Morphological and population changes of the cockle *Cerastoderma glaucum* in Lake Qarun, Egypt, may be driven by accelerated environmental deterioration

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ABSTRACT

The cockle *Cerastoderma glaucum* represents a significant element of the benthic community in Lake Qarun, Egypt. The population of this species has severely declined in the lake. For stock position assessment, cockles were collected in the winter of 2008 and 2018. The body size (length-frequency) distributions considerably varied among the collections. A single peak at 14 mm was present in 2008, and two peaks appeared ten years later (one at 13 mm and another at 19 mm). The total weight, shell weight and flesh weight of individuals collected in 2008 exhibited isometric increase with shell length (SL). For samples collected in 2018, flesh weight increase was slower than shell length, indicating negative allometric growth. When 50% of the population achieved maturity (SM50), their sizes were 7.7 and 7.4 mm of SL for 2008 and 2018 collections, respectively, consistent with the sexual maturity being related to size rather than age. The overall sex ratios did not significantly differ from unity (1:1). The results of this study suggest that the species is sensitive to salinity changes and water pollution, with larger individuals being more resistant than the smaller ones. This may have implications for species’ long-term reproduction and density.

1. Introduction

The cockle, *Cerastoderma glaucum* (Bruguière, 1789) [1], a bivalve mollusk, with a wide distribution [2–4] inhabits closed brackish-water lagoons and estuaries. This species is dioecious, suspension and deposit feeder and tolerates a wide range of salinity values and thermal conditions [5,6]. Like other filter feeders, *C. glaucum* may significantly contribute to water quality by reducing particulate organic load. In addition to the ecological role, this species is consumed by the local human population.

This cockle is considered as a key species of macrobenthic assemblage at temperate latitudes [7]. In the food chain, cockles link primary producers (phytoplankton, phyto-benthos) and consumers, including shrimps, crabs, fishes, and birds that feed on them [8]. The cockle *C. glaucum* can be used as a bioindicator for metal pollution monitoring programs [9,10], which, combined with its broad tolerance of ecological conditions, makes it particularly suitable for monitoring and reducing organic loads in estuaries [11].

Lake Qarun acts as a natural sink for agricultural drainage water in the Fayoum Depression [12]. As such, it is vulnerable to natural and anthropogenic changes in the terrestrial environments as well as changes in climatic regime. The lake is fed by approximately 450 million cubic meters of water with inflow from across 12 drains, with “Bats” and “Wadi” dominating the supply [13]. This inflow of drainage water roughly balances the amount of lake water lost annually by evaporation [14]. A major consequence of this evaporation is a gradual upsurge of salts, heavy metals and pesticides in addition to other pollutants [13,15–17]. For example, since 1901 salinity increased from 12 g/L to about 34–39 g/L in the late nineties (1995–2000) [18,19]. Between 2002 and 2013, salinity fluctuated about the mean of 36.1 g/L, without any positive or negative trend [20]. High salinity had a highly negative impact on the lake’s faunal composition and diminished its standing biomass [21,22].

*Cerastoderma glaucum* is one of the most common macrobenthos species that jointly affect the benthic ecosystem processes in Lake Qarun [21–23]. The annual average weight (AAW) of *C. glaucum* fluctuated between 0.440 and 0.845 g in 1989–2000, and between 0.115 and 0.261 g in 2006–2013 [20]. At the same time, the standing crop and biomass of the cockle were in the range of 11–93 ind.m-2 and from 6.9 to 70.7 g fresh weight.m-2, respectively [21,24]. Kandeel et al. [25,26] studied reproductive biology and population dynamics of the cockle in the lake. Nevertheless, available information on the biology and ecology of *C. glaucum* in Lake Qarun is scarce.
Considering the importance of *C. glaucum* to the ecology of the Lake Qarun, its population dynamics, individual growth rates and reproductive potential need further insights for stock position assessment. The present study aimed to analyse the population structure, length–weight relationships, condition index, size at onset of sexual maturity and sex ratio of the cockle *C. glaucum* of Lake Qarun. Furthermore, we attempt to compare the current status of the population with that established ten years ago. Ultimately, insights into the population size structure and growth will help improve our understanding of heavy metal pollution in the lake.

