

**Evaluation of Grazer Mediated Algal Bloom Control  
Method: a Bucket Experiment Using *Daphnia Cf. similis*  
from Teghane and Mai Gassa II Reservoirs of Tigray, Ethiopia**

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**Abstract:** Freshwater ecosystems are often viewed as highly productive biological systems. But currently these water bodies are affected by phytoplankton blooms as a result of eutrophication. *Daphnia* species is an important component of many zooplankton communities and takes a central position in food webs. The objective of this study was to provide experimental evidence for the impact of *Daphnia* grazing on phytoplankton composition. Phytoplankton species collected from two freshwater reservoirs, namely, Teghane and Mai Gassa II of Ethiopia were treated in presence and absence of *Daphnia Cf. similis* respectively. The experiment contained six treatments with three replicates. Results were statistically analyzed using Stata version 11 software. The results showed significant negative association between *Daphnia Cf. similis* and phytoplankton biomass across treatment ( $F=270.7$ ;  $P=0.0001$ ). Mortality of *Daphnia Cf. similis* was found high in Mai Gassa II while they reproduced and attained high density in Teghane where phytoplankton biomass was low. Our results confirm that *Daphnia Cf. similis* can suppress growth of phytoplankton, if the abundance of phytoplankton is low, but they cannot control an already existing bloom.

**Key words:** *Daphnia Cf. similis* • Algal Bloom Control • *Cyanobacteria* • Reservoir

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## INTRODUCTION

Freshwaters as natural resources provide important ecosystem services such as the delivery of water for consumption, fishing, recreation, irrigation and wide range of other domestic and agricultural purposes to human population. Therefore, adequate management of water resources is of utmost significance to the growing world population [1].

Currently, most of the fresh water bodies found in Tigray, Ethiopia are dominated by phytoplankton bloom forming species [2]. Cyanobacteria or blue green algae are among the oldest and ubiquitous phytoplankton and *Microcystis*, *Anabaena* and *Aphanizomenon* increasingly dominate such phytoplankton assemblages. They are common members of marine, brackish and freshwaters. Factors such as selective zooplankton grazing, nutrient

concentrations (P and N), temperature, light, pH, hydrologic and meteorological conditions have all been implicated as factors contributing to Cyanobacteria bloom [3]. Phytoplankton blooms have a marvelous impact on the socio-economic and ecological value of fresh-water body, due to the changes they provoke in the biotic and a biotic environment. These blooms also have a great effect on the food web structure of fresh-water ecosystem, because they are often inedible and even toxic [4]. Understanding of the different factors that control the survival, growth and reproduction of cyanobacteria would improve our ability to control nuisance and toxic algal blooms, maintain the esthetics of surface water bodies, protect drinking water supplies and improve fisheries production. Thus, it is preferable to prevent these blooms in the first place rather than to treat their consequences.

Grazer-mediated bloom control by herbivores like *Daphnia* can be considered among the different ecological processes involved in decreasing the abundance of phytoplankton bloom [5]. However, whether *Daphnia* can control grazing-resistant phytoplankton like cyanobacteria or not continue to be a controversial question. There is considerable evidence that *Daphnia* may promote the dominance of grazing-resistant cyanobacteria, but there are also several well-documented examples in which *Daphnia* have greatly reduced the abundance of Cyanobacteria [6]. Therefore, this study supplements information on the controversial issue as well as evaluate the bloom control ability of *Daphnia Cf. similis* on phytoplankton. It might contribute to a better understanding of biomanipulation. Thus, the objective of this research is to evaluate the grazing impact of *D.cf.similis* on phytoplankton bloom using a bucket experiment.

## MATERIALS AND METHODS

**Study site and Experimental setup:** The samples were collected from two reservoirs of contrasting trophic state viz. Teghane and Mai Gassa II found in Northern Tigray, Ethiopia. Teghane is renowned for its water transparency and considered quite turbid (Secchi disc transparency, 20 cm; chl a, 340.77 µg l<sup>-1</sup>). Mai Gassa II, is the second reservoir which has macrophytes around the shore and considered as clear and less turbid (Secchi disc transparency, 35.5 cm; chl a., 34.17 µg l<sup>-1</sup>). Tadesse *et al.* [2] identified several cladoceran species in these reservoirs and the genus *Daphnia* is reported as the most abundant. Besides among the local phytoplankton community cyanobacteria, chlorophytes and cryptophytes were reported as dominating.

