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PLANKTON DIVERSITY AS MIRRORED BY SILVER CARP (*Hypophthalmichthys molitrix*) GUT CONTENTS IN RUPA LAKE, POKHARA, NEPAL

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ABSTRACT

A study was conducted in Rupa Lake from February to April of 2023 to investigate plankton diversity and gut content in silver carp (*Hypophthalmichthys molitrix*) in the lake. Samples were collected from 25 locations using a <75 µm mesh plankton net. The samples were preserved in Lugol's solution, and quantitatively analyzed using a Sedgewick Rafter Counting Chamber. Environmental parameters like temperature, pH, dissolved oxygen, and water transparency were examined. Silver carp samples were collected from daily catch of the lake and gut sample was preserved in 70% ethanol. The gut contents were analysed qualitatively using microscope. Altogether 28 genera, 13 belonging to phytoplankton and 15 belonging to zooplankton were recorded in the lake. The phytoplankton belonged to 4 groups with Chlorophyceae, Bacillariophyceae, Cyanophyceae, Zygnematophyceae based on dominance. The zooplankton belonged to 3 groups with Rotifera, Copepoda, Cladocera based on dominance. The highest number of phytoplankton and zooplankton were recorded in the deeper region, and lowest in the outlet area. The dominant phytoplankton in the gut of silver carp was Chlorophyceae, Cyanophyceae and Bacillariophyceae. The dominant zooplankton in the gut of silver carp was Cladocera, Copepoda and Rotifera. Diet of silver carp mostly corresponded with phytoplankton and zooplankton available in the Lake.

Keywords: Silver carp, phytoplankton, zooplankton

INTRODUCTION

A large number and high diversity of plankton including zooplankton and phytoplankton exhibit in water bodies, despite the fact that competitive species require the same types of resources (Hutchinson 1961). Thus, lakes are highly productive environments, characterized by complex food web networks and high levels of diversity (Barbier et al. 1997). Planktons are drifting aquatic organisms along the current in water bodies. They directly or indirectly support fish populations and other various aquatic organisms including nektons. They are of two types: phytoplankton and zooplankton. Phytoplankton are minute, microscopic chlorophyll-bearing organisms (Kushwaha 2012) that live suspended in the water column. One of the major consumers of phytoplankton is zooplankton, which is comprised of animals in the origin of the planktonic community. In the complex food web network, phytoplankton serves as the foundation of the food chain and contributes significantly to the productivity of the water body (Mahaseth 2013). They produces oxygen through photosynthesis, which improves habitat quality for fish growth and production (Gurung et al. 2019). However, sudden excessive growth limits the penetration of sunlight into the deeper regions, inhibiting photosynthesis by submerged and emergent plants (Gurung et al. 2019; Bastakoti and Timilsina 2020).

Phytoplankton standing abundance in aquatic environments represents a balance between production and loss. The loss factor of phytoplankton in lakes is highly associated with predation by several types of grazers (microplankton and metaplankton) including Pisces, silver carp (*Hypophthalmichthys molitrix*). Silver carp are surface feeders, they collect suspended food items from the water mechanically rather than selectively (Spataru and Gophen 1985). They do not breed naturally, but occur in Rupa Lake due to annual stocking by local fishers as they subsist on plankton and grow fast and bigger with no additional feed from outside, instead by consuming planktons, especially phytoplanktons (Gurung 2007). However, some studies have suggested that silver carp may be an opportunistic consumer and may consume small zooplanktons as well (Spataru 1977; Spataru and Gophen 1985; Wu et al. 1997; Smith 1989; Tang et al. 2002). The gill rakers of silver carp are known to be capable and suitable for filtering phytoplankton and zooplankton (Spataru 1977; Spataru and Gophen 1985; Xiao et al. 2010). The silver carp are captured using gill nets almost every day by fishers as food, to sell in the local market. The silver carp fishery has been supporting the local livelihood of hundreds of fishers since last early 1980 in Rupa Lake (Gurung 2007; Chaudhary et al. 2015; FAO 2019; Husen et al. 2019; Pant 2019).

