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Kelsie Meystedt , Bommanna G Loganathan * , Susan P. Hendricks , David S. White *

Posted Date: 9 October 2023

doi: 10.20944/preprints202310.0535.v1

Keywords: Dissolved Calcium; Kentucky Lake; Streams; Ohio River; Zebra Mussel doi:10.20944/preprints202310.0535.v1

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Dissolved Calcium in Kentucky Lake and Its Watershed: Trends and Possible Sources and Implications for Zebra Mussel Colonization

Kelsie Meystedt [1], Bommanna G. Loganathan [1*], Susan P. Hendricks [2] and David S. White [2]

[1] Department of Chemistry and Watershed Studies Institute, Murray State University, 1201 Jesse D. Jones Hall, Murray, KY42071, USA,
[2] Hancock Biological Station, Murray State University, 561 Emma Drive, Murray, KY 42071, USA.
* Correspondence: bloganathan@murraystate.edu (B.G.L); dwhite@murraystate.edu (D.S.W.).

Abstract: Dissolved calcium (Ca^{2+}) concentrations in freshwater ecosystems are of growing concern as increasing levels have been implicated in altering the environmental conditions and biodiversity. Elevated Ca^{2+} levels and sporadic re-emergence and disappearance of invasive zebra mussels in Kentucky Lake in recent years served as a motivation for this study. The objective of this study was to determine if Ca^{2+} spatial and temporal patterns in Kentucky Lake, selected tributary streams, and the Ohio River were related to the zebra mussel invasion. Over 1000 water samples were collected and analysed for dissolved calcium during 2018-2022. Approved analytical methods were followed for sampling and measuring dissolved calcium levels. Results revealed significant spatial and temporal patterns. Kentucky Lake Ca^{2+} levels varied between 15-25 mg/L depending on the sampling location and month/year. Kentucky Lake channel sites exhibited comparably higher concentrations of Ca^{2+} than did most embayment and/or stream sites, indicating that tributary streams did not serve as primary sources of calcium to the lake. Dissolved calcium levels at main lake sites had exceeded the threshold for zebra mussel growth and reproduction in 2018 during the time when zebra mussels were present. Calcium in lake water samples collected from 2019 through 2022 was at or just below the threshold. Temporal trend data showed a gradual increase in Ca^{2+} in Kentucky Lake throughout the study period but remaining at or below the threshold level considered critical for zebra mussel reproduction and development. Calcium levels in the Ohio River site at Paducah were similar to Kentucky Lake reflecting the predominance of Tennessee River water, while levels at the Brookport site were consistent with values known to support zebra mussel populations. The elevated calcium levels in Kentucky Lake waters during the late winter and early spring months may be due to natural sources (mineral weathering) as well as human activities in the Tennessee River basin. This study emphasizes the need for continued calcium monitoring in the watershed to determine the potential for future zebra mussel outbreaks and potential influences on the lake ecosystem and its functions.

Keywords: dissolved calcium; kentucky lake; streams; Ohio river; zebra mussel

1. Introduction

Calcium (Ca) is an essential element in ecosystems at many levels of the food chain to maintain ecosystem health and sustain biodiversity. In general, calcium is abundant in rivers, lakes, and coastal waters. Calcium is a key structural component in building the inner and outer skeleton of invertebrates and vertebrate animals [1-7]. Freshwater and marine mollusks depend on dissolved calcium for reproduction and growth [8-11]. Mollusk shells are predominantly composed of calcium carbonate. Many species of bivalve mollusks survive, reproduce, and maintain normal populations in rivers, lakes, and oceans only where calcium concentrations are sufficient. For many bivalve species, dissolved calcium concentrations in water can be a limiting factor for survival, growth, and reproduction. Although calcium concentration data are available for major rivers throughout the world [12-14], they are still comparatively rare for numerous lakes and reservoirs [13], The present study examined spatial and temporal changes in dissolved calcium that may have led to the establishment of a reproducing zebra mussel population in Kentucky Lake, the terminal and largest reservoir in the Tennessee River system.

The zebra mussel (*Dreissena polymorpha* (Pallas); Dreissenidae, Bivalvia) is an exotic invasive aquatic mollusk that came to the United States from the Caspian Sea via ballast waters discharged into the Laurentian Great Lake System in 1985-1986 [15, 16]. The mussel quickly colonized the Great Lakes, moving down the Mississippi River and up the Ohio River in the early 1990s, eventually arriving in the Tennessee River and several other freshwater ecosystems in the southeastern and mid-western United States [16,17,18,19]. By 1994, the following states had reported zebra mussels within their borders or in water bodies adjacent to their borders: Alabama, Arkansas, Illinois, Indiana, Iowa, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, New York, Ohio, Oklahoma, Pennsylvania, Tennessee, Utah, Vermont, West Virginia, and Wisconsin [19].

Numerous studies have documented that zebra mussels can displace native aquatic species, disrupt energy flow through ecosystems, and negatively affect people's quality of life. Zebra mussels hinder boating, swimming, fishing, and other recreational activities leading to ecological and economic impacts [16,20,21]. Zebra mussels are notorious for their "biofouling" capabilities costing billions of dollars in their removal from industrial and power plant systems and public water supply lines [16,20,21]. The adverse effects of dreissenid mussels on freshwater systems have led to their ranking as one of the world's most invasive aquatic species [22, 23]. Zebra mussels are now found abundantly in the lower Ohio River, but only sporadically in the Tennessee River. The Cumberland, Green, and Tennessee rivers (including Kentucky Lake) have the highest diversity of unionid mussel species in the world [24, 25]; thus, zebra mussels have the potential for disrupting their populations [26, 27].

