Feeding response of *Daphnia* cf. *similis* to different concentration gradients of *Microcystis* and its implication for preventing algal blooming

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ABSTRACT

Daphnia are important components of zooplankton communities in lakes, ponds and reservoirs. Currently freshwater ecosystems are affected worldwide by Cyanobacterial blooms through the process of eutrophication. The objective of this study was to provide experimental evidence to the response of *Daphnia* to various concentrations of *Microcystis*. The experiment contained four treatments and two controls each with three replicates. The first control contained *D.* cf. *similis* without *Microcystis* and the second control contained *Microcystis* without *D.cf. similis*. The remaining four treatments contained both *D.* cf. *similis* and *Microcystis* at different concentration of *Microcystis*.. The results showed a significant negative relationships between *D.* cf. *Similis* and *Microcystis* across the treatments ($F=294.5$; $p<0.00$). From the four *Microcystis* concentration gradients, we found mortality of *D.* cf. *Similis* in HMC (high *Microcystis* concentration) and 67% of the original HMC gradient while in the other treatments, in 43% and 22% original HMC treatment, *D.* cf. *Similis* reproduce and attain high density. Therefore, from these results it is concluded that *D.* cf. *similis* can control the growth of *Microcystis* if the concentration is low but they cannot reduce an already existing bloom.

Keywords: Concentration gradient, *D.* cf. *Similis*, Feeding-treatment, *Microcystis*, Ethiopia.

1. INTRODUCTION

Understanding, managing and learning about freshwater ecosystems has become increasingly significant throughout the world as the development of land continues to expand and as knowledge of the impact increases. Due to the frequency of algal blooms in the world, particularly as a result of intense, hot summers, there has been an increasing awareness of the associated issues including the creation of anoxic conditions, and risk of intoxication for those exposed to toxic cyanobacteria (Codded et al., 2005). Freshwaters such as lakes, rivers and reservoirs are the most important resources, especially in the tropics, where they are often viewed as highly productive biological systems. In Tigray Regional State, there are more than 70 reservoirs (Tsehaye et al., 2007; Tadesse et al., 2008). They provide water for fishing, irrigation and a variety of other domestic and agricultural purposes. But currently these freshwater reservoirs are affected by Cyanobacterial blooms as a result of eutrophication and global warming (Tsehaye et al., 2007; Tadesse et al., 2008). Cyanobacteria blooms have a tremendous

impact on the socio-economic and ecological values of freshwater bodies by threatening human and ecosystem health (Anderson, 2012). Prevention of this bloom is better than curing since there is a possibility for harmful metabolites to be leached that are potentially dangerous to human health. Grazer-mediated bloom control is among the different methods that are used for controlling blooms, *Daphnia* is an excellent candidate for this task, because it is a natural component of freshwater ecosystems, ubiquitously present and since there are strong grazers.

In the absence of fish predation, the biomass of large species of *Daphnia* increases and the biomass of filamentous cyanobacteria often decreases (Paterson et al., 2002). Some studies have provided evidence for ingestion of cyanobacteria by *Daphnia* and suggested that grazing can provide a control mechanism for cyanobacterial (*Microcystis*) blooms (Boon et al., 1994), or that cyanobacteria can be a complementary resource for zooplankton (*Daphnia*) (Kurmayer, 2001). On the contrary several studies have shown a different scenario where *Daphnia* are not good grazers of cyanobacteria compared to other algal species, highlighting their insufficiency to control *Microcystis* proliferation (Lurlingr, 2003; Ghadouani et al., 2004).

Experiments showing that *Daphnia* can suppress cyanobacteria in eutrophic lakes have been limited to cases where *Daphnia* were able to achieve high densities before cyanobacteria became dominant (Lurlingr, 2003). Thus, it is not clear whether *Daphnia* can overcome the inhibitory effects of high cyanobacterial abundance on their competitive ability and invade a cyanobacteria-dominated assemblage.

