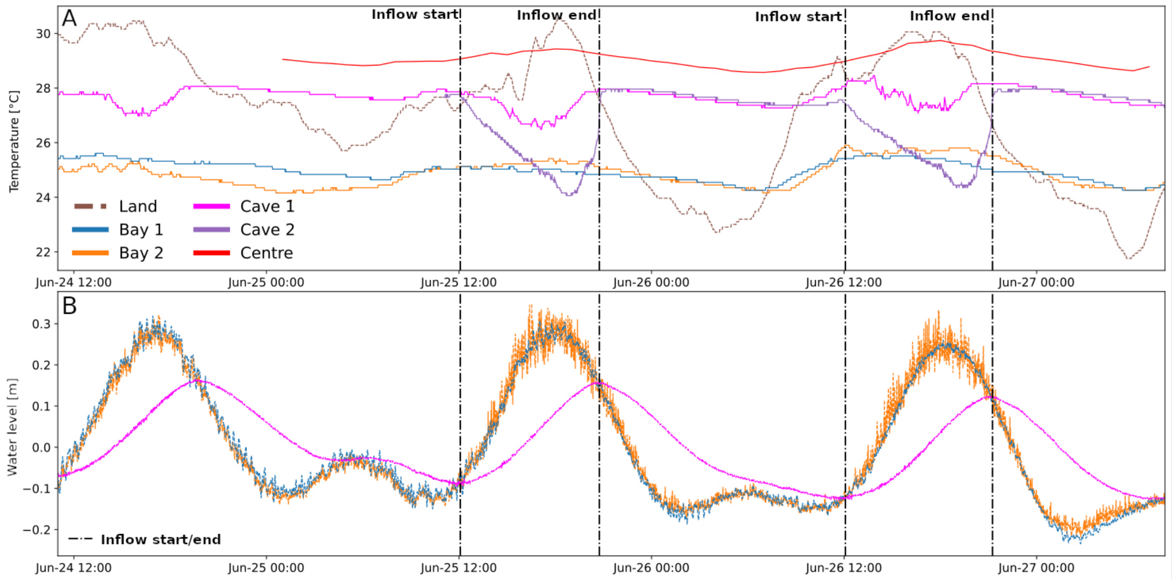

Fig. 4. Air and water temperatures (**A**) and water level (**B**) for 24 – 27 June 2021. "*Inflow start*" marks the time when the rising tides at sea exceed the water level at ZO, i.e., when tidal inflow to the lake is expected. "*Inflow end*" marks the end of the inflowing period. Locations and colours as shown in Fig. 1.