Kentucky Lake (Figure 1) was created by the impoundment of the Tennessee River by the Tennessee River Authority (TVA) in 1944. Kentucky Lake is mesotrophic to oligotrophic (based on N, P, and chlorophyll concentrations). It is a moderately turbid reservoir that drains north into the Ohio River. Kentucky Lake covers an area of about 65,000 ha. It is the last and the largest of a series of nine mainstem reservoirs on the Tennessee River and is the largest reservoir in the southeastern United States [28, 29]. Kentucky Lake was created specifically for flood control, power generation, and commercial navigation. Surrounding communities also depend on Kentucky Lake for recreational activities, regional development and growth, and domestic and industrial water supplies [30]. Tourism and sport fisheries are important contributors to the economy. The Tennessee River basin encompasses portions of seven southeastern states with a land use comprised primarily of row crop agriculture and smaller portions of second-growth forests. Population density is 19 people km^{-2} concentrated primarily in Knoxville and Chattanooga, both of which are several hundred river km upriver from Kentucky Lake [29-30]. The Tennessee River empties into the Ohio River at Paducah, Kentucky, approximately 25 km downstream from the Kentucky Lake dam, thus the portion of Kentucky Lake actually in Kentucky is relatively small (Figure 1).

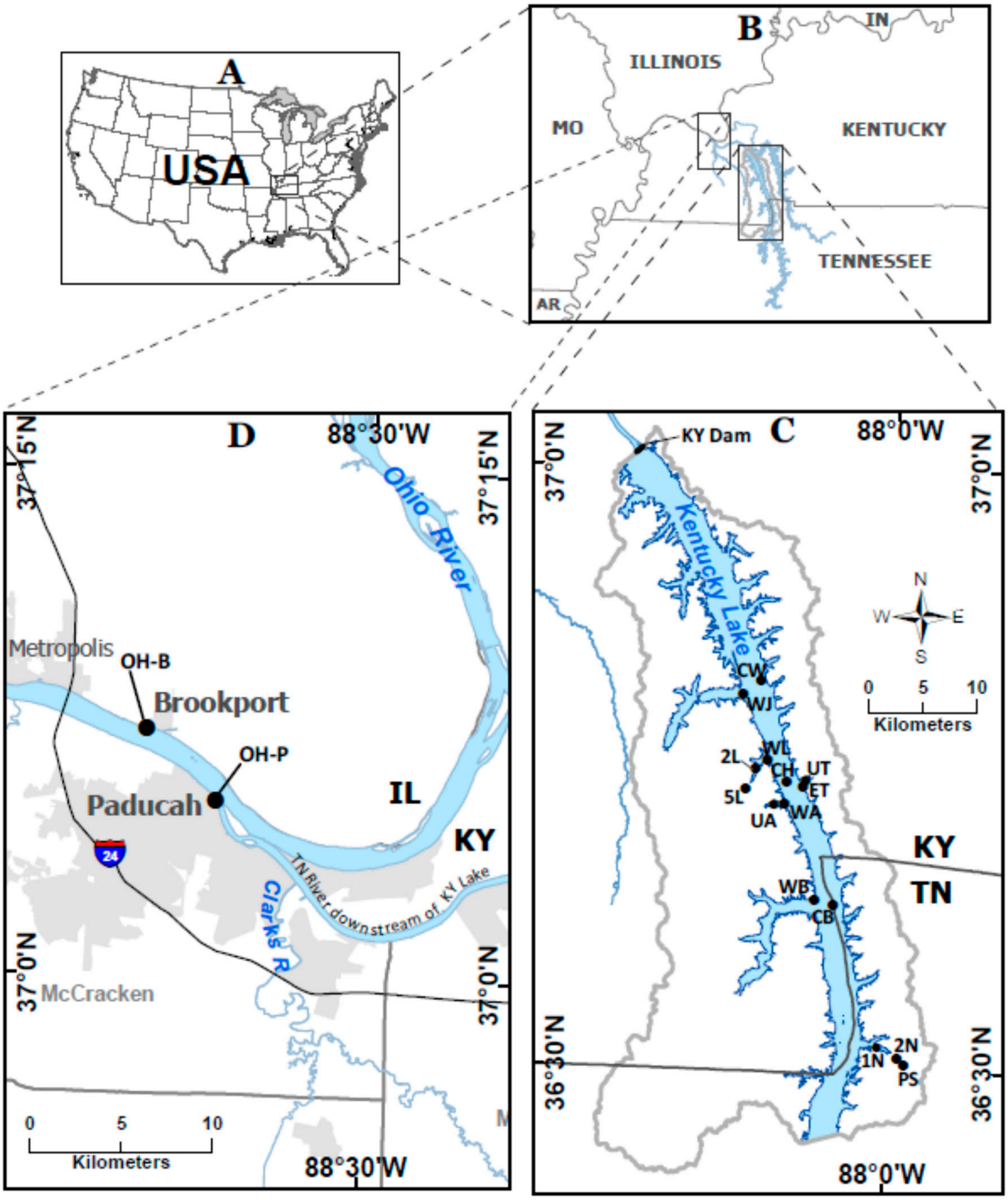

Figure 1. Map showing study area including Kentucky Lake and Ohio River. A: Map of the USA, identifying the general sampling area with a rectangle. B: Close-up view of the general area, including surrounding states. C: Selected sites from the Kentucky Lake Monitoring Program (KLMP) that were monitored for calcium. Kentucky Lake sites monitored included the main channel (CW, CH, CB), tributary mouths (WJ, WL, WA, WB, UA, UT), Ledbetter Creek embayment and stream 2L, 5L), and the Panther Creek embayment and stream (1N, 2N, PS). D: Ohio River sites monitored include OH-P (Ohio River at Paducah) and OH-B (Ohio River at Brookport). Refer to Table S1 for more details on these sites.