Recent studies have shown that the water quality in Rupa Lake is poor with a Water Quality Index (WQI) score of Grade C (Sahadev et al. 2020). Such changes in water quality affect aquatic life (Biro and Vörös 1982; Verma et al. 2020). Phytoplankton has short carbon turnover rates and is sensitive to water quality conditions (Makarewicz 1993). So, it has been recognized as a significant bio-indicator of water quality, reflecting the pollution levels in aquatic ecosystems, as well as indicating the ecological quality of water through its biomass (Fakioglu 2013; Mahaseth 2016; DC et al. 2022). Since, it is empirically not well examined which plankton are abundantly consumed by silver carp and what could be the possible implication of plankton grazing in subtropical Lake Rupa. Therefore, this study attempted to analyse what types of plankton might be preferred by silver carp (*Hypophthalmichthys molitrix*) and how the plankton consumption might be impacting plankton diversity and lake water quality. The types of phytoplankton and zooplankton consumed has been attempted to be mirrored through examining the gut contents of silver carp in Rupa Lake.

MATERIALS AND METHODS

Study area

The study was conducted in Rupa Lake (28°08'58.61" N and 84°06'39.90" E), located in the Kaski District of Gandaki Province Nepal. It is the third largest lake after Phewa and Begnas of Pokhara Valley which is located at an altitude of 600 masl. The lake has an area of 1.35 km^2, an average depth of 3 m, and a maximum depth of 6 m (Rai et al. 1995). The lake has a catchment area of 28 km^2.

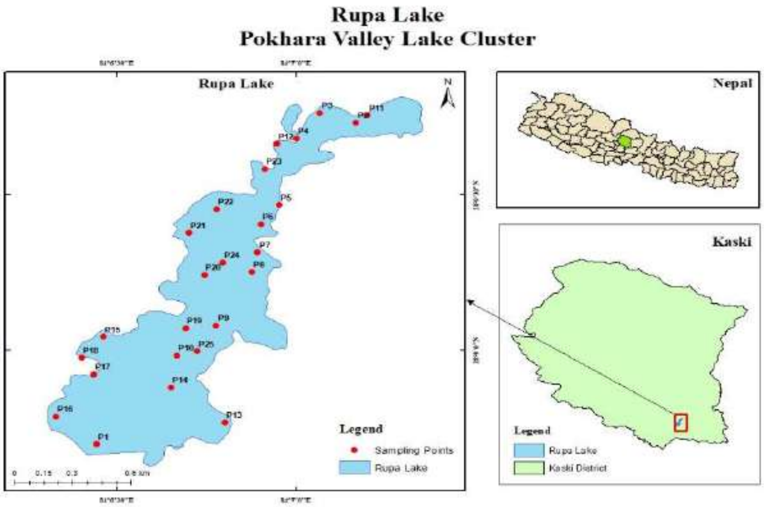

Figure 1: Map showing study area of Rupa Lake, Pokhara, Kaski

Rupa Lake is irregularly populated by several plankton species (Rai 2000). Therefore, five sampling sites (Site 1 to Site 5) were selected strategically including outlet area, settlement area, deeper region, shallow region and inlet area as shown in (Table 1). Total of 25 sampling points (P1 to P25) as shown in (Figure 1) were selected from the periphery of sampling sites, to perform a comprehensive understanding of the phytoplankton distribution and diversity across the different parts of the Lake Rupa.

Table 1 : Sampling location with geographical coordinates and site descriptions

Sample sites	Coordinates	Site descriptions
Site 1	28°08'41.712" N and 84°06'26.676" E	An outlet area with minimal human intervention.
Site 2	28°08'45.924" N and 84°06'48.204" E	The settlement area with the highest level of human activity.
Site 3	28°09'4.68" N and 84°06'46.728" E	The deeper region of the lake near Devithan temple.
Site 4	28°09'39.852" N and 84°06'56.916" E	The shallow region of the lake with barren area.
Site 5	28°09'40.932" N and 84°07'0.12" E	An inlet area characterized by cattle grazing and agriculture practices.

Water quality analysis

Among various water quality parameters, Temperature, pH, Dissolved Oxygen (DO) were measured using multi-parameter probes. Transparency was measured using Secchi disc in different sampling points. The parameters were analysed during the month of February, 2023.