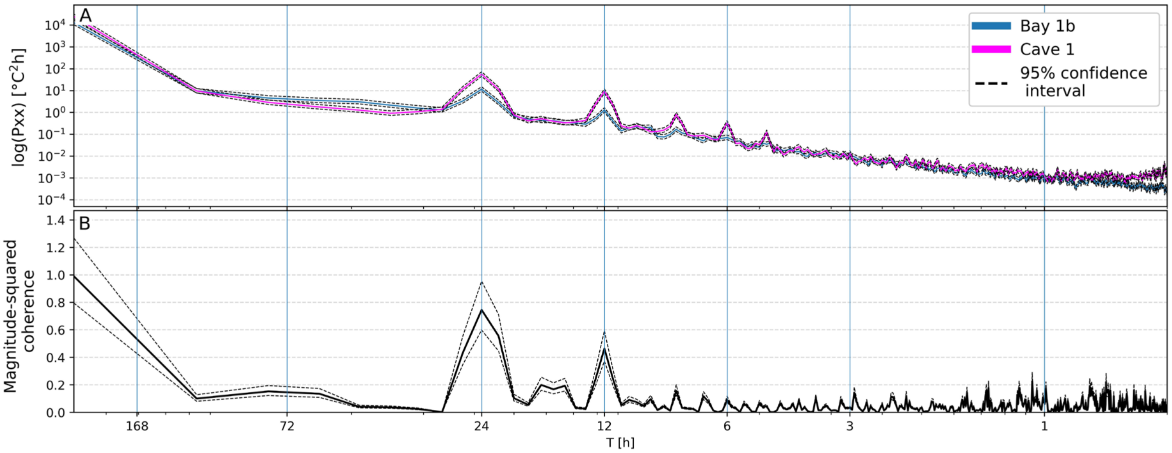

Fig. 5. Spectral analysis of the 2023 temperature series recorded simultaneously in ZO (*Cave 1*) and the nearby sea (*Bay 1b*): power density (**A**) and magnitude-squared coherence (**B**) spectra. Locations and colours as shown in Fig. 1.

much stronger (not shown), while the daily temperature drop in *Cave 1* resembles a half sine wave (Fig. 4A). The magnitude-squared coherence shows a high coherence in the 24-hour (diurnal) and 12-hour (semi-diurnal) periods. In other periods the coherence is not recognisable, except for the longer periods that describe the lake-sea connections at synoptic and longer temporal scale.

The spectral and cross-spectral analysis of the water level data from ZO (*Cave 1*) and the Adriatic Sea (*Bay 1b*) from 2023 (Fig. 6) again shows the coherent energy maxima around the diurnal and semi-diurnal tidal periods, with higher energies toward longer periods, i.e., at storm surge periods. However, there are two smaller peaks around 8.2 and 6 hours, possibly resembling seich-