Zebra mussels are commonly found in the lower Ohio River, with well-established colonies. While small numbers of primarily adult zebra mussels have been present in the Tennessee River and its impoundments since 1992, only one short-lived reproducing colony had been recorded in Kentucky Lake [31]. The recent infestation in Kentucky Lake most likely began at least as early as

2016 based on mussel size distributions. Unfortunately, mussels were not observed until late 2017, Calcium levels in Kentucky Lake had not been routinely measured before 2018; therefore, we were limited to analyses during the 2018-2022 period when mussels were present and after the infestation subsided. Zebra mussels largely disappeared by the end of 2018, and by 2022, only occasional adults were found on monitoring equipment.

Understanding calcium patterns and potential causes may help predict the occurrence of this notorious biofouling exotic mollusk in the future. Previous studies had reported that mussels can survive in waters with calcium concentrations as low as 12 mg/L but that reproducing populations usually occur at 25-28 mg/L [32], we were motivated to investigate calcium dynamics in Kentucky Lake waters focusing on spatial and temporal changes during and after zebra mussels were present.

We took advantage of an already existing Kentucky Lake Monitoring Program (KLMP) [33] and added dissolved calcium sampling at several of the regular monitoring sites. Sites were monitored monthly from early 2018 through 2022 (Figure 1C). Sites included the Kentucky Lake main channel (CH, CW, CB,), several tributary embayments (WL, 1N, UA), and two tributary streams (5L, PS, 2N). Two sites on the Ohio River (Figure 1D) were added, one at the confluence of the Tennessee and the Ohio Rivers at Paducah, Kentucky (OH-P) and one in the Ohio River at Brookport, Illinois (OH-B) that represents primarily Ohio River water and known for zebra mussel infestation. The objectives of our study were to (i) determine spatial and temporal variations in dissolved calcium levels in Kentucky Lake and Ohio River waters during and after zebra mussel colonization (ii) evaluate whether calcium concentrations reached a threshold level that would allow for zebra mussels' growth and reproduction in the Kentucky Lake, and (iii) determine if we could identify potential sources of increased dissolved calcium concentrations.

2. Materials and Methods

2.1. Sampling Site Selection

Site selection, sampling date, and sampling protocols used in this study were aligned with the Murray State University Kentucky Lake Long-Term Monitoring Program (KLMP). The KLMP was established on Lower Kentucky Lake in 1988 [33]. Monitoring cruises occur every 16 days with over 670 cruises completed to date. Every second cruise is termed extended and includes Panther Creek and Panther embayment sampling. For the present study, we participated in monthly extended cruises (10-16 sites, cruise numbers 578-667) covering 2018-2022. Stream sites were usually sampled a day before the cruise date. Two sites in the Ohio River one at Paducah (OH-P), Kentucky, and one at Brookport (OH-B), Illinois, (Figure 1D) were monitored monthly for calcium beginning in 2018 as a comparison with Kentucky Lake. Some of the cruises were truncated or canceled due to weather, and others were canceled during the COVID-19 pandemic; however, we felt that there were sufficient data for our analyses

2.2. Sampling Methods

Kentucky Lake water samples were collected one meter deep from the surface and one meter from the bottom of the lake using a Nansen water sampler. About 200 mL from each sample were transferred to pre-cleaned I-CHEM glass bottles (pre-cleaned with trace metal grade concentrated nitric acid, detergent, and distilled water) and stored in a cooler containing ice. Surface water from streams and the Ohio River was collected by grab samples using pre-cleaned I-CHEM bottles. The samples were held in a cooler containing ice and transported to the laboratory. Samples were filtered and acidified within 24 hr after collection and stored at 4°C until further analysis.

2.3. Chemical Analysis

Calcium occurs in freshwaters in solution as the free ion (Ca^{2+}), as calcium carbonate ($CaCO_3$), and in colloidal complex with sediments and organic matter. Standard analytical methods define “dissolved calcium” as calcium measured in a sample after filtration through a 0.45μ filter and “total calcium” as calcium measured in an unfiltered sample after acid digestion. Because the dissolved

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fraction is readily bioavailable for uptake by organisms, we analyzed only the dissolved calcium fraction. The samples were processed and analyzed for dissolved calcium following the standard procedures with some modifications [34-37]. 40 mL were filtered using a Nylon 30-mm, 0.45µm filter (F2502-1, Thermo Scientific, USA) and transferred into clean 50 mL polypropylene conical tubes (CAT# REF 352098). The filtrate was acidified to a final concentration of 0.32 M using 800 µL trace metal grade nitric acid.

Calcium analyses were performed using a PerkinElmer PinAAcle 900F Atomic Absorption Spectrometer (AAS). Calcium measurements were made at a wavelength of 422.67nm and a slit width setting of 0.7nm. Compressed air and acetylene were used as oxidants with flow rates of 10.00 mL/min and 2.70 mL/min respectively. The flame AAS was calibrated using calcium standards with concentrations ranging from 1 to 100 mg/L. Seven-point calibration analysis was performed to evaluate the AAS response to different amounts of calcium (r^2 = >0.98).