An increasing number of studies report tolerance of *Daphnia* clones to toxic *Microcystis* (Boon et al., 1994; Sernelle and Wilson, 2005). Gustafsson et al. (2005) conducted a study which exposed *D. magna* individuals to toxic *Microcystis* and they found that *Daphnia* were able to develop and pass the defense mechanisms to their offspring. From such study it was concluded from the difference in the time taken to reach maturity and smaller numbers of offspring per clutch between individuals previously exposed and those that were not. This means *Daphnia* has the ability to adapt to environmental conditions by passing information through maternal effects (Gustafsson et al., 2005). Similarly, Sarnelle et al. (2010) concluded that *Daphnia* populations with prior experience with toxic cyanobacteria show positive population growth even at high concentrations of cyanobacterial toxins.

However, interactions between bloom forming cyanobacteria and *Daphnia* are controversial issue in literatures (Lurlingr, 2003; Sarnelle et al. 2010). Many studies reported that a decline in

biomass of *Daphnia* as a result of *Microcystis* (DeMott et al, 1999; Paterson et al., 2002; Sarnelle et al., 2010). Study on this controversial issue is important to understand the interaction between *Daphnia* and bloom forming cyanobacteria. Therefore, this study intends to look on the effect of *D.* cf. *similis* grazing potential on *Microcystis* at different concentration gradients of *Microcystis*.

2. METHODS

2.1. Culturing of *D.* cf. *similis*

Daphnia samples were collected from Adi Abagie reservoir, located in Eastern Tigray, using 64μm mesh size in November, 2011. *D.* cf. *similis* were isolated from the sample and were transferred individually into plastic jars using a pipette with a large tip opening. The mothers producing the experimental animals were cultured individually in 250 ml jars of spring water supplemented with *Scenedesmes,* brought from KU Leuven Aquatic Laboratory, Belgium. Every two days interval the water was changed and *Scenedesmes* added as food. First we prepare the new water in a new jar then we transfer the *Daphnia* after the *Scenedesmes* added as food. *D.* cf. *similis* were cultured for two generations before using them in the experiment according to the method recommended by Sarnelle and Wilson (2005). This is crucial since it gets rid of maternal effects of previous exposure and also ensures enough representation of individuals for the experiment.

2.2. *Microcystis* Collection and Dilution

Microcystis were collected from Gereb Mihiz, one of the reservoirs located in southern Tigray. We were lucky to get bloom of *Microcystis*, thus we did not do further process of multiplication for *Microcystis.* Different concentration levels of *Microcystis* were prepared by adding different milliliters (100mL, 50mL, 25mL and 12.5mL) of concentrated sample from the reservoir into 1 liter of spring water from the initial source. The starting sample, which we refer as high *Microcystis* concentration (HMC), was our original sample collected from Gereb Mihiz dam which was further concentrated by repeatedly filtering it with 30μm sieve and the colonies were counted and it was 103,860 colony/l. The first sample was 900ml of HMC, the second sample consisted of 50ml of HMC and 950ml of water, which when we count the colony was found to consist 69,600 colony/l, hence the colony count was 67% of the original sample, thus it is referred as 67% of the original HMC. Dilution for the third sample was done by adding 25ml of HMC and 975ml of water and the fourth sample consisted of 12.5ml of HMC and 987.5ml water.

The colony counts for the third and fourth samples were 44,600 colony of *Microcystis* per liter (43% HMC) and 23,000 colony of *Microcystis* per liter (22% of the original HMC) respectively.

2.3. Experimental Design, Sampling Method and Analysis

The experiment comprised of four treatment groups and two control groups [*D.* cf. *similis* without *Microcystis* and *Microcystis* without *D.* cf. *similis*] each with three replicates. As the experiment is done in 1000ml of sample of phytoplankton, 20 individuals (as the volume of water is only 1 liter greater than this after reproduction will create crowded condition) of *D.* cf. *similis* were added into *Microcystis* containing 1 liter spring water with four different concentrations gradients (HMC, 67% HMC, 43% HMC, and 22% HMC). The purpose was to test whether *D.* cf. *similis* can invade *Microcystis* dominated plankton community or they fail to graze on *Microcystis*.