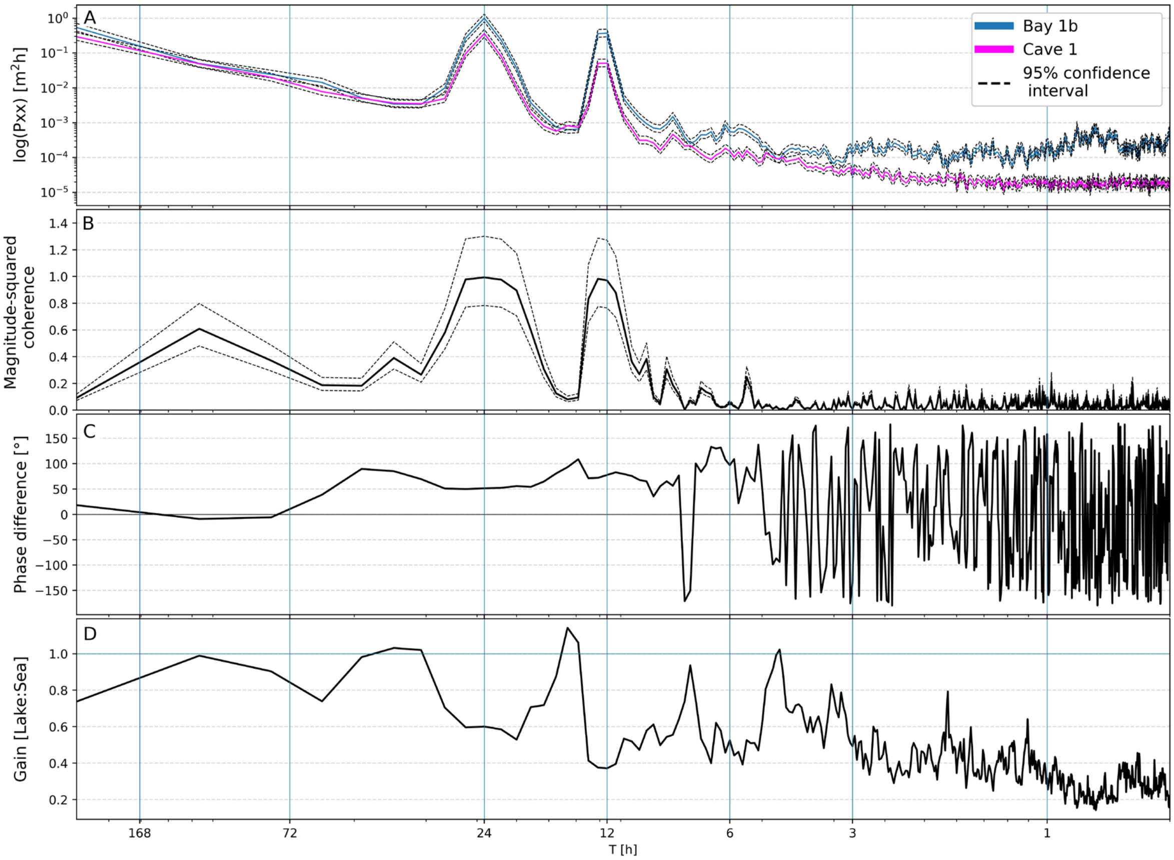

Fig. 6. Spectral analysis of the 2023 water level series recorded simultaneously in ZO (*Cave 1*) and the nearby sea (*Bay 1b*): power density (**A**); magnitude-squared coherence (**B**); phase difference (**C**); gain (**D**) spectra. Locations and colours as shown in Fig. 1.

es, which occur in the northern and central Adriatic (e.g. Šepić *et al.*, 2022). In comparison, the ratio of accumulated sea and lake energy at tidal frequencies follows the observed greater damping of processes at higher frequencies than at lower ones. That is, when corresponding peak energies (Fig. 6A) are extracted from the graph, the lake-to-sea energy ratios are 0.14 and 0.36 for the ~12h and ~24h component, respectively. The damping ratio can be clearly seen in the gain spectra, where the signal damping is progressively increasing from periods below about 36 h, where only 20-30 % of the signal is still present in ZO at sub-hourly periods. The phase difference is 51° for the diurnal components and 78° for the semi-diurnal components (i.e., 3.4 h and 2.6 h, respectively), again revealing a classical damping tidal system with limited water exchange, as observed, for example, in the Mljet Lakes (Peharda and Vilibić, 2008). No high-frequency water level fluctuations are observed in the lake, even if such fluctuations, which occasionally reach the tidal range, occur outside the lake (Fig. 7). Strictly speaking, on 15 March 2023, a weak low-pressure area was located over the Adriatic Sea, associated with strong mid-tropospheric winds that maintain propagating instabilities in the lower troposphere capable of generating extreme non-seismic water level fluctuations on tsunami timescales and meteotsunamis (Vilibić and Šepić, 2009; Zemunik *et al.*, 2022). This period was followed by a (weather-wise) stable period, and from 21 to 24 March, a high-pressure system was located over southeastern Europe with fair weather conditions, which meant that no high-frequency sea level fluctuations were to be expected in ZO.

Based on the gain water level spectrum in Fig. 6D, it can be assumed that oscillations shorter than 2 hours can be neglected when it comes to the impact of the Adriatic Sea water level on the ZO water level. After applying the low-pass filter (2-hour moving average) to the 2023 water level series, the relative change in lake volume was calculated (Fig. S2). Shorter segments of the signal were then examined, which showed that the average 24-hour water exchange in the lake was 1594.73 m^3 (1.76% V_{lake}) during the period March 2023 - July 2023. The maximum change in water volume in 24 hours was 2785.88 m^3 (3.1% V_{lake}; 22 May, 10:43 – 23 May, 10:42), while the minimal volume change was 441.81 m^3 (0.5% V_{lake}; 29 March, 01:43 – 30 March, 01:42).