Study of planktons

A plankton sample was collected using a plankton net with a mesh size of <75 µm. The samples were preserved with Lugol's solution upon collection, and brought to the lab for analysis. The sampling was done in the month of February, 2023. The quantitative analysis of plankton was done using Sedgewick Rafter Counting Chamber. The UNESCO phytoplankton manual was referred for counting and freshwater algae key and image based key was used for the identification (Caspers 1980; APHA 1998; Haney 2013). Similarly, the silver carp gut samples were obtained from the daily catch of fishes by RLRFC (Rupa Lake Restoration and Fishery Cooperative) in the month of April, 2023. A dead specimen of silver carp was randomly selected from the entire catch early in the morning, and this process was repeated daily for a week. Then, with a single incision at ventral side in between the pectoral fins at the anterior end, the gut was removed. The foregut (from the anterior part of the oesophagus to the first bend) was sampled (Yu et al. 2019; Tumolo and Flinn 2019). The dataset for the sampled fish was recorded on site (weight of fish, weight of full and empty gastrointestinal tract, and weight of the gut contents). The sample was preserved in 70% ethanol solution, stored in an ice box and carried to lab for examining the contents eaten by the fish. The gut contents were examined qualitatively by recording and identifying the features of remaining body parts, as have been reported in several earlier studies (Yu et al. 2019; Tumolo and Flinn 2019; Rai 2000). Plankton species were observed under 10X and 40X magnifications using OLYMPUS CX 41 microscope. Likewise, the plankton biomass was determined by using Ash Free Dry Weight Method (AFDW) following APHA (1998).

Dry weight, mg/L = [(*A*-*B*) × 1000] / (sample volume, mL)

Where,

A = weight of filter + dried residue, mg

B = weight of filter, mg

AFDW, mg/L = [(*C*-*D*) × 1000] / (sample volume, mL)

Where,

C = weight of filter + residue before ignition, mg

D = weight of filter, mg

RESULTS AND DISCUSSION

Water quality analysis

Some of the water quality parameters measured on various sampling points during February, 2023 has been shown in (Table 2). Water temperature varied between 18.4 ºC to 24.6 ºC during the study period, with high and low temperatures both in the outlet area. Temperature appears to be relatively stable, with only a few sites showing variations of more than 2º C. The highest value of pH i.e. 8.5 was recorded in sampling point (P10) at the settlement area and lowest value of pH i.e. 7.5 in sampling point (P11) at the inlet area. Such difference in the pH may be due to human activities such as agriculture or sewage discharge, which may contain alkaline substances in the settlement area. Moreover, the pH values align with previous studies by Jones (1989) that have noted the neutral to alkaline nature of Nepalese lakes. Similarly, the findings for DO concentrations are consistent with the findings of Gautam et al. (2016) i.e. 4.0 mg/L to 9.8 mg/L. The highest value of DO i.e. 9.5 mg/L was recorded in the deeper region and lowest value of DO i.e. 4.1 mg/L was recorded in the outlet area. The Secchi disc transparency was observed to be lowest in the outlet area i.e. 45 cm and highest in the settlement area i.e. 1.0 meter. The low transparency in the outlet area could be due to the sediments runoff from upstream. The maximum Secchi depth was recorded as 3.5 meter in this study.

Table 2: Mean and range of water quality parameters measured during February 2023 in Rupa Lake

Parameter	Mean ± SD	Range
Temperature (℃)	20.4 ± 1.2	18.4 - 24.6
pH	8.0 ± 0.2	7.5 - 8.5
Dissolved Oxygen (mg/L)	8.4 ± 1.3	4.1 - 9.5
Transparency (cm)	73 ± 13	45 - 100

Phytoplankton composition and distribution

A total of 1161 phytoplankton cells were enumerated, which comprised of 13 genera of 4 groups (i.e. Chlorophyceae, Bacillariophyceae, Cyanophyceae and Zygnematophyceae). The maximum number was counted for *Staurastrum spp.* (262 cells) and a minimum for *Tetraedrongracile* (2 cells). The highest number (88 cells) was recorded in the deeper region (Site 3), whereas a poor number (17 cells) was recorded in the inlet area (Site 5) as shown in (Figure 2).