Quality assurance and quality control protocols used for the analysis of calcium included procedural blank analysis using deionized water, duplicate analysis, and matrix spike recovery (100±20%). Calibration and calibration verification (seven-point calibration with r^2 = >0.98) were checked routinely at the beginning and end of each batch of monthly samples. Analytical precision (reproducibility) was checked by performing a triplicate analysis of the sample, and %RSD was calculated using the formula: Standard Deviation/Mean)*100. Detection limits were calculated following approved procedures [37]. The standard deviation of baseline noise was determined from a seven-time analysis of matrix blank (2% trace metal grade nitric acid) spiked with the lowest concentration of the calibration standard. The instrument's limit of detection (LOD) was three times the standard deviation of the baseline noise divided by the slope of the calibration curve. The limit of quantification (LOQ) was ten times the standard deviation of the baseline noise divided by the slope of the calibration curve. The data generated were subjected to a t-test in order to determine if there were significant variations among sites.

3. Results and Discussion

3.1. Dissolved Calcium Concentrations in Tributary Streams and Embayments

Streams can be important sources of runoff for reservoirs that result in input of organic and inorganic matter (metals). Sources of metals include the weathering of benthic substrates and substances and the runoff from the surrounding lands. Ledbetter Creek (site 5L) drains a geology of clays and chert. Water sources are runoff from agriculture and rural development and from groundwater seepage [38]. Discharge from this 2-3rd order tributary averages 0.1 m^3/sec. and its contribution to the embayment is minimal. 5L recorded the greatest number of non-detectable (<0.2 mg/L) concentrations as well as the lowest yearly mean (1.62 mg/L in 2020) calcium levels. (Tables S2a, S2b). Thus, it was assumed that this and similar western tributaries were not significant sources of calcium. Site 2L is in the middle of the Ledbetter Creek embayment that primarily contains Kentucky Lake water. Calcium levels were similar to the main lake (Tables S2a-b and S3a-e).

Panther Creek drains a forested limestone and dolomite landscape [33]. While significant runoff may occur following rainstorms, the primary water source is a series of perennial karst springs, as is true for many other small tributaries entering on the eastern side of Kentucky Lake. The mean discharge of approximately 0.1 m^3/sec is similar to Ledbetter Creek. In 2018 concentrations at the embayment site (1N) ranged from 15.6 to 35.4 mg/L with an annual mean of 26.4 mg/L ± 6.50. Panther Creek site 2N had greater variations in dissolved calcium concentrations both spatially as well as temporally (Table S2a, S2b). In 2018, calcium concentrations at stream site 2N ranged from 5.0 mg/L to 49.7 mg/L with an annual mean of 28.4 mg/L ± 15.8. Site 2N exhibited unusually larger fluctuation in calcium concentration over several months (Table S2b). The fluctuation was seen throughout each year of the study.

To better understand the patterns at 2N, the Panther Creek Spring site (PS) was added to the study in 2021 (Figure 2). Consistently, greater concentrations of dissolved calcium were found in discharge at PS, with concentrations ranging from 38.9-46.9 mg/L and an annual mean of 42.8±2.77.

The primary source of calcium for 2N appears to be from upstream spring sites, with the large fluctuations being caused by the varying amounts of runoff from the watershed. Although very high annual mean concentrations (>40 mg/L) of dissolved calcium were found in the spring (PS), the discharge of dissolved calcium entering Panther Creek embayment (1N) was trivial and determined not to be a significant source to the Kentucky Lake. Zebra mussels may have been present at 1N, but no survey was conducted.

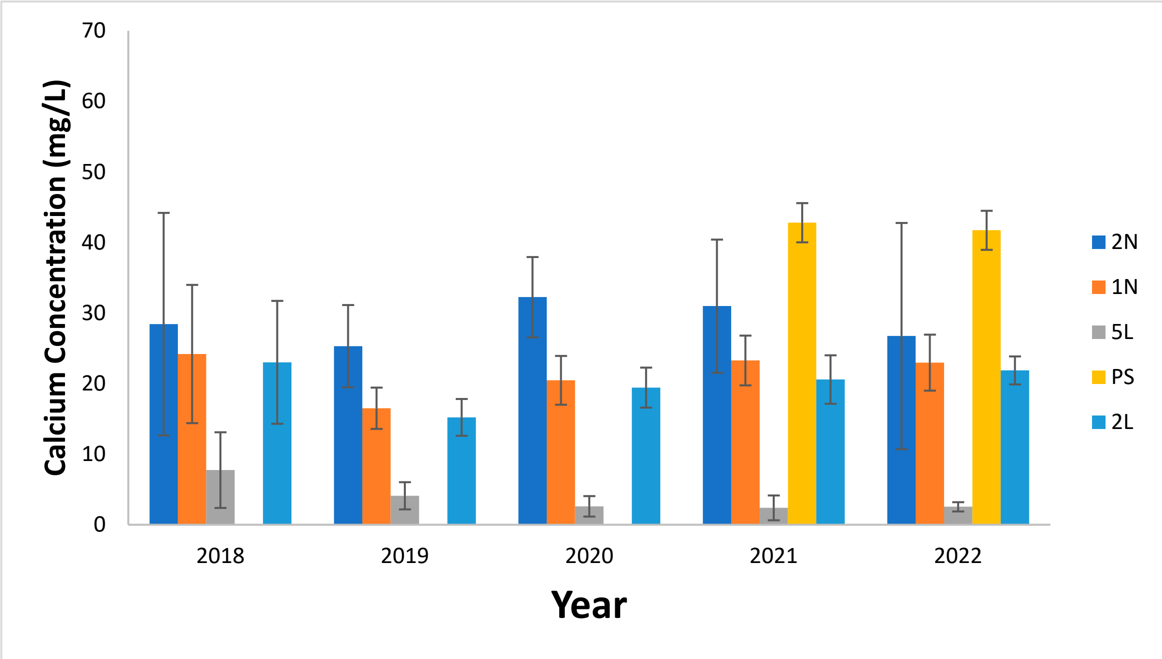

Figure 2. Spatial and temporal variations in dissolved calcium concentrations (mg/L) in Ledbetter and Panther Creek sites (Figure 1). Error bars indicate the standard deviation from the mean.