In addition to the temperature changes recorded with data loggers, a sampling campaign was carried out on 29 May 2023, during which salinity, DO and nutrient concentrations were measured in the samples taken at the beginning and end of the inflow period. Based on the temperature time series (Fig. 2 and 4), sites *LB E* and *LB W* were disregarded as ZO-sea contact points, and the *Centre* site was unaffected by the lateral inflow water. The results show a difference between the tidally influenced seawater and the ZO water (Table 1). Temperature measurements follow what was observed in Fig. 4: at *Cave 1* site, the effects of the inflowing water are diminished by the end of the tide, while at *Cave 2 back* water is still colder than the water at reference point *Centre.* Salinity was exceptionally low in the nearby sea (34.3), while it was expectedly lower at a reference point in the centre of the lake (25.3), possibly due to increased precipitation and the resulting surface and subsurface freshwater runoff. One may expect that the inflow of saltier waters (in this case, the difference was approximately 10) may induce a lake to overturn, however, salinity in the bottom layer is high and close to sea values (above 35), preventing – jointly with the temperature effect – frequent overturning of ZO (Dominović *et al*., 2023). In general, salinity was lower in *Cave 1* (19.5 - 20.6) than in *Cave 2* back (25.4 - 25.8). Surprisingly, the trends at these two sites were different, i.e. a gradual increase was observed in *Cave 2* towards the end of the inflow period, while the opposite was true for *Cave 1*.

The highest DO concentration was measured at the ZO reference point (15.2 mg L^{-1}; *Centre*), while 10.4 mg L^{-1} was measured at the marine reference point (*Bay 2*). However, the inflowing water is characterised by slightly lower DO concentrations in both caves, suggesting that the high DO levels in the lake are due to the local processes in ZO rather than lateral water advection. The processes that can generate high DO levels – e.g. primary production, which usually has a maximum in spring (Ciglenečki *et al*., 2005, 2015; Marguš *et al*., 2015) - are usually weaker in places where there is no sunlight or where oxygen-consuming microbial communities might reside (such as in caves). The PO_4^{3-} and SiO_4^{4-} values in the caves are comparable to the values determined at the *Centre* reference point, but the tides change these values considerably. SiO_4^{4-} increases in *Cave 1* and even

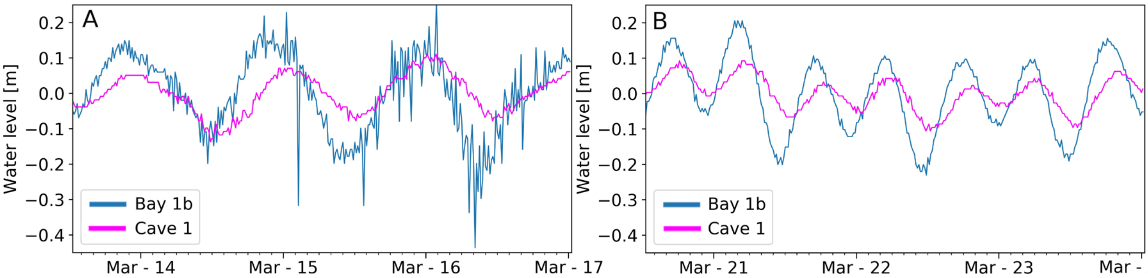

Fig. 7. Water level showing high frequency oscillations in the sea signal (14 – 17 March 2023) (**A**) and water level in the sea during calm weather (21 – 24 March 2023) (**B**).

Table 1. Temperature, salinity, dissolved oxygen, and nutrients measured on 29 May 2023 shortly after the beginning (“*Inflow start*”) and at the end (“*Inflow end*”) of the lake water level rise period at three locations inside the lake and one outside, i.e. in the Adriatic Sea (Fig. 1). LOD stands for *Limit of Detection*.