2. Materials and methods

2.1. Study area

Lake Qarun (Figure 1) represents the third largest lake in Egypt [27], located in the northern part of El-Fayoum Depression in the Western Desert (approx. 90 Km to the southwest of Cairo). The lake occupies an area between 30°34′ and 30°49′E longitude and 29°25′ and 29°34′N latitude at 43.5 m, lies below sea level [28] and contains 924 million m³ of water, with a surface area of 243 km² [18].

Lake Qarun consists of two main basins; the main (western) basin and the shallower (eastern) basin, having a maximum depth of 8.4 m and not more than 5 m, respectively [29]. Lake Qarun basin provides a reservoir for water drained from agricultural lands via El-Bats and El-Wadi drains [30]. The lake is now saline, turbid (Secchi disk transparency typically < 40 cm) and without a surface outflow [28]. The lake bottom consists of coarse sand, fine sand, silt and clay, and the organic matter share by weight falls in 2.2–7.5% range. Average water temperature reached a maximum in summer (30.1°C) and a minimum value (14.6°C) in winter, with an annual average of 23.8°C [30].

2.2. Sampling procedure

Collecting *C. glaucum* samples was conducted from the Shakshuk area (Figure 1) during the Winter season of the two years; 2008 and 2018. At the collection site, a beam trawl with a 1 mm mesh is deployed at a depth of almost 2 m, then dragged to assemblage the cockles. The catch was carefully washed on site. The collected cockles preserved in 5% formaldehyde-seawater.

2.3. Measurements

The SL of each cockle was determined to the nearest 0.1 mm by using of a Digital caliper (precision = 0.05 mm). SL measurements were then used to generate length-frequency distribution for samples obtained during the two years. We adopted class intervals of 1 mm. Subsequently, the cockle soft body was removed from the shell using a scalpel. Total weight (TW, shell + flesh), shell weight (SW) and flesh weight (FW) were recorded with precision of 0.0001 g using toploader digital balance.

2.4. Weight–length relationships

The following allometric equation described the relationship between body weight (W; in grams) and SL (in mm) [31]:

\[ W = aSL^b \]

where \( W \) is a dependent variable; \( L \) is the independent variable; \( a \) and \( b \) are intercept (initial growth coefficient) and slope (relative growth rate of variables) values, respectively. Weight and length were converted to natural logs, and a linear regression equation was determined:

\[ \log W = \log a + b \log L \]

Using this approach, we evaluated the relationship between SL and total weight (log TW), shell weight (log SW) and flesh weight (log FW). The degree of association among variables was measured as the determination coefficient (\( R^2 \)). In particular, we sought to determine whether they differed from the isometric type (i.e. when \( b = 3 \)). Here, we used a Student’s t-test (Ho: \( b = 3 \)) with a confidence level of ±95%, following the equation [32]:

\[ t_s = (b - 3)/s_b \]

where \( t_s \) = t-test value, \( b \) = slope and \( s_b \) = standard deviation of \( b \). This test helped to determine whether the slopes are not different from the isometric (\( b = 3 \)) or allometric relationship (negative allometry: \( b < 3 \) or positive allometry: \( b > 3 \)). To determine whether regression slopes of length–weight relationships differed between the study periods, we ran an analysis of covariance (ANCOVA) between SL and square-root transformed for TW, SW, and FW. These analyses were performed on both years’ data sets by MINITAB software (version 13, 2000) and Statistica (StatSoft, version 10), with levels of statistical significance set at \( P < 0.05 \).

2.5. Condition index

Body condition index (C.I.), previously used by Leite et al. [33] and Smaoui-Damak et al. [34], was determined on an individual basis as a percentage between flesh weight and total weight of cockles (FW/TW × 100). C.I. was expressed as mean ± S.D. and calculated for samples collected in 2008 and 2018. The variation between the mean values of C.I. among the two collections was tested by paired t-test.

2.6. Size at the onset of sexual maturity

The gonadal smear of each specimen was examined under the microscope to identify the mature animals
Figure 1. Sampling site (∗) at Lake Qarun, Egypt.

Figure 2. Gonadal smear of *Cerastoderma glaucum* (Bruguière, 1789) [1] showing growing oocyte (Go) and mature ovum (Mo) in females and sperm in males.