3.2. Dissolved Calcium Concentrations in Kentucky Lake

Over 750 water samples from 7 to 10 different sites during the period from 2018 through 2022 were analyzed and examined for spatial and temporal trends. Table S3a-e shows the details on the cruise numbers, sampling date, sample ID, and dissolved calcium concentrations in surface and bottom samples from each site. Main channel sites (CH, CB, and CW) have depths of about 14-17m depending on winter and summer pool, whereas embayment sites Ledbetter (L-sites) and Turkey Bay (ET and UT), West Anderson (UA, WA) sites have depths of about 2- 9m. In 2018, bottom water samples from channel sites (CH, CB, and CW) contained slightly higher concentrations of dissolved calcium than surface water samples. However, during the subsequent years, there were only very small differences recorded between surface and bottom waters. The shallow nature and continuous mixing of waters by wind and current produced a rather well-mixed water column might have resulted in little to no differences in dissolved calcium levels between surface and bottom waters. (Table S3a-e). Therefore, dissolved calcium data only from surface water samples were used in figures and further discussions.

3.2.1. Spatial and Temporal Variation in Dissolved Calcium Concentrations in Kentucky Lake

In general, dissolved calcium concentrations varied at all sites during different months in a year. For the channel sites, yearly average dissolved calcium concentrations revealed a steady state and did not reflect any significant variations among them except for the year 2018 (Figure 3), during which elevated concentrations of dissolved calcium were evident. Further, in terms of temporal trend, there was a gradual increase in dissolved calcium concentration in Kentucky Lake from 2019 through 2022 (Figure 3). Although absolute concentrations of dissolved calcium varied with the site, Kentucky Lake main channel sites (CH, CB, and CW) contained comparatively higher concentrations than embayment sites. For example, average dissolved calcium concentrations at the Upper Anderson (UA) site were consistently lower than the channel site (CB) during the years 2018-2021. Similar trends were also evident for other channels and embayment sites (Figure 4). This observation revealed that the source of calcium was primarily from upstream Tennessee River rather than tributary streams. Overall, average dissolved calcium concentrations at channel sites ranged between 17 mg/L to 25 mg/L (Figure 3). Zebra mussel colonies were present in Kentucky Lake in 2018 when dissolved calcium concentrations had reached as high as 37.2 at CB (monthly mean concentrations during April 2018), but live specimens except for a few adults were not found in Kentucky Lake during the year 2019 through July 2022. The exact cause of the spike in calcium concentration in 2018 remains unknown.

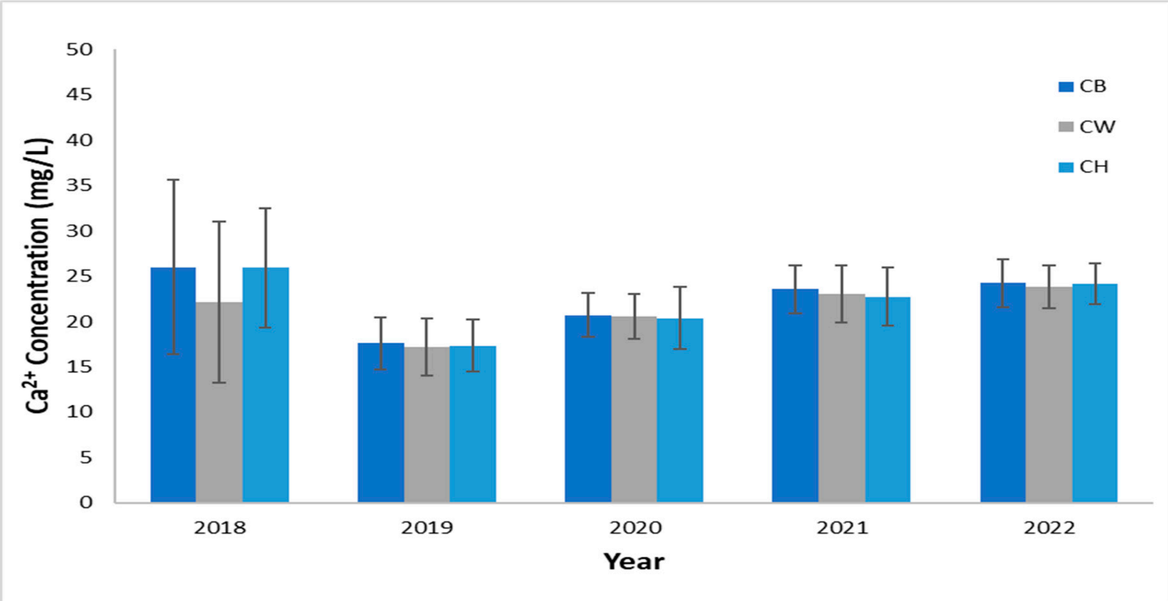

Figure 3. Spatial and temporal variation in dissolved calcium concentrations in surface water samples from channel sites in Kentucky Lake. Error bars indicate standard deviation from the mean.