Parameter	Unit	Sea			Lake		
		Bay 2	Centre	Cave 1	Cave 1	Cave 2 back	Cave 2 back
		Ref.	Ref.	Inflow start	Inflow end	Inflow start	Inflow end
T	°C	22.14	27.96	23.96	25.70	26.68	24.83
S	-	34.3	25.3	20.6	19.5	25.4	25.80
DO	mg/L	10.37	15.22	7.03	7.64	10.72	11.23
c(PO_4^{3-})	µM	0.113	0.317	0.334	0.298	0.457	0.448
c(NH_4^+)	µM	0.575	6.279	2.960	6.195	1.293	3.135
c(NO_2^-)	µM	<LOD	0.038	0.114	0.200	0.206	0.231
c(NO_3^-)	µM	1.006	0.898	20.773	5.584	10.449	4.713
c(SiO_4^{4-})	µM	2.640	18.422	16.754	19.436	16.052	13.569

exceeds the reference value of the lake, while in *Cave 2* there is a clear downward trend. The NO_2^- and NO_3^- values are much higher in the caves than in the centre of ZO. The tides drive water with higher NO_2^- concentrations from the karst into the lake, while a notable decrease in NO_3^- is observed between the beginning of the inflow (similar to the lake water located in the caves) and the end of the inflow (similar to the inflowing lake water). Finally, the concentrations of ammonia (NH_4^+) are much lower in the caves than in the centre of ZO, with their concentration tripling towards the end of the inflow, indicating the importance of local processes for the biogeochemistry of the lake.

DISCUSSION

ZO is an isolated, small lake that is only weakly connected to the nearby Adriatic Sea through the karstic underground. Apart from rare overturning events, ZO is stably stratified most of the time. Due to these characteristics, its upper layer immediately reflects the changes in its surroundings. Observations of WTs measured at different times of the year (summer-autumn-winter 2020 and summer 2021) at five locations in the lake and three locations in the nearby sea show that WTs in the sea and in the lake are not identical and can differ substantially. The inflowing water during the tides contributes to lateral cooling in the warmer season, while in the autumn and winter the inflowing water promotes the warming of ZO. This effect could be due to the mixing of seawater and fresh or brackish water in the karst rocks, where this mixture takes on the ambient temperature and consequently enters the lake. Similar phenomena have also been observed in other related karst formations (Benović *et al.*, 2000; Blanchette *et al.*, 2020). In such systems, the greatest differences in diurnal cycles are observed near the connecting channels, to which the two cave sites (*Cave 1, Cave 2*) are most similar in the case of ZO. Indeed, in the summer months, when solar radiation is at a maximum and one would expect a maximum daytime temperature, there is a visible, steep drop in WT. This drop coincides with the time when the sea tide exceeds the ZO water level, i.e. when a tidally induced flow towards the lake is expected. This cooling can be substantial, with a maximum daily amplitude of 6.6 °C (August 2020) at the *Cave 2 back* site, so the WT difference between ZO and the sea can be quite high. Although it could be argued that the different depths of the data loggers could influence the conclusions, this was generally not the case in this study. Indeed, all loggers placed at the same depth (2.8 m; *Cave 2 mouth, LB E*, and *LB W*) indicated that the diurnal temperature range was greater at the back of *Cave 2.* It was found that occasionally no daily temperature trend could be detected at some locations in the lake. In some locations (e.g. *Centre*) this could be due to high cloud cover. However, the *Cave 2* back site is completely submerged and located in a cave, so this absence is more likely to be explained by inflowing water with similar WT to the ambient water or perhaps a temporary reduction in water exchange. Since the ZO-sea transport through the connecting channels can take 2-3 hours (as shown here and in Dominović *et al.*, 2023), it should also be considered that the seawater exchanges heat with its surroundings while being pushed through the karst, which has already been reported for other sites in the region (Stroj *et al.*, 2020). Figures 4 and 5 also show non-negligible WT oscillations in the sea. As the positions of the data loggers were fixed, the temperature fluctuations in the sea can mainly be explained by the vertical movements of seawater during the tides, which periodically exposed the fixed recording instruments to deeper and shallower layers with different temperatures. In contrast, the horizontal water movements seem to be a more important process when it comes to the lateral WT trends in ZO. Therefore, simultaneous WT measurements at any location in ZO and in the sea for at least 1 day can be used to detect water inflows, as opposed to (or together with) visual detection of optically distinct layers during diving.