A bucket experiment was carried out in Aquatic and fishery laboratory of Biology department from September to December 2013. The enclosure consists of 18 synthetic polyethylene plastic cone shaped buckets (Top diameter: 35 cm, bottom diameter: 30 cm, depth: 60cm, volume: 60 L). Before filling, the 18 buckets were randomly assigned to six treatments: 1) Phytoplankton from Mai Gassa II with *Daphnia Cf. similis* Mai Gassa II (PM-DM), 2) Phytoplankton from Mai Gassa II with *Daphnia Cf. similis* Teghane (PM-DT), 3) Phytoplankton from Mai Gassa II without *Daphnia Cf. similis* (PM-ND), 4) Phytoplankton from Teghane with *Daphnia Cf. similis* Teghane (PT-DT), 5) Phytoplankton from Teghane with *Daphnia Cf. similis* Mai Gassa II (PT-DM) and 6) Phytoplankton from Teghane without *Daphnia Cf. similis* (PT-ND).

All treatment and control units (without *D. cf. similis*) are replicated three times. Phytoplankton was collected from the above two dams using 30 µm mesh size phytoplankton net whereas. *Daphnia Cf. similis* was collected with a zooplankton net (64µm) and introduced into the *Daphnia* treatments equally across all experimental units at the starting of the experiment. To a measured quantity of phytoplankton, twenty individuals of *Daphnia Cf. similis* were added to all treatment units.

**Analysis of Samples:** Turbidity and chlorophyll *a* were measured using flurometer. From each bucket *Daphnia Cf. similis* was directly counted using a counting chamber after filtering 10 L of water sample over a 64 µm net. All samples were taken once per month and the experiment lasted for four months. Water samples for phytoplankton were collected with a tube sampler and preserved with acid Lugol's solution, then phytoplankton species were counted using Olympus inverted microscope. Bengal Rose B dye solution was added to facilitate distinction between detritus particles and phytoplankton cells. Colonies were considered as a single 'unit' during the counts and counting was continued until at least 300 units were counted [7]. Biovolume calculations were made by measuring the linear dimensions of 50 units of each taxon in a sample and fitting the different taxa to geometric forms. Phytoplankton biomass was estimated from cell biovolume measurements using previously published biovolume [8].

$$HH = \sum_{i=1}^n (HN_i \times SH_i)$$

HH = Total biovolume of phytoplankton (Cells/l)

HN<sub>i</sub> = the number of organisms belongs to i. species/l

SH<sub>i</sub> = Average cell volume of i. species.

**Statistical Analysis:** Results from the experiment were statistically analyzed with multivariate statistical analysis using Stata version11 software. Multivariate analysis provides tests of overall treatment. Multivariate regression was used to investigate the relation between phytoplankton taxon composition and experimental treatment. The effect of *Daphnia Cf. similis* on phytoplankton was assessed by comparing the phytoplankton biomass between control and treatment. Log transformed variables were used in the analysis to homogenize treatment variances and to linearize relationships. The statistical analysis was performed at 0.05 significant level.

**RESULT**

**Phytoplankton Taxa Composition:** At the starting of the experiment, phytoplankton community composition was different in the two-source waters. The communities were dominated by cyanobacteria (Derived mainly from Mai Gassa II) and chlorophytes and cryptophytes (Mainly derived from Teghane); some dinoflagellates were encountered in both Teghane and Mai Gassa II water sources. At the end of the experiment, cyanobacteria were the dominant taxa in Mai Gassa II whereas dinoflagellates biomass showed increase in both Mai Gassa II and Teghane treatments. In contrast, the biomass of chlorophytes and cryptophytes showed decrease in all Mai Gassa II treatment whereas in Teghane showed decrease only in the *Daphnia* treatment, but in the control showed increase at the end of the experiment.