(morphologically mature spermatozoa for males and growing oocyte or ripe ovum in females). The share of mature individuals (%) was evaluated for both sexes. This was accomplished by fitting a logistic curve to the data. We then expressed the fit as the sexual maturity (SM50) index—the length, L, at which 50% of individuals were mature.

2.7. Sex ratio

First, each specimen was sexed by smearing the individual’s sexual products (Figure 2) that were then inspected for histological indicators of maturity in a compound microscope at a magnification of 100x. Next, we determined the sex ratio (defined as a ratio of the number of females to males, M:F). Deviations from the
null sex ratio of 1:1 were evaluated by Chi-square ($\chi^2$) test with one degree of freedom [35].

3. Results

3.1. Population structure

The length of collected individuals varied broadly from 7.5 to 21.9 mm (13.18 ± 2.76) and from 6.5 to 30.2 mm (16.59 ± 5.69) mm in 2008 and 2018, respectively. The mean value of SL for animals collected in 2008 was significantly less ($t$-value = 6.93, $P$ = 0.000) than for cockles collected in 2018 Table 1. One frequency peak occurred at 14.0 mm shell length in 2008 and two peaks at 13.0 and 19.0 mm shell length in 2018 (Figure 3). Most of the cockles were in size classes of 10–14 and 12–20 mm, representing 50.0% and 48.4% of individuals from 2008 and 2018, respectively. Large individuals ($> 19.0$ mm) constituted 2.2 and 10.1% of all samples collected during the two sampling years, respectively. The size of either male or female cockles does not differ between 2008 and 2018 collections (Males: $t$-value = 0.04, $P$ = 0.972, ***; females: $t$-value = 0.25, $P$ = 0.808).

3.2. Weight–length relationships

3.2.1. Total weight–shell length relationship

In both sampling years, total weight (TW) and SL were strongly related (regression analysis, $P < 0.0005$; Table 2), indicating isometric growth pattern in the two sampled years The relationship between SL and square root transformed TW was the same in 2008 and 2018 (ANCOVA, $P = 0.673$).

3.2.2. Shell weight–shell length relationship

The relationships between shell weight (SW) and SL were also strongly positive. Shell weight (SW) increased isometrically with SL in the two collection years (Table 2). Relationships for specimens of similar size ranges (up to 21 mm) follow the same pattern in both collection years (ANCOVA, $P > 0.2$).

3.2.3. Flesh weight–shell length relationship

The relationships between logarithmically transformed data of flesh weight (FW) and SL were derived from linear regression equations in the following form:

$$\log FW = \log a + b \log SL$$

Student’s $t$-test results showed that flesh weight increase did not differ from the null model, which supports an isometric growth of weight with length 2008 population. However, a different pattern emerged for the population sampled in 2018: Flesh weight failed to keep up with shell length, indicating a negative allometric growth pattern in that period (Table 2). Differences between the slopes of the relationship among the two collection years were insignificant (ANCOVA, $P > 0.2$).

3.3. Condition index

The mean value of CI for cockles collected in 2008 (35.31) is significantly greater (paired $t$-test, $t$-value =
Table 3. Condition index of Cerastoderma glaucum (Bruguière, 1789) [1] at different two years in Lake Qarun.

| Year | Mean | S.D. | Min. | Max. | N  |
|------|------|------|------|------|----|
| 2008 | 35.31| 8.16 | 6.74 | 89.16| 158|
| 2018 | 28.81| 7.68 | 3.24 | 77.78| 117|

Figure 4. The percent of maturity plotted versus shell length (mm) of Cerastoderma glaucum (Bruguière, 1789) [1] assembled during 2008 and 2018 from Lake Qarun. The size at 50% maturity (SM50) is calculated. N = number of cockles examined.

12.10, $P = 0.000$) than for cockles collected in 2018 (28.81) (Table 3).

3.4. Size at onset of sexual maturity

Cockles reached sexual maturity at a smaller size in 2018 than in 2008. This is indicated by the relationship between shell length (SL, mm) and the share mature C. glaucum have in the population. Population collected in 2008 reached sexual maturity at SM50 at 7.7 mm while SM50 in 2018 population was 7.4 mm (Figure 4). Although the minimum size of an adult was similar (7 mm SL) in both temporal sets, the maximum size of a juvenile was consistent with the SM50 pattern of differences among mature sizes: it was 11 mm in 2008 and 8 mm in 2018.