Jars (modified 2 liter plastic bags) were sampled in three days interval for 16 days. We measured dissolved oxygen, pH, temperature and conductivity *in situ* with a WTW Multi 340 I electrode. Turbidity and concentration of chlorophyll was measured using fluorometer (Turner Aquafluor; average of three measurements). *D.* cf. *similis* were directly counted using a pipette with a large tip opening at 0 and 4 days, but as the number of *D.* cf. *similis* increased with time (8-16 days) for 43% HMC, 22% HMC treatment and control, 100ml sub samples were taken and filtered using 64µm mesh size and the filtrate was preserved in 10% sugar saturated formalin. Then individuals were counted using stereomicroscope according to the method recommended in Fernando (2002). Five ml of *Microcystis* sample was taken with a pipette and preserved by lugol's solution and colonies were counted using inverted microscope according to the method used in John et al. (2002).

Results from the experiment were statistically analyzed with repeated-measures ANOVA and student *t*-test using SPSS version 16. Repeated measures analysis provides tests of overall treatment. Association between *D.* cf. *similis* and *Microcystis* at each treatment was analyzed with paired *t*-test at 0.05 significant levels.

3. RESULTS

The comparisons of the colony counts of *Microcystis* and individual survival counts of *D.* cf. *similis* on the start of the experiment, day zero, and end of the experiment, day 16 across the concentration gradients showed variation among the different concentration gradients (Tables 1).

Table 1. Comparison of the average count of *D. cf. similis* and *Microcystis* at day 0 and at the end of experiment (16 day) across the concentration gradient of *Microcystis*.

Treatment	**Taxa**	**Time**	
		Av. count at day 0	**Av. count at day 16**
HMC	*Microcystis*	103,860 colony/l	96,373colony/l
	D. cf. *similis*	20 individuals/l	0 individuals/l
67%HMC	*Microcystis*	69,600 colony/l	57,461 colony/l
	D. cf. *similis*	20 individuals/l	4 individuals/l
43%HMC	*Microcystis*	44,600 colony/l	22,451 colony/l
	D. cf. *similis*	20 individuals/l	86 individuals/l
22%HMC	*Microcystis*	23,000 colony/l	5,266 colony/l
	D. cf. *similis*	20 individuals/l	155 individuals/l
Control (with no *Microcystis*)*[1]	*Microcystis*	0 colony/l	0 colony/l
	D. cf. *similis*	20 individuals/l	302 individuals/l
Control (with no *D.* cf. *similis*) *[2]	*Microcystis*	104,153 colony/l	138,924 colony/l
	D. cf. *similis*	0 individuals/l	0 individuals/l

Note: *[1] The Control (with no *Microcystis*) was supplemented with *Scenedesmes.*

*[2] The nutrient source for the control (with no *D. cf. similis*) is dam water.

Microcystis concentration gradients had effect on growth, reproduction and survival of *D. cf. similis* compared to that of the control with no *Microcystis* (which contain only *Scenedesmus* as food). Repeated measure of ANOVA revealed significant treatment - time interaction for *D. cf. similis* and *Microcystis* ($F=294.5$; $p<0.000$). Analysis using paired t-test also revealed the negative correlation between *D.* cf. *similis* and *Microcystis* across the treatment along time ($p< 0.05$).

In addition to the comparisons of the colony count of *Microcystis* and survival of *D.* cf. *similis* across the concentration gradients of *Microcystis*, percentage population growth of *D.* cf. *similis* relative to control was compared to assess the extent at which *D.* cf. *similis* potential to control *Microcystis* (Tables 2 and 3). At the highest concentration of *Microcystis*, all *D.* cf. *similis* died while in the other *Microcystis* treatments (43% and 22% HMC) *D. cf. similis* grow and reproduce and able to control the *Microcystis* until the *Microcystis* remain 50.3% in 43% HMC and 2.28% in 22% HMC treatments (Table 3).