Since the Adriatic has a mixed tidal regime (Cushman-Roisin *et al.*, 2001), there is a lower high tide and a higher high tide in the day most of the month, which is why Fig. 4 shows one steep drop in WT in the caves and another barely perceptible (note that these measurements were taken during neap tide, i.e. when the lower high tide was much weaker than the higher high tide). As the intensity of tides changes substantially between neap tides and syzygy, affecting the lake-sea exchange magnitude, the steepness of the temperature curve is also expected to change. The signals are strongly correlated at 24 h and 12 h, while the energy of the lake water level signal is consistently lower than that of the sea level. This is true for periods from about 36 h to 1-2 h, below which the oscillations are completely absent, indicating that the karstic environment acts as a natural low-pass filter. However, diurnal temperature signal in the lake is stronger than in the sea, reflecting its fast response to atmospheric forcing, i.e. diurnal thermal cycle in the lake. The phase lag in tidal frequencies (3.4 and 2.6 hours for the diurnal and the semi-diurnal component, respectively) confirms that the water forced through the karstic environment takes a considerable time to enter the lake. Convincingly, the damping has progressed over time since the first sea level measurements (Dominović *et al.*, 2023). The spectral analysis also suggests that the lake is protected from the hazards of high-frequency oscillations ($T < 2$-3 h), which can otherwise make a substantial contribution to the water level signal (Ruić *et al.*, 2023), especially during the extreme high-frequency events (Šepić *et al.*, 2015).

Although it is important to take temperature and water level into account when considering the effects of incoming water, these parameters are not sufficient to fully describe the exchange between ZO and the sea. Indeed, salinity is a crucial parameter that maintains the vertical stability of ZO (Dominović *et al.*, 2023). However, before discussing its effects, it should be noted that the salinity results presented in this paper are based on sporadic measurements during opportunistic field campaigns. Salinity was measured at three sites in the lake (*Cave 1*, *Cave 2 back*; and *Centre* for reference; Fig. 1) and at one site in the sea (*Bay 2*; Fig. 1), but not continuously in time like temperature, i.e. by CTD or similar loggers. These measurements, although limited, indicate unusually low salinity levels at all sites. Generally, low salinity values in the sea away from obvious sources (e.g. rivers, streams, etc.) can indicate submarine groundwater springs, that may be active even after minor precipitation events (Benjanni *et al.*, 2020). In the case of sea near ZO, due to ZO water having lower salinity and possible mixing with freshwater in the karst, it is possible that the water flowing out to the sea contributes to lower salinity in the surrounding bays. Since May 2023 was extremely wet (precipitation was almost three times the average for May; CMHS, 2023), low salinity in *Bay 2* is most probably the result of the combined effects of the springs and the ZO/karst outflowing water. The salinity of the lake is lower than the salinity of the sea. This is a common situation in the lake due to the freshwater load which takes place during and after precipitation events (Ciglenečki *et al.*, 2005, 2015), and most likely due to the drainage of the lake and underground discharges (Dominović *et al.*, 2023). Both samples from *Cave 2 back* indicate that the lake water is possibly mildly mixing with seawater, as the salinity was slightly higher than in the *Centre*, a difference that became even more apparent during the second sampling. However, contrary to what was suspected at the beginning of the opportunistic experiment (i.e. the sampling on site was not fully adjusted for this), the salinity of the seawater was not observed at the end of the sea-to-lake flow direction (i.e. at the end of the water level rise in the lake). However, the slight increase in salinity in the inflowing water suggests a more direct connection between ZO and the sea through the *Cave 2* contact point.