*Daphnia Cf. similis* had effect on growth of phytoplankton compared to that of the control without *Daphnia Cf. similis*. Multivariate regression revealed significant treatment-time interaction for phytoplankton (F=270.7; P=0.0001). The effects of *Daphnia Cf. similis* on phytoplankton were assessed by comparing the phytoplankton biomass with control and treatment. In Mai

Gassa II treatment *Daphnia Cf. similis* did not survive up to the end of the experiment, but in Teghane treatments, they managed to live and increase in density up to the end of the experiment.

**Controlling ability of *Daphnia Cf. similis* on Phytoplankton:** In Teghane water treatment where the initial phytoplankton population was low, the number of *Daphnia Cf. similis* was high and they grew and reproduced well. This provides clue for the ability of *Daphnia Cf. similis* to control and graze the phytoplankton population. In our experiment, most phytoplankton groups tended to show lower biomass in the presence of *Daphnia* than in the control (Absence of *Daphnia Cf. similis*). Effect of *Daphnia Cf. similis* on phytoplankton biomass was observed and phytoplankton biomass tended to decrease during the course of the experiment in Teghane treatment (Figure 1). In contrast, we observed a rapid increase of *Daphnia Cf. similis* biomass across the experiment in Teghane treatment (Figure 2).

Multivariate analysis showed that the presence/absence of *Daphnia Cf. similis* results in a stronger differentiation of phytoplankton community

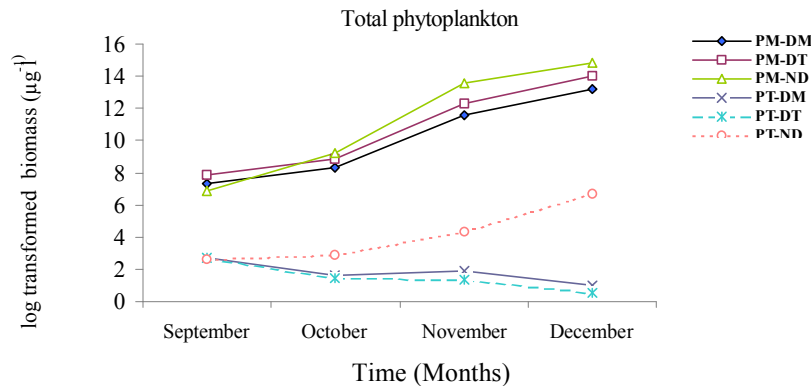


Fig. 1: Phytoplankton biomass in the presence and absence of *Daphnia Cf. similis* across the treatments

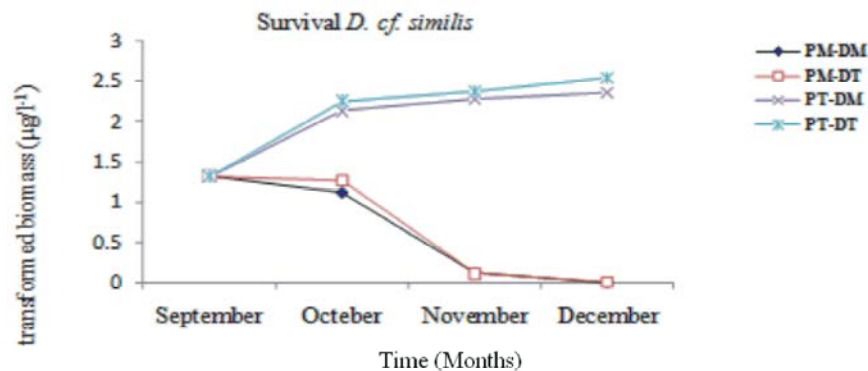


Fig. 2: Survival of *Daphnia Cf. similis* in Mai Gassa II and Teghane during the experiment