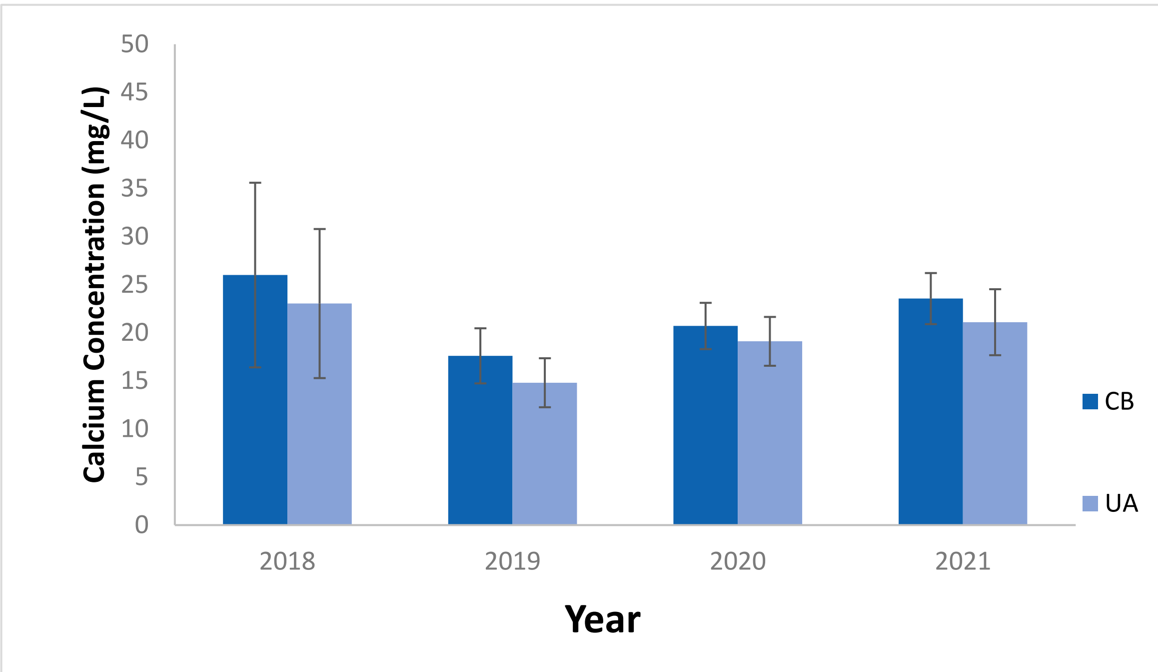

Figure 4. Comparison of average dissolved calcium concentrations in surface water samples from the Kentucky Lake Channel site (CB-1) and an embayment site (UA-1) during the years 2018-2021. Error bars indicate the standard deviation from the mean.

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3.3. Dissolved Calcium Concentrations in Ohio River

Details of dissolved calcium concentrations in the Ohio River sites are presented in Table S4, and yearly average concentrations are shown in Figure 5. Water samples (n= 85) from the Ohio River at the Paducah site contained significantly lower dissolved calcium concentrations than samples from the Brookport site (Illinois side). At the Paducah site, the yearly mean concentrations ranged from 17.2- 30.0 mg/L similar to the concentrations found for Kentucky Lake indicating that water at this site was predominantly from the Tennessee River water rather than the Ohio River (Figure 5). A gradual increasing trend in concentration during the years 2018-2022 was evident at the Paducah site, reflecting the steady rise of calcium levels in Kentucky Lake water that is being discharged to the Ohio River (Figure 5). No zebra mussels (shells) were found at this site during the study period. Yearly mean dissolved calcium concentrations at the Brookport site ranged from (32.4 - 46.1mg/L), indicating expected higher calcium levels. Zebra mussels are known to be abundant in this part of the Ohio River.

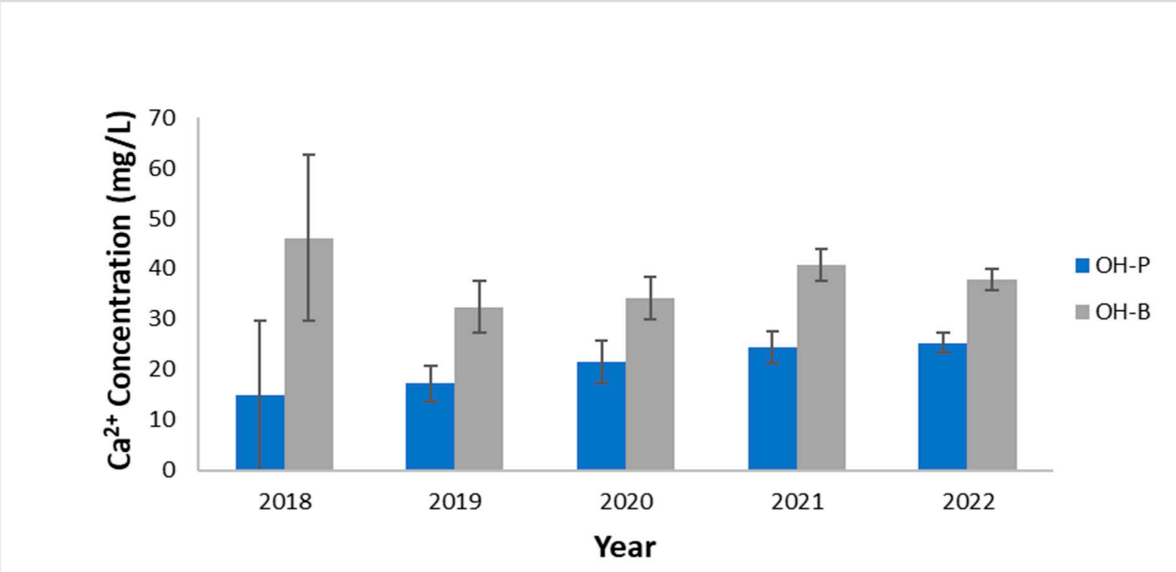

Figure 5. Yearly mean dissolved calcium concentrations in surface water samples from the Ohio River at Paducah, Kentucky (OH-P) and the Ohio River at Brookport, Illinois (OH-B). Error bars indicate standard deviations.