In terms of chemical parameters, DO and nutrient levels are higher for the surface layer in ZO compared to concentrations in the surrounding sea (Ciglenečki *et al.*, 2005, 2015), which can be particularly pronounced during more severe meteorological events (e.g. heavy rainfall bringing nutrients through surface runoff and underground discharges advecting water with quite different chemical properties - Matić *et al.*, 2013 - or direct input of dust by winds - Mifka *et al.*, 2022). Although nitrate concentrations at the contact points in ZO decreased as the tide progressed, these values were closer to the values determined in freshwater karstic springs than to the concentrations measured in the ZO centre and especially in seawater, which is characterized by low levels of inorganic nitrogen species (Bejannin *et al.*, 2020). This also indicates considerable freshwater load which presumably mixes with Adriatic Sea water in the porous karst area around ZO. In addition, the limited connection between ZO and the sea – with increased nutrient concentrations – makes the lake a hotspot of primary production with extreme DO concentrations (Ciglenečki *et al.*, 2017; Mifka *et al.*, 2022).

Having defined the effects and properties of the incoming water, it is logical to discuss the quantities of water that ZO exchanges with its surroundings. On average, 1.8% of the lake volume was exchanged in the 24-hour intervals between March and June 2023, while the minimum and maximum amounts were 0.5% (end of March) and 3.1% (second half of May), respectively. However, due to the temperature and salinity, this water is unlikely to penetrate the deep ZO layers. So, if the exchanged water interacts only with the upper layers, it can make a substantial contribution there, up to 50% of the upper-layer volume. The question arises as to whether the seawater entering the lake could considerable influence its trophic state, including oxygen depletion, stratification and possibly mixing. Based on the results presented, it appears that this influence is only weak, much weaker than the influence that most likely comes from the freshwater pollution (precipitation – especially during extreme events - and groundwater). However, the calculated quantities only apply to the observed period from March 2023 to July 2023, i.e. a warmer season and the current climate conditions. It is questionable what would happen if suddenly larger amounts of water (e.g. twice the currently exchanged volume) with different physico-chemical properties (e.g. saltier, Verri *et al.*, 2024) were to enter the lake. This scenario could be represented in the more extreme coastal flooding that is possible in the future climate, often associated with the salinization of coastal aquifers (Paldor and Michael, 2021), which would lead to further stratification of coastal systems (Ladwig *et al.*, 2021). Moreover, a long-term Adriatic Sea level rise would cause ZO level rise, which would change its volume substantially due to its small area and shallowness. Further, as construction projects are expected around ZO in the coming years (e.g. a tourist complex planned north of ZO, in addition to the already existing marina complex northeast of ZO; Fig. 1), there could be an additional impact on the exchange between ZO and the sea. Namely, adding more concrete in the area around the lake could a) increase the surface run-off that ends in the lake or in the sea, adding to the stratification and b) clog the small, yet unknown, karstic pores on the side of *Bay 2* through which part of the daily water exchange is carried out. Furthermore, there have been considerations to create artificial connections (tunnels) between ZO and the sea, which could affect the natural physico-chemical properties of ZO as well as its biotic communities. That is, the ecosystem of ZO is different than the one found in *Bay 1* and *Bay 2* (both in species present and their abundances, e.g. Bakran-Petricioli *et al.*, 1998; Pjevac *et al.*, 2015; Malešević *et al.*, 2015; Ciglenečki *et al.*, 2017) and if it is meant to be preserved in the current state, the location, size, and the maintenance of the tunnels must be carefully planned and conducted. In addition, the stratification of the lake could change in the future climate due to a change in the precipitation regime in the region (Branković *et al.*, 2013; Tojčić *et al.*, 2024) - an increase in precipitation is predicted in winter, while a decrease is expected in summer. Thus, the immediate effects would be stronger stratification in winter and a decreased one in summer. A weakened stratification in late summer and early autumn would allow strong cooling events (air temperature drops and strong winds as part of the pressure lows) to cause the overturning events more often. In fact, such conditions may have already occurred during the last decade, when a much higher frequency of overturning events was reported for ZO (Dominović *et al.*, 2023; Simonović *et al.*, 2023). Due to scarcity of data, assessing all possible scenarios and their cumulative effect on the future hydrodynamics of ZO should be concentrated on sensitivity modelling experiments (by varying the thermohaline properties of the lake, the surrounding sea, and the variable amounts and salinities of water exchanged through the porous karstic environment of ZO), thus quantifying all possible pathways of the ZO system stratification.