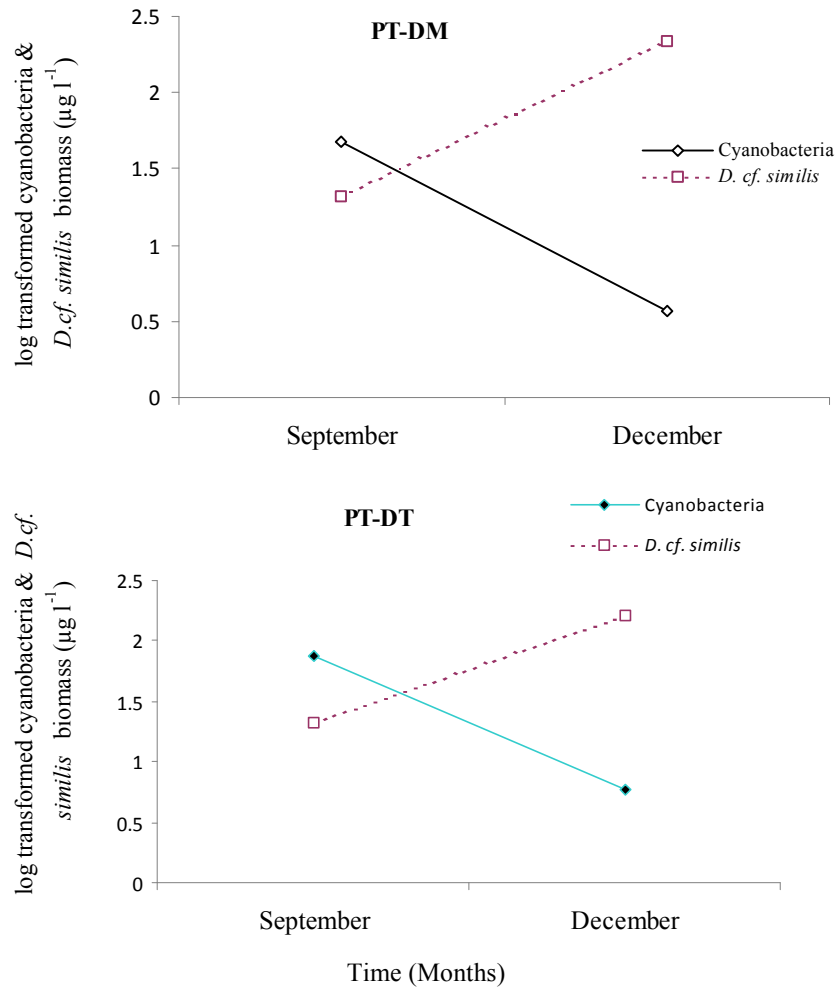


Fig. 3: Interactions between *Daphnia Cf. similis* and cyanobacteria in Teghane water treatments

structure than whether medium of Mai Gassa II or Teghane was used. *Daphnia Cf. similis* tends to suppress the abundance of phytoplankton in our experimental units, but the impact differs strongly depending on the taxon considered. Of the phytoplankton, significant top-down impact of *Daphnia Cf. similis* was observed for chlorophytes ( $F= 119.1$ ;  $P= 0.0004$ ) and cryptophytes ( $F= 43.7$ ;  $P= 0.003$ ).

At the end of the experiment, phytoplankton biomass significantly decreased when compared to the first month of the experiment. The effects of *Daphnia Cf. similis* on phytoplankton were assessed by comparing the phytoplankton biomass of control and treatment. Significant negative effect of *Daphnia Cf. similis* on total phytoplankton biomass was observed in the treatments across the experiment ( $F= 33.7$ ;  $P= 0.004$ ). We can observe the relationship between *Daphnia Cf. similis* and *Cyanobacteria* biomass in Teghane water treatments at the initial and end of the experiment (Figure 3).

**Effects of Phytoplankton on *Daphnia Cf. similis*:**  
 Over all, the phytoplankton biomass tended to increase during the course of the experiment in most of the treatments except in PT-DT and PT-DM treatments, which showed a decrease in phytoplankton biomass during the experiment (Figure 1). In contrast, we observed a major decline in *Daphnia* biomass in Mai Gassa II treatments. The biomass of *Daphnia Cf. similis* rapidly declined and became similar across all treatments from November onwards (Figure 2).

*Cyanobacteria* were the dominant taxa in Mai Gassa II treatment which contained the highest phytoplankton biomass at the end of the experiment (Table 1). In this treatment the growth and reproduction of *Daphnia Cf. similis* was affected by cyanobacteria abundance. As phytoplankton biomass increased, survival of *Daphnia Cf. similis* was decreased and we observed a massive decline of *Daphnia Cf. similis* during the experiment, including a complete collapse of *Daphnia*.