3.4. Sources of Calcium in Kentucky Lake Waters

Calcium is one of the major elements on the Earth's crust and occurs abundantly as limestone ($CaCO_3$), gypsum ($CaSO_4 \cdot 2H_2O$), fluorite (CaF_2), apatite (calcium chloro- or fluoro-phosphate) [39]. Natural weathering processes including, the breakdown of deep-layer silicates, formation of clay minerals, etc., can contribute calcium to aquatic ecosystems. The ionic form of calcium is present in most natural waters (dissolved phase) due to the universal presence of carbonate rocks and minerals. Calcium also enters the water bodies due to increasing atmospheric CO_2 concentration. CO_2 dissolves in rainwater and lowers its pH. Acidic rainwater breaks down calcium silicate that resides in rocks into calcium ion (Ca^{2+}) that adds to dissolved calcium in natural waters [40].

Examining the source of calcium via tributary streams (Table S2a, b), our results showed that dissolved calcium concentrations varied in different streams. These variations indicated fluctuating amounts of input from its sources. In the case of Ledbetter sites, the upstream site, viz 5L consistently reported lower dissolved calcium levels than other sampling sites, suggesting that calcium contribution from 5L to the Kentucky Lake is minimal or even negligible. Considering the embayment site 2L (downstream of 5L), dissolved calcium concentrations were consistently higher than 5L and, comparable to the calcium levels in Kentucky Lake channel sites, indicating that calcium levels found in 2L were mainly from Kentucky Lake waters. Therefore, Ledbetter Creek is not a significant source of calcium to the Kentucky Lake. Unlike Ledbetter, Panther Creek sites exhibited significantly higher dissolved calcium concentrations than other embayment sites (UA, UT) and Kentucky Lake channel sites (Table S2b, Figure 2). Examining the calcium concentrations at 2N and 1N sites (downstream of PS), it appeared that Panther Spring (PS) did not contribute a significant amount of calcium to Kentucky Lake.

In addition to natural sources, rivers, and lakes receive calcium input via a multitude of human activities. Recent reports have revealed an increasing trend of salinization of hundreds of streams, rivers, and lakes across North America, Australia, China, Europe, and Russia [41, 42-44]. One of the major causes for this surge is attributed to road salt/brine applications during the winter season and the salinization of freshwater ecosystem from road salt is considered an emergent, anthropogenic disturbance [41, 45, 46]. In the United States, road salt usage has increased about 100-fold from 0.20 million metric tons/yr. in 1944 to 24.5 million metric tons/yr. in 2014 [47,48]. In addition, sewage and agricultural fertilizers release potassium salts, acidic rainfall dissolves limestone and concrete structures leading to the release of calcium and bicarbonate ions, and mining activities deliver several different salt ions into waterways through runoff. According to Canedo-Arguelles et al. (2016), runoff of salts from roads, agricultural lands, and terrestrial ecosystems contributes to increasing total concentrations of dissolved inorganic salts in freshwater causing adverse effects on human health, increasing the cost of water treatment for human consumption, reducing freshwater biodiversity, and affecting economic well-being by altering ecosystem goods and services [41]. For the Tennessee River basin, we do not have statistical data on the quantity of brine ($CaCl_2$) applied before or during this study period. Therefore, slightly higher dissolved calcium concentrations (>25 mg/L) observed during winter and early spring months (Figure 6), may or may not be due to contributions from road salt runoff. Records of agricultural liming of fields are sporadic, but it may not be an issue because there have been no major changes in cultivation, e.g., switching from corn to soybeans. Further study is warranted to evaluate other sources of calcium in Kentucky Lake.