3.5. Sex ratio

The sex ratios did not appear to change during the study period: in 2008, 50.57% of the cockles examined microscopically were sexed as males and 49.43% as females (Table 4). In the 2018 data set, 47.33% were males and 52.67% were females. The overall ratios of females to males for the two data sets (1 M: 0.98 F and 1 M: 1.11 F, respectively) did not statistically differ from the theoretical sex ratio of 1:1 ($X^2 = 0.02$ and 0.43, respectively; $P > 0.05$). The variation of sex ratio at different SL (2 mm size group) for 2008 population ranged between 100% males vs zero females in the largest size group (21–22 mm SL) and 42.86% males vs 57.14% females in (17–18.9 mm SL) (Table 5). Whereas the 2018 population showed that females outnumbered males in all size classes except in the 21–22.9 and 27–28.9 mm SL (Table 6). Deviation at a 95% level of significance from the anticipated 1:1 ratio was not recorded for all size classes of the two years of collections.

4. Discussion

In the present study, length-frequency distribution between the two collections changed significantly, primarily in relation to growth rate differences. The increasing trend in the maximum size of cockles appears to continue. For example, individuals larger than 22.0 mm were recorded only in the 2018 collection. The largest animal found was 21.9 mm of SL in 2008 collection analysed in this study and 27.0 mm reported in another study [26] also based on material collected in 2008. The most recent collection contained a much larger individual (SL 30.2 mm). This is not exceptional as the largest recorded animal had SL 32.0 mm in a similar Lake Timsah [26]. The length-frequency distribution affords several inferences; for example, they allow estimating the stock biomass, growth, mortality and dynamic rates of recruitment in a specific ecosystem [36, 37].

The significant differences in the mean shell length (3.41 mm SL, $P = 0.0000$) between the two years of collection may return to more appropriate conditions to grow and reach the large size groups (22–30.9 mm SL) in the 2018 population. Predators play an important role in the regulation of population dynamics of the cockles [38]. Many types of fishes in Lake Qarun such as Solea vulgaris, Tilapia zilli, sparus aurata, and Mugil sp. have a great effect on structuring the adult cockle population [26]. The lake’s fish production reduced from 3184 tons in 2008–878 tons in 2016 due to a salinity increase [39, 40]. This may be the reason for the appearance of the cockle large size classes among the 2018 population. Presently, the level of salt concentration in Lake Qarun overtakes the international permissible limit [40]. The average salinity increased from 32.45% in 2015 to about 34.3% in 2016 leading to a great decline in the fish stock and the disappearance of many fish species [41]. However, Rygg [5] stated that the cockle C. glaucum can tolerate a wide range of salinity from 11‰ to 45‰.

The small size classes in the two years of collections represent a great percentage of the population in lake Qarun. The abundance of small-size groups indicates high and probably year-round recruitment [42]. The pattern of recruitments of C. glaucum in Lake Qarun is throughout the year [26]. Tarnowska et al.
Table 4. Percentage and ratio of males and females of *Cerastoderma glaucum* (Bruguière, 1789) [1] in different years in Lake Qarun

| Year  | Sample size (N) | Percentage | Ratio |
|-------|-----------------|------------|-------|
|       | Males | Females | Total number | $X^2$ value | Males | Females | Males | Females |
| 2008  | 88    | 86     | 174        | 0.02     | 50.57 | 49.43 | 1     | 0.98    |
| 2018  | 71    | 79     | 150        | 0.43     | 47.33 | 52.67 | 1     | 1.11    |

Note: The critical value for $X^2$ goodness of fit test of equal numbers of males and females (1 df) at 95% significance is 3.841.