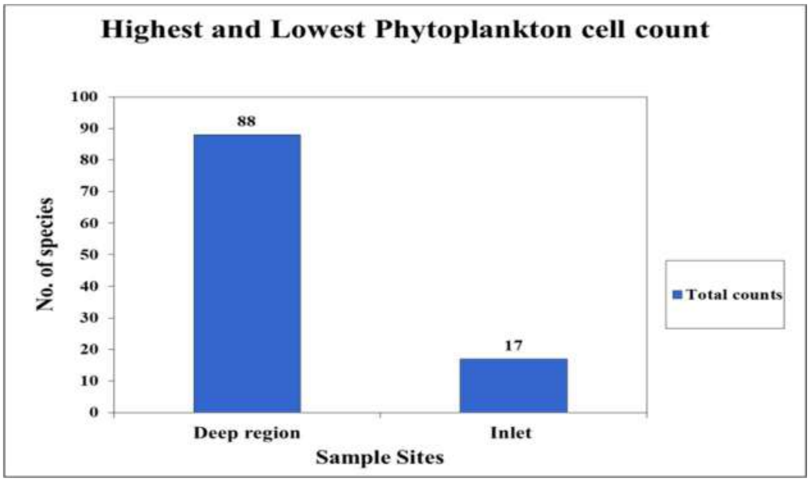

Figure 2: Sampling sites with highest and lowest phytoplankton cell count

The most dominating group was Chlorophyceae (677 cells), consisting of *Staurastrum spp*., *Ankistrodesmus falcatus*, *Pediastrum duplex*, *Scenedesmus spp*., *Sphaerocystis schroeteri* and *Tetraedrongracile* followed by Bacillariophyceae (396 cells), comprising of *Melosira granulate*, *Thalassionema*, *Rhizosolenia erienis*, *Surirella sp*. and *Fragilaria sp*. and by Cyanophyceae (72 cells), comprising of *Merismopedia tennuissima* and by Zygnematophyceae (16 cells), comprising of *Spirogyra sp*. In previous studies, *Tabellaria fenestrate* and *Melosira granulate* were dominant species within Bacillariophyceae (Rai, 2000; Dhakal et al., 2011); whereas *Staurastrum spp*. was within Chlorophyceae dominated throughout this study. Similarly, *Cyclotella spp*., *Melosira granulate*, and *Synedra ulna* were identified as major species by Dhakal et al. (2011), while this study identified *Staurastrum spp*., *Ankistrodesmus falcatus*, and *Pediastrum duplex* as the most dominant species. Also, this study identified *Spirogyra sp.* within Zygnematophyceae as a group of phytoplankton present, whereas it was absent in the previous study by Dhakal et al. (2011). The *Staurastrum spp.* was present during the month of March-April but was not recorded during February by Dhakal et al. (2011). But in this study, *Staurastrum spp*. was present with maximum phytoplankton count (262 cells) in the month of February, 2023. Such an interesting shift in species may be due to environmental variables and seasonal differences in sample collection.

Zooplankton composition and distribution

A total of 424 zooplankton individuals were enumerated in this study, which comprised of 15 genera of 3 groups (i.e. Rotifera, Copepoda and Cladocera). The maximum zooplankton was counted for *Bosmina longirostris* (73 individuals) and a minimum for *Leptodiaptomus tyrrelli* (3 individuals). The highest count (42 individuals) was recorded in the deeper region (Site 3) throughout the study period, whereas the lowest count (3 individuals) recorded in the outlet area (Site 1) as shown in (Figure 3).