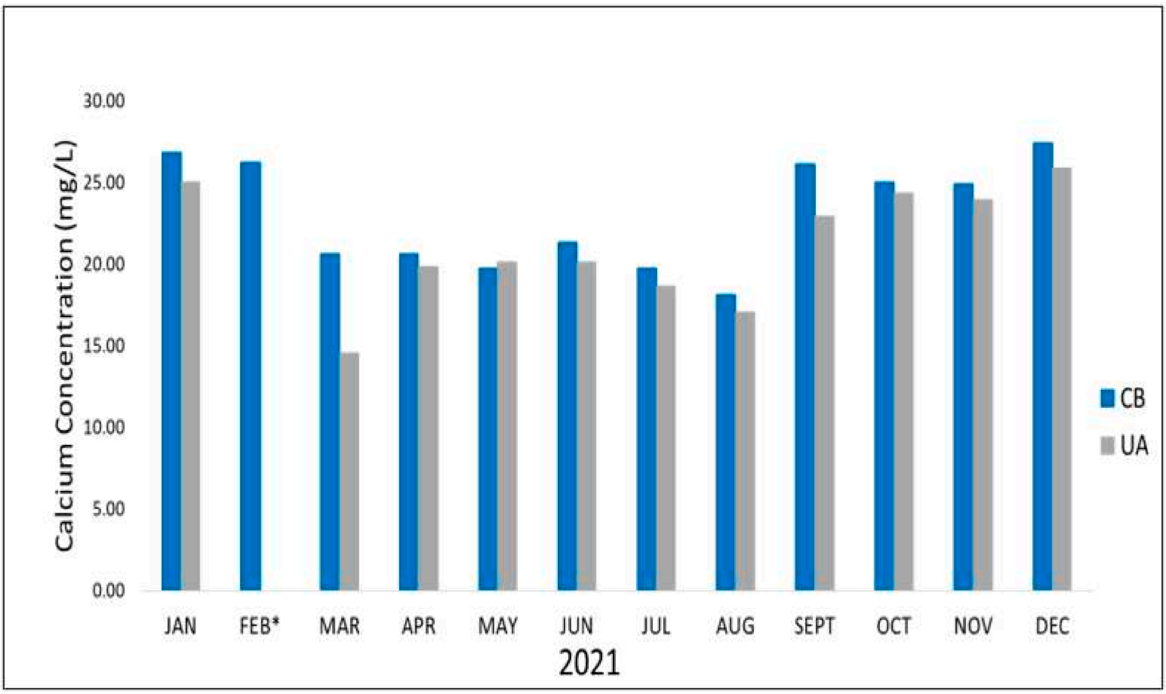

Figure 6. Dissolved calcium concentrations (mg/L) in surface waters from selected sites (CB and UA) during the year 2021. .

3.5. Zebra Mussel Occurrence and Calcium Threshold Levels

Several studies have recognized ambient calcium concentration as a key factor affecting zebra mussel distribution in aquatic environments [49,50,51]. These authors estimated the minimum calcium concentration needed to support a reproducing population which they refer to as the mussels' "calcium threshold" [50,51]. Studies conducted in Europe or North America reported different thresholds. In an analysis of over 500 European Lakes, zebra mussels were found only in waters with greater than 25-28 mg/L of calcium [52,53]. In more than 500 lakes studied in Belarus, zebra mussels were present only in lakes with more than 25.4 mg/L calcium [54]. In North America, zebra mussel populations have been reported from several sites with calcium concentrations between 13 and 25 mg/L [50, 55]. Some studies have estimated that the calcium threshold could be as low as 8.5 mg/L [56]. Zebra mussels' calcium requirements may vary with other environmental factors. For example, several studies have reported that zebra mussels' calcium threshold varies with pH, usually declining with increasing pH [52, 56]. Considering the spatial and temporal profile of dissolved calcium in the Kentucky Lake watershed and the various threshold levels reported in the literature, it appears that Kentucky Lake remains near the tipping point of a zebra mussel outbreak.

4. Conclusions

A recent global assessment report by the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) has documented that the number of alien species is growing across the globe, leading to dramatic changes to biodiversity and ecosystems and costing the global economy US $423 billion annually [57]; and furthermore, number of established alien species projected to increase by 36% in the next three decades [58, 59]. In order to reduce economic and ecological impacts from aquatic alien species, studies are warranted across major taxonomic groups, geographic regions habitat types. Our study addressed the dissolved calcium profile in a man-made freshwater lake in the southeastern United States and its implications for one of the top-ranked invasive species, the zebra mussel, and its colonization. The results revealed several important findings with respect to spatial and temporal variations in calcium levels in streams, Kentucky Lake, and the Ohio River. Possible sources of this element to these water resources, as well as its relevance for the prevalence of zebra mussels in Kentucky Lake, were explored. At this time, we can only speculate on possible sources. The findings are as follows: (i) detectable concentrations of dissolved calcium were found in all selected sampling sites in tributary streams, Kentucky Lake, and Ohio River; (ii) results provided evidence that dissolved calcium levels varied with location and year(s) of sampling; (iii) tributary streams from rural (Ledbetter) sites had relatively lower calcium levels and did not serve as sources of calcium to the Kentucky Lake; (iv) the forested site (Panther Spring) consistently had the highest dissolved calcium levels, however, was not a significant source of calcium to Kentucky Lake because of low discharge rates. Future studies may be focused on the Panther Spring chemistry and geological makeup of this region as well as loading estimates of calcium into the Kentucky Lake, and (v) dissolved calcium in Kentucky Lake water exceeded the threshold level for establishing zebra mussel colonies of > 28 ppm in 2018. Calcium monitoring did not begin until 2018 when zebra mussel populations were already present in Kentucky Lake. It appears that zebra mussel reproduction could have begun as early as 2016 and that populations had gone undetected. Therefore, we can assume that calcium probably had exceeded the threshold at least by 2016.

The results of this study emphasize the value and need for future monitoring of Kentucky Lake and its watershed to detect any abnormal spikes in calcium as well as monitoring for zebra mussel populations. Kentucky Lake is an integral part of the landscape and economy of Western Kentucky and Tennessee. Understanding the calcium levels and their influence on zebra mussel's biological cycles could provide useful information about their relationship within a man-made freshwater reservoir ecosystem such as Kentucky Lake and predict zebra mussel outbreaks.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, BL, SH., and DW.; Chemical analysis, KM., and BL; Orginal draft preparation, KM and BL.; Review and editing, SH and DW.; Supervision, BL and SH.; Funding acquisition, SH, DW and BL. **All authors have read and agreed to the published version of the manuscript.**

Funding: This research was funded by a National Science Foundation IDBR grant (PI: SH), WSI Summer Research Grant (KM), and a MSU Committee on Institutional Studies and Research Grant (CISR) (PI: BL).

Institutional Review Board Statement: Not applicable.

Informed Content Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request.

Acknowledgements: The authors thank Dr. Michael Flinn, Mr. Clark Hendrix, and the students at the Hancock Biological Station for their help during the sampling for this study. The authors also are thankful to Ms. Jane Benson for her meticulous work in preparing the study area map and Dr. Daniel Johnson for his support and for allowing us to use the facilities at the Chemistry Department's Ross/Jones Research and Instrumentation Center. This research was funded by the National Science Foundation IDBR grant, the WSI Summer Research Grant, and the Murray State University Committee on Institutional Studies and Research.

Conflict of Interest: The authors declare no conflict of interest.

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