Table 5. Percentage and ratio of males and females at different shell lengths (2 mm size classes) of *Cerastoderma glaucum* (Bruguière, 1789) [1] collected in winter 2008 from Lake Qarun.

| Sample size (2 mm size groups) | Sample size (N) | Percentage | Ratio |
|---------------------------------|-----------------|------------|-------|
|                                 | Males | Females | Total number | $X^2$ value | Males | Females | Males | Females |
| 7.0–8.9                         | 5     | 2       | 7         | 1.29     | 71.43 | 28.57 | 1     | 0.40    |
| 9.0–10.9                        | 10    | 10      | 20        | -        | 50.00 | 50.00 | 1     | 1       |
| 11.0–12.9                       | 21    | 21      | 42        | 0.02     | 49.25 | 50.75 | 1     | 1.03    |
| 13–14.9                         | 33    | 34      | 67        | -        | 50.00 | 50.00 | 1     | 1       |
| 15–16.9                         | 10    | 10      | 20        | -        | 42.86 | 57.14 | 1     | 1.33    |
| 17.0–18.9                       | 6     | 8       | 14        | 0.33     | 66.67 | 33.33 | 1     | 0.50    |
| 19.0–20.9                       | 2     | 1       | 3         | 1        | 100   | -     | 1     | -       |
| 21.0–22.9                       | 1     | -       | 1         | 1        | 50.57 | 49.43 | 1     | 0.98    |

Note: The critical value for $X^2$ goodness of fit test of equal numbers of males and females (1 df) at 95% significance is 3.841.

[43] reported continuous spawning patterns for *C. glaucum* and *C. edule* in the Mediterranean sea and Atlantic coasts, respectively. Del Norte-Campos [44, 45] determined that this pattern of spawning is significant to tropical bivalves, which are fast-growing and short-lived species. The multiple spawning with an interval between batches plays an important role in terms of the survival of the species by reducing intraspecific competition for food resources [46].

The results of this study agree with the prevalence of isometric growth over negative allometric growth for 25 bivalve species from the Algarve coast in southern Portugal [47]. Kandeel et al. [26] reported isometric growth for *C. glaucum* in Lake Qarun (significant to length, height, breadth, and weight) in different seasons. Since the majority of bivalvia is a typical inhabitant for sandy or sandy-mud bottom. This phenomenon could be illustrated by the fact that the density of substrata is not a limiting factor on firmer sediment. In addition, the slope value $b$ may exceed 3 in some species [48]. The overall positive allometry for *C. glaucum* and fairly negative allometric growth for *C. edule* (significant to height, breadth, and weight) reported by Mariani et al. [49]. Bivalvia are characterized by a wide range of morphological diversity [51]. This wide variation of allometric patterns enables *C. glaucum* to resist unstable and extreme environmental factors [43]. Table 7 shows various weight–length parameters for different families of bivalvia.

In Lake Qarun, the condition index (CI) suggests a significant deterioration in the growth conditions of the cockle in the recent decade. The limited *C. glaucum* growth is consistent with the observed decline in condition index (28.81) and may be caused by pollution. Condition indices are generally regarded as useful information on the nutritive status of bivalves. The health of cockles was previously assessed with a condition index, which may also serve as an assay for monitoring various pollutants and diseases [52]. Also, relative weight deficiency suggests food resources that are insufficient
towards meeting the metabolic requirements of organisms [53, 54].

Agricultural sewage input into Lake Qarun caused severe water pollution and bottom sediments primarily when trace metals (Cu, Zn, Pb, and Cd) concentration increase. Sediment metals such as Fe, Mn, Cr and Ni also represent a probable threat to the aquatic environment [28]. The survival of M. balthica has been affected by the high metal concentration in the sediment [55]. Bryan and Gibbs [56] stated that heavy metal contamination can cause mortality for adult cockles over the long run. Toxicant plays an important role in reduction of the activation of the immune system of marine bivalves against parasites and pathogens increasing the incidences of the diseases [57]. In this study, the parasites' cysts were observed with high numbers during examination of the smear especially for large cockles (personal observation). Kandeel et al. [25] indicated that the tendency of infection with digenese parasites increases with size. Trematode manifestation is a castrating agent for the adult cockles population [57]. The combination of sub-lethal effects of environmental pollution with other stressors such as pathogens may result in cockles mortality [58]. This may illustrate the low representation (2.2%, 10.1%) of large cockles (> 19 mm SL) of all samples collected during the two sampling years, respectively, and seeing a high percentage of dead cockles on the shore of the lake.