CONCLUSIONS

The aim of this study was to identify the points of contact between the Adriatic Sea and ZO where water is exchanged, to describe the type of water exchanged qualitatively and quantitatively and to discuss the causes and possible effects of this water exchange on the ecosystem of ZO. Of the five sites investigated in the lake, the sites in the two caves were confirmed as contact points. The central point in the lake, where most measurements have been taken in the last 30 years of ZO research, was not found to be significantly affected by the lateral inflow of water. All measured parameters (temperature, salinity, DO and nutrients) show that the exchanged water is neither pure seawater nor lake water, but a mixture of both with a possible addition of freshwater from the underground. The *Cave 2 back* – sea connection point is probably more direct and faster, with the predominant influence of the Adriatic Sea, while the *Cave 1* – sea connection point seems to bring water that has a longer residence time in the surrounding karst, originating from a low-oxygen environment. The *Cave 1* contact point brings less phosphates and NO_2^- into the lake compared to *Cave 2*, while the water from *Cave 2* brings more DO and less NH_4^+, NO_3^- and SiO_4^{4-}. However, the incoming water at both locations is enriched with nutrients compared to the sea and even the centre of the lake when it comes to PO_4^{3-}, NO_2^- and NO_3^-. The inflowing water has a different effect on the temperature of the lake at the lateral boundaries: In the colder season (October – December) it is warmer than the lake water at a similar depth, and in the warmer season (June – September) it has a cooling effect.

The water exchange is governed by the difference in water level between the Adriatic Sea and ZO. The karst between the sea and the lake represents a natural low-pass filter: the semi-diurnal tide is more damped than the diurnal tide, and there is a different phase shift for each of the oscillations found in the signal. This signal attenuation could also affect the lateral WT. Indeed, depending on the time in the lunar month and the meteorological conditions, the high tide in the sea may not penetrate the lake or its effects on the WT may be masked. Due to the filtering effect of the karst, the lake is shielded from the higher frequency oscillations ($T > 2$-$3h$) that may occur strongly in the sea around the lake. For the observation period March – July 2023, the volume of exchanged water in the 24-hour intervals was estimated to be between 0.5% and 3.1% of the total lake volume. Although this is not a large amount, further research is needed to identify and quantify the impact of the lateral inflow of water with varying salinity, temperature, dissolved oxygen and nutrients in future climates when more extreme rainfall and coastal flooding is expected.

Lastly, it is clear from the analyses that there is a need for continuous and systematic measurements rather than relying on opportunistic campaigns, especially when it comes to thermohaline and biogeochemical variables that describe the dynamics of ZO. Indeed, ZO has been studied for almost 30 years and a wealth of interdisciplinary knowledge has been gained (a review of the 1994-2015 period in Ciglenečki *et al.*, 2017, and the newest research in Dominović *et al.*, 2023; Simonović *et al.*, 2023; Marguš *et al.*, 2023; Mateša *et al.*, 2024), on which the design of the field sampling described in this research was based. Now that new technologies have emerged and become more accessible at reasonable costs, it may be time to establish permanent stations in ZO. Such stations should also be used for reliable prediction of overturning events – the most important goal for proper management of this unique physical and natural ecological laboratory.

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AUTHOR CONTRIBUTIONS

Iva Dominović – Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing (original draft); Marija Marguš – Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Writing (original draft); Tatjana Bakran-Petricioli – Data curation, Resources, Writing (review & editing), Donat Petricioli – Data curation, Resources, Writing (review & editing); Irena Ciglenečki – Funding acquisition, Project administration, Resources, Supervision, Writing (review & editing); Ivica Vilibić – Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing (original draft).

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