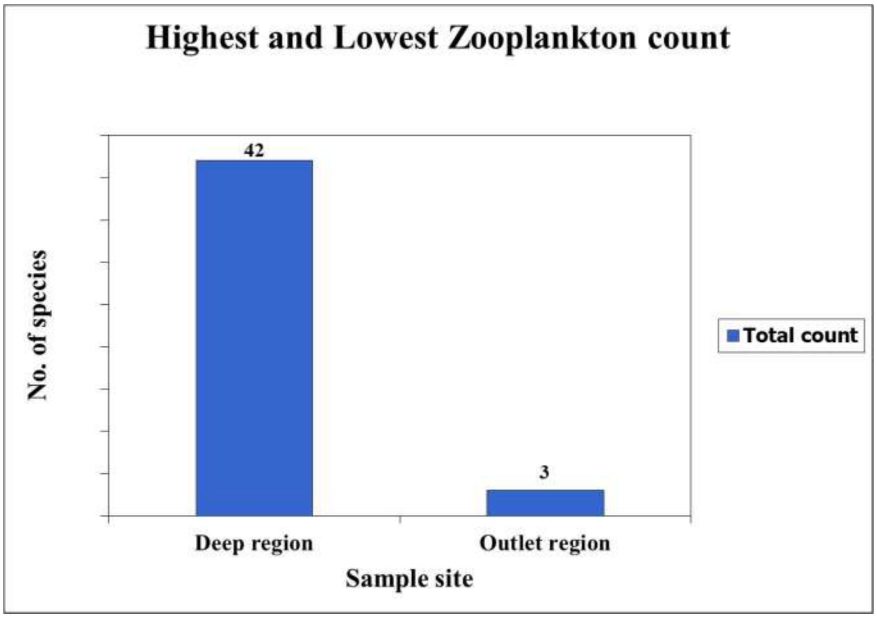

Figure 3: Sampling site with highest and lowest zooplankton count

The most dominating group Rotifera (199 individuals), consisted of *Trichocerca cylindrica, Branchionus havanaensis, Polyarthra trigala, Keratella cohleans, Keratella valga, Keratella tecta,* and *Branchionus angularis* followed by Copepoda (139 individuals), comprising of *Cyclopoid nauplius, Nauplius, Microcyclops rubellus, Mesocyclops leukarti, Leptodiaptomus tyrrelli* and *Cyclops vicinus* and by Cladocera (86 individuals) comprising of *Bosmina longirostris* and *Eubosmina tubicen*. In previous study, Copepoda were the most abundant species in Rupa Lake, followed by Rotifera (Rai 2000). However, Rotifera was most dominant followed by Copepoda including species like: *Keratella cohlearis*, *Polyartha trigala*, and *Nauplius* (Husen et al. 2011; Gautam et al. 2016). In this study, Rotifera were dominant over Copepoda and Cladocera including species like: *Bosmina longirostris*, *Keratella valga*, *Cyclopoid nauplius*, and *Branchionus havanaensis* that were present abundantly. Genus *Branchionus* is linked to eutrophic water, while *Trichocerca* is almost exclusively found in oligotrophic water (Sládeček 1983). So, establishing a *Branchionus*: *Trichocerca* quotient $Q_{B/T}$.

$Q_{B/T}$ = Number of species of *Branchionus* / Number of species of *Trichocerca*
(Oligotrophic if $Q_{B/T} < 1$, Mesotrophic if $\leq Q_{B/T} < 2$, Eutrophic if $Q_{B/T} \geq 2$)

The number of species of *Branchionus* species is 2 (i.e. *Branchionus havanaensis* and *Branchionus angularis*). Similarly, the number of species of *Trichocerca* is 1 (i.e. *Trichocerca cylindrica*). Therefore, the $Q_{B/T}$ quotient is (2/1) which gives 2. So, based on this quotient, the trophic status of Rupa Lake may rank as eutrophic water body. The differences in number of zooplankton species reported by different authors may be due to seasonal differences in sampling and locations.

Biomass of plankton

The mean biomass of plankton in the lake was found to be 0.18 ± 0.27 mg/L (Figure 4). The highest biomass was found to be 1.0 mg/L in sampling point (P25) at settlement area and lowest was found to be 0.02 mg/L in sampling points (P6, P8 P10, P24 and P23) at shallow, deep, settlement and inlet region. From the result it can be inferred that the change in biomass may be associated with several factors, such as predation, food availability, environmental stressors, and the presence of submerged macrophytes etc. (Dembowska et al. 2018; Gurung et al. 2019; Takamura 2003). During the field visit, there was absence of submerged macrophytes in the sampling point (P25) at settlement area and (P18) at outlet area whereas, submerged macrophytes were observed in the sampling point (P6) at shallow region and (P23) at inlet area. According to Takamura (2003) and Gurung et al. (2019), plankton biomass decreases may show poor value if lakes are dominantly covered by submerged macrophytes in lakes. This is because submerged macrophytes provide refugee for zooplankton, which increases their survival and grazing pressure on phytoplankton, ultimately leading to a reduction in biomass. The other reasons explained is that in shallow lakes the rooted vegetation can outcompete phytoplankton in nutrients uptake, as rooted vegetation has the advantages to uptake nutrients directly from sediments (Gurung 2007).

Plankton species mirrored in gut of silver carp

In this study, the major phytoplankton species in the gut of the silver carp were *Pediastrum duplex*, *Ankistrodesmus falcatus*, *Staurastrum sp.* of Chlorophyceae; *Melosira granulate* of Bacillariophyceae and *Merismopedia tennuissima* of Cyanophyceae. Among these species *Pediastrum duplex*, *Ankistrodesmus falcatus*, and *Merismopedia tennuissima* was observed in almost all the gut samples. The dominant phytoplankton group observed in the gut of silver carp was in the order, Chlorophyceae > Cyanophyceae > Bacillariophyceae.