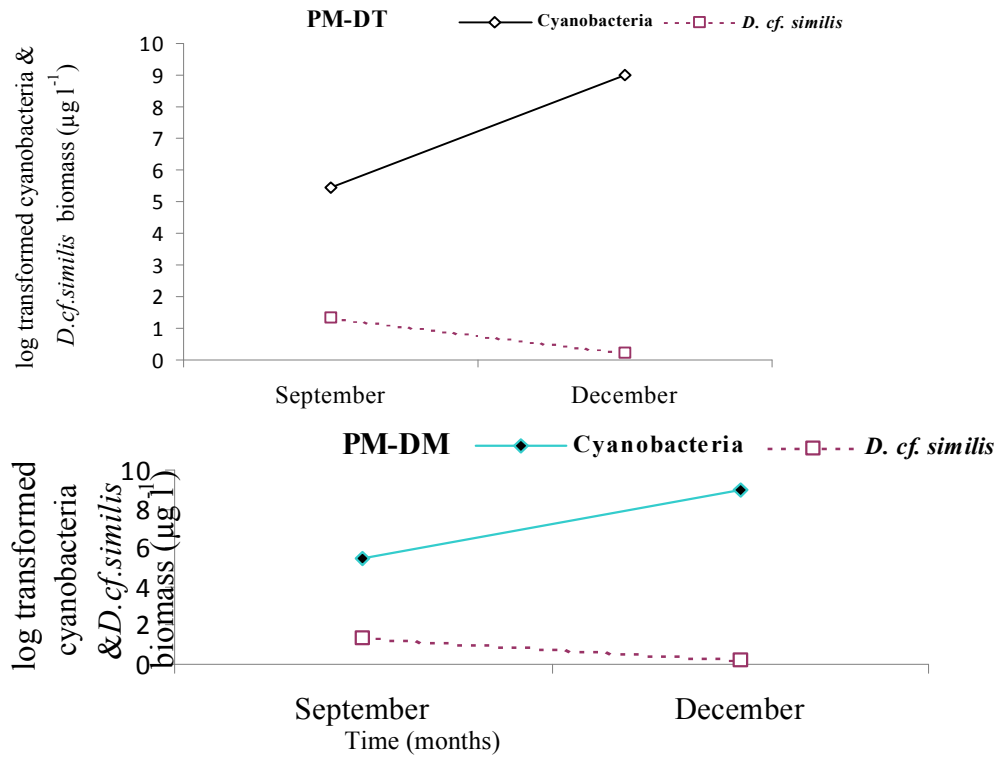


Fig. 4: Interactions between *Daphnia Cf. similis* and *Cyanobacteria* in Mai Gassa II treatments

Table 1: Log transformed biomass ( $\mu\text{g l}^{-1}$ ) of phytoplankton species at the start and end of the experiment

Time	Taxa	PM-DM	PM-DT	PM-ND	PT-DM	PT-DT	PT-ND
Initial (September,2013)	Cyanobacteria	5.45	5.47	5.41	1.67	1.87	1.79
	Chlorophytes	2.82	2.97	2.87	5.97	5.77	5.99
	Cryptophytes	2.68	2.79	2.97	4.59	4.68	4.88
	Dinoflagellates	4.13	4.13	4.13	1.67	1.38	1.38
End (December,2013)	Cyanobacteria	8.98	8.99	8.83	0.56	0.77	2.45
	Chlorophytes	0.52	0.49	0.48	2.23	2.32	7.88
	Cryptophytes	0.47	0.59	0.64	3.45	3.58	7.54
	Dinoflagellates	9.35	7.56	8.34	5.35	3.57	4.68

Table 2: Results of Multivariate regression testing for the association *Daphnia Cf. similis*, water source and their interaction on log-transformed biomasses of different phytoplankton taxa in the experiment

Taxa	Sources	F	P
Cyanobacteria	D	33.7	0.004
	W	11.42	0.005
	D*W	7.89	0.003
Chlorophytes	D	119.1	0.004
	W	73.07	0.002
	D*W	6.25	0.041
Cryptophytes	D	43.7	0.003
	W	48.74	0.004
	D*W	6.73	0.034
Dinoflagellates	D	3.05	0.155
	W	6.38	0.03
	D*W	8.76	0.035
Phytoplankton biomass	D	270.7	0.001
	W	15.91	0.001
	D*W	11.4	0.001

Note: D (*Daphnia Cf. similis*), W (Water sources) and D\*W (Interaction between both treatments)

We can observe the effect of *Cyanobacteria* on *Daphnia Cf. similis* at higher *Cyanobacteria* population in Mai Gassa II treatment (Figure 4).