Not all individuals of a species reach maturity at the same length, and several species become sexually mature at a specific age, including individuals with a wide range of lengths. When shell length at maturity SM50 is available, it provides a convenient insight into wide range of lengths. When shell length at maturity mature at a specific age, including individuals with a similar length, and several species become sexually mature at a specific age, including individuals with a similar length, and several species become sexually mature. In the present study, length at maturity indicates that C. glaucum can produce and discharge gametes at the age of one year. A similar precocious sexual maturity was recorded by Yankson [62].

A previous study on the Lake Qarun population has shown that C. glaucum reached the first maturity at 9.6 (females) and 8.5 (males) mm. For comparison, SM50 was 16.8 mm for females and 15.1 mm for males at Gulf of Gabes, southern Tunisia [63], which is much larger than those of the Lake Qarun population. High temperature in the latter, with monthly means between 14.6 and 30.1°C, as compared to 12.3 – 26.8°C for the Gulf of Gabes, encourages early recruits into the breeding population. The filtration rate that bivalves exhibit upsurges with temperature increase [64], in anticipation of accelerating gamete development and earlier maturation.

In the present study, sex ratios were not significantly divergent from unity. A similar result was recorded in C. glaucum population from the north coast of Sfax Gulf of Gabe, Tunisia [63], and three non-Mediterranean populations: the Baltic Sea, the North Sea [43]. Departure from the 1:1 sex ratio is not expected for most aquatic species [65, 66]. However, a male-biased sex ratio was recorded for C. glaucum at Lake Qarun, Egypt [25] and two populations at Sussex, north-east coast of England [67]. Sex ratio could reflect some impacts like thermal effects on sex determination and predator factor on mortality [65, 68, 69]. The prevalence of males in another study may be explained by elevated female sensitivity towards unfavourable environmental conditions [25]. An alternative explanation might be sex-specific mortality that was recorded in other bivalves, such as Mytilus trossulus [70] and Modulus modulus [71].

In conclusion, as the specific environmental conditions of Lake Qarun affect the stock position of C. glaucum, our baseline research may serve as a reference for assessing and managing the species. Further, this information will assist future research on employing C. glaucum as a bio-indicator of organic loading. Because bivalves bioaccumulate metals, the insights provided by this study will assist any further studies required to determine the concentration of heavy metals in biotic and abiotic components of Lake Qarun and to assess

### Table 7. Total weight-length relationship parameters for different bivalve species.

| Family/Species | Log α | b | c² | N | Relationship (r-test) | Country | Locality | References |
|----------------|-------|---|----|---|-----------------------|---------|----------|------------|
| F: Cardiidae C. glaucum | −3.49 | 2.75 | 0.747 | 158 | Isometric | Egypt | Lake Qarun | Present study (2008) |
| | −3.23 | 2.50 | 0.898 | 117 | Isometric | Egypt | Lake Qarun | Present study (2018) |
| | −2.60 | 2.44 | 0.870 | 688 | -allometry | Algeria | El Mellah Lagoon | Lamia et al. [50] |
| | −3.04 | 2.59 | 0.975 | 38 | Isometric | Egypt | Lake Timah | Kandeel et al. [26] |
| | −3.44 | 2.98 | 0.974 | 82 | Isometric | Egypt | Lake Timah | Kandeel et al. [26] |
| | −3.00 | 2.76 | 0.724 | 210 | +allometry | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| F: Acanthocardia Acanthocardia A. Aculeata | −4.30 | 3.42 | 0.976 | 210 | +allometry | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| F: Mactridae Mactra corallina | −4.05 | 3.13 | 0.009 | 172 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| | −3.70 | 3.04 | 0.990 | 172 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| F: Veneridae Callista chione | −4.00 | 2.76 | 0.724 | 210 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| | −3.47 | 3.38 | 0.945 | 164 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| F: Donacidae Donax variegatus | −4.70 | 3.38 | 0.945 | 164 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
| F: Anomia ephippium | −4.30 | 3.10 | 0.964 | 73 | Isometric | Southern Portugal | Algarve coast | Gaspar et al. [47] |
and provide assurance of the quality of the aquatic environment of this lake.

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Authors’ contributions

MA collecting samples, Laboratory measurements, interpretation, and writing the original draft. AE idea and formulation of the research goal. JK writing, review and editing. KK Application of statistical techniques to analyse study data, review and editing. All authors read and approved the final manuscript.

Availability of data and materials

The data sets used and/or analysed during the present study are available from the corresponding author on request.

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