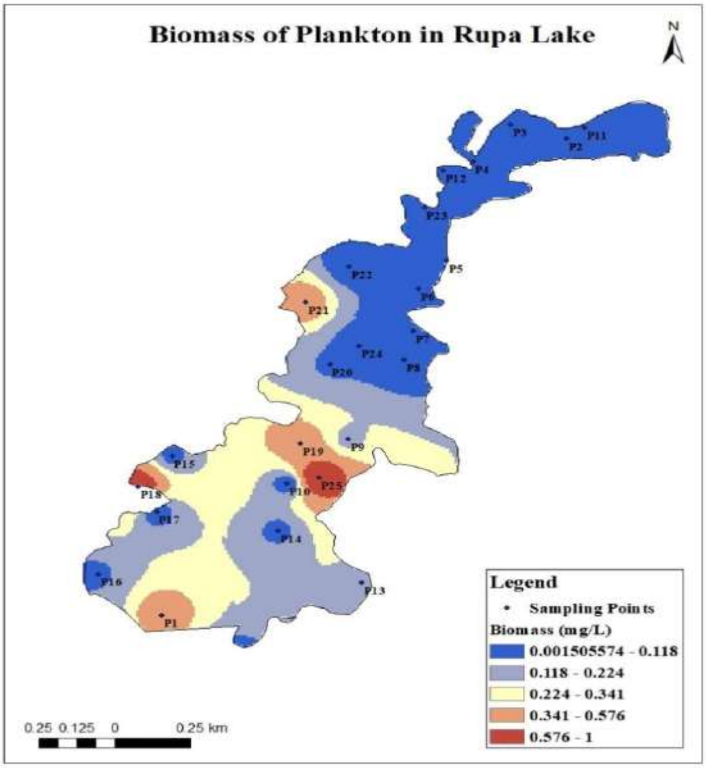

Figure 4: Biomass of plankton in Rupa Lake

The major zooplankton species in the gut contents of the silver carp were *Bosmina longirostris*, *Eubosmina tubicen*, of Cladocera and *Cyclopoida spp.* (*Microcyclops rubellus* and *Cyclops vicinus*) of Copepoda. Among these species *Bosmina longirostris* was found to be dominant throughout the study followed by *Cyclopoida spp.* and *Eubosmina tubicen.* The dominant zooplankton group in the gut of silver carp was in the order, Cladocera > Copepoda > Rotifera.

Pediastrum duplex of (Chlorophyceae) which was dominant in gut was recorded with (82 cells) in the lake whereas; *Staurastrum spp.* of (Chlorophyceae) which was least in gut was recorded with (262 cells) in the lake. So, it may be assumed that the quantity of food available for silver carp in the lake as limited. Also, many of the *Pediastrum duplex* was found undigested in the gut i.e. the species were identified distinctly. We can also say that the phytoplankton fed by silver carp may be compromised in quality due to their poor digestibility. The cause of *Staurastrum sp.* for being least in the gut may be due to better digestibility or least preference by the silver carp because *Staurastrum spp*. was dominant in the lake.

Bosmina longirostris (Cladocera) which was dominant in the gut was recorded with (73 individuals) in the lake whereas; *Cyclopoida spp*. (*Microcyclops rubellus* and *Cyclops vicinus*) of (Copepoda) which was least in the gut was recorded with (30 individuals) in the lake. *Eubosmina tubicen* of (Cladocera) was also consumed dominantly by silver carp, which was recorded with (13 individuals) in the lake. So, the least species of *Eubosmina tubicen* in the lake can be inferred to its high consumption by silver carp.

CONCLUSION

The dominant phytoplankton species were *Staurastrum spp.* of (Chlorophyceae) and zooplankton species was *Bosmina longirostris* of (Cladocera) in the lake. Among phytoplankton species, *Pediastrum duplex* of (Chlorophyceae) was considered dominant and preferred food of silver carp. Among zooplankton species, *Bosmina longirostris* of (Cladocera) was considered dominant and preferred food of silver carp. During the study, similar groups of plankton species were observed in lake as well as in gut of silver carp. Therefore, the gut contents of silver carp (*Hypophthalmichthys molitrix*) corresponded with the plankton in the Lake Rupa. The feeding preference of silver carp corresponded with the plankton diversity in the lake. So, it implies that feeding preference of silver carp directly as well as indirectly impact over the water quality of the lake. As silver carp are harvested every day by RLRFC, the removal helps to remove N, P and organic carbon in the form of phytoplankton and zooplankton from the lake. That means the removal of phytoplankton feeder fish controlling the lake to be overloaded with inorganic nutrients such as N, P and organic carbon in the form of planktons from the lake.

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