Multivariate regression revealed significant effect of source water was observed for cyanobacteria, chlorophytes, cryptophytes and dinoflagellates (Table 2), but not for euglenoids ( $P = 0.09$ ).

## DISCUSSION

Our results indicated that the presence of *Daphnia Cf. similis* has a very important impact on phytoplankton composition. The presence/absence of *Daphnia Cf. similis* results in a stronger differentiation of phytoplankton community structure. Similarly the growth of phytoplankton was not the same as that of control which contained no *Daphnia Cf. similis*. This indicates that *Daphnia Cf. similis* tended to suppress the abundance of phytoplankton in our experimental units, but the impact differs strongly depending on the taxon considered. This is in agreement with the finding of Muylaert *et al.* [9] that provides considerable evidence that *Daphnia* can have large negative effects on phytoplankton abundance despite the relative grazing-resistant species.

The top-down impact of *Daphnia Cf. similis* was significant for chlorophytes, cryptophytes and cyanobacteria but not for Dinoflagellates. It is well known that phytoplankton species varied strongly in their resistance to *Daphnia* grazing [10]. The top-down impact of *Daphnia Cf. similis* on phytoplankton biomass was strong in Teghane treatments in which chlorophytes and cryptophytes were the dominant taxa at the starting of the experiment. As expected from previous studies cryptophytes and chlorophytes were the most vulnerable taxa [11] and our results are generally in line with expectations.

*Daphnia Cf. similis* were found to be grown and reproduced, this provides clue for the ability to control phytoplankton community in Teghane treatment. Chlorophytes were strongly suppressed by *Daphnia Cf. similis* at the end of the experiment. Bednarska *et al.* [12] reported that algae species varied in fatty acid composition, both in terms of quantity and quality and algal food quality. Therefore, this depends on the algal species included in the *Daphnia* diet. In many laboratory studies *Daphnids* are fed with green algae (*Scenedesmus*, *Chlamydomonas*, *Chlorella*), which in most cases seem to be adequate food [13]. In addition, higher growth rate, reproduction and survival of *Daphnia* with *Scenedesmus*

as food were reported by Lurling [14]. Similarly, Kasprzak and Lathrop [6] found that green algae *Chlorella* and *Scenedesmus obliquus* are enriched with vitamin B group and can increase *Daphnia magna* fecundity and population growth rate. Maximum cryptophytes biomass was observed in the control than in the presence of *Daphnia Cf. similis*. This finding is in agreement with the work of Ruckert and Giani [15] that cryptophytes are small edible species and have been shown to be highly susceptible to grazing filter feeder zooplankton. This is because cryptomonas are generally regarded as a food of high nutritional quality for zooplankton that contains high levels of fatty acids [16].

Large dinoflagellates like *Peridinium* species are known not to be grazed efficiently by filter feeding zooplankton [14]. It may be true in case of this study because less top-down impact of *Daphnia Cf. similis* on dinoflagellate was observed. In the treatments where dinoflagellates were increased, other phytoplankton taxa such as chlorophytes and cryptophytes showed decrease in biomass in our experiment. The work of Wu *et al.* [17] suggests that different phytoplankton taxa have different resistance to grazing by zooplankton.

Additionally, in the treatments where cyanobacteria were dominated (Mai Gassa II), other phytoplankton showed decrease in biomass in our experiment. This finding is in agreement with the work of Lehman *et al.* [18] that toxic *Cyanobacteria* can affect phytoplankton community composition. Similarly, Paerl *et al.* [3] reported that *Cyanobacteria* species like *Microcystis* contain gas vesicles that allow them to float on the surface of the water column where they can decrease light availability below the surface. This may partly explain the decreased density of other phytoplankton taxa that enables them to adjust their position in relation to light in the water column.

The controlling effect of *Daphnia* on cyanobacteria is controversial [19]. However, the result of this study showed two important conditions: 1) *Daphnia Cf. similis* was able to grow and reproduce at lower cyanobacteria biomass in Teghane treatments and 2) *Daphnia Cf. similis* showed complete collapse at higher cyanobacteria in Mai Gassa II treatments. This condition was also reported by Sarnelle [20] who conducted enclosure experiment using *Daphnia pulicaria* and *Microcystis* at different initial conditions. Probably the reason is as the toxic cyanobacteria abundance increase production of secondary metabolites, such as polyunsaturated fatty acid or protease inhibitors that might result in the death of *Daphnia* [21].

In Teghane treatments, where the initial *Cyanobacteria* biomass was low, *Daphnia Cf. similis* were found grown and reproduced. This provides clue for the ability to control and graze the cyanobacteria population. This can be justified by the fact that cyanobacteria are among the complementary food items of *Daphnia* [22]. There are also other supporting evidences that indicate ingestion of cyanobacteria by *Daphnia* species [23]. This study indicates that *Daphnia Cf. similis* were able to feed on *Cyanobacteria* and thereby bring out a decrease in them especially at low cyanobacteria biomass. This is in agreement with the finding of Paterson *et al.* [24] that provides considerable evidence that *Daphnia* can have large negative effects on cyanobacterial abundance despite the relative grazing-resistance.

The result of this study showed how cyanobacteria abundance affects the ability of *Daphnia* to suppress cyanobacteria. This is in agreement with the majority of studies that have reported the effect of *Cyanobacteria* on *Daphnia* [14]. From this study, higher mortality of *Daphnia Cf. similis* was recorded in the higher cyanobacteria biomass (Mai Gassa II) treatments. We observed a massive decline of *Daphnia Cf. similis* across the experiment, including a complete collapse of the *Daphnia Cf. similis*. This likely reflects cyanobacteria affecting *Daphnia Cf. similis* rather than the reverse; this is in agreement with Arnold [25] that reported higher mortality of *Daphnia* when cyanobacteria are the dominant taxa or when they are the only food source.

The reason behind the *Daphnia* population decline in the cyanobacteria dominated treatment may be related to several reasons. Lampert [26] reported that large colonies or filaments cyanobacteria may mechanically interfere with grazers by blocking their filter apparatus. In addition, the ingested small colonies or parts of filaments may be poorly digested and assimilated or may lack essential nutrients required for *Daphnia* [27] and several strains of cyanobacteria like *Microcystis* have been reported to be toxic to *Daphnia* [28].

In addition to the toxic effect of cyanobacteria, colony/filament size also affects the survival probability of *D. cf. similis*. Even though the size of *Daphnia* is enough to feed on colony [23] at higher cyanobacteria (In Mai Gassa II) treatment there were attachment of many colonies/filaments into one size and the movement of *Daphnia Cf. similis* was highly affected. Most of *Daphnia Cf. similis* remains in the bottom side of the bucket and the long chain of cyanobacteria did not even allow them to move. Due to this, they were not active in

their movement and spend most of their time at the base of the bucket. This effect of cyanobacteria was also observed in the work of Debenardi and Guissani [27] who found the morphological effect of cyanobacteria on *Daphnia*. Cyanobacteria abundance had great effect on the collection of available food of *Daphnia* [16]. As the abundance of *Cyanobacteria* increases their mechanical interference with the collection of available food sources also increases. Therefore, at higher phytoplankton biomass, *Daphnia* may not be able to filter the available food and this may lead to starvation and death.

Our finding indicated that *Daphnia Cf. similis* can control the growth phytoplankton if the abundance is low, but they cannot control an already existing bloom. Depending on the phytoplankton abundance, *Daphnia Cf. similis* showed both abilities to suppress (Teghane) or not able to suppress phytoplankton biomass (Mai Gassa II) treatments. This indicates better control of blooming algae by *Daphnia* in less eutrophic water bodies.

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