The growth and reproduction of *D. cf. similis* showed significant differences between the control (with no *Microcystis*) group and the other treatments. At the end of the experiment maximum density of *D.* cf. *similis* (302 individuals per liter) was recorded in the control with no

Microcystis, but in *Microcystis* treatment *D.* cf. *similis* did not attain this density. At low concentration of *Microcystis* (22% HMC) the density of *D.* cf. *similis* reached high (155 individuals per liter) compared to the 67% HMC (4 individuals) and 43% HMC (86 individuals).

Table 2. Percentage growth of *D. cf. similis* at different gradients of *Microcystis* concentration relative to control (with no *Microcystis*).

	***D.* cf. *similis* growth**	
Treatment	**Relative to control**	**Remark**
HMC	0%	All dead
67% HMC	0%	80% dead
43% HMC	28.48%	Reproduce and grow
22% HMC	51.3%	Reproduce and grow
Control (with no *Microcystis*)	100%	Highly Reproduce and grow

Table 3. Percentage of *Microcystis* left at the end of experiment relative to day zero.

Treatment	**Microcystis left relative to day zero**
HMC	93.1%
67% HMC	82.5%
43% HMC	50.3%
22% HMC	2.28%
Control (with no *D.* cf. *similis*)	100%

Microcystis showed significant differences between the controls (with no *D.* cf. *similis*) and the two treatments (43% HMC and 22% HMC). Density of *Microcystis* remains high in the control with no *D.* cf. *similis* but significantly decreased in 22% and 43% HMC treatment (Table 3).

3.1. Interactions between *Microcystis* and *D.* cf. *similis*

HMC treatment contains the highest concentration of *Microcystis* which is more than 103,860 colonies per liters and 20 individual of *D.* cf. *similis*. However, at the end of the experiment all *D.* cf. *similis* died. In the second treatment, 67% HMC **(**69,600 colonies), 80% of *D.* cf. *similis* died (Table 2).

The response of *Microcystis* with *D.cf. similis* at HMC and 67%HMC treatment is depicted in figure 1.

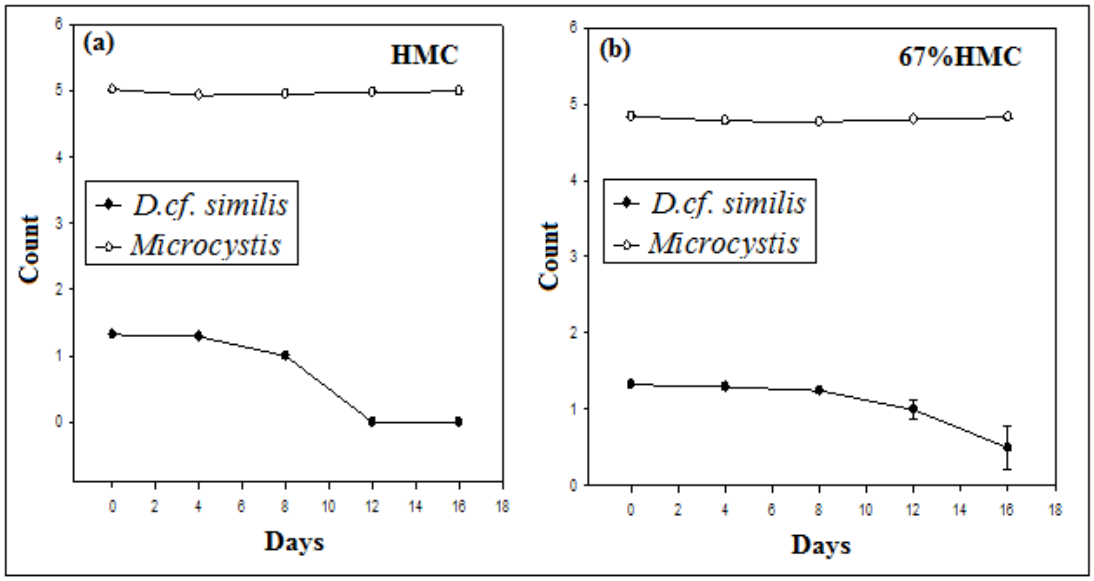

Figure 1. Interactions of *D. cf. similis* and *Mycrocystis* at a) HMC and b) 67% HMC (Log transformed count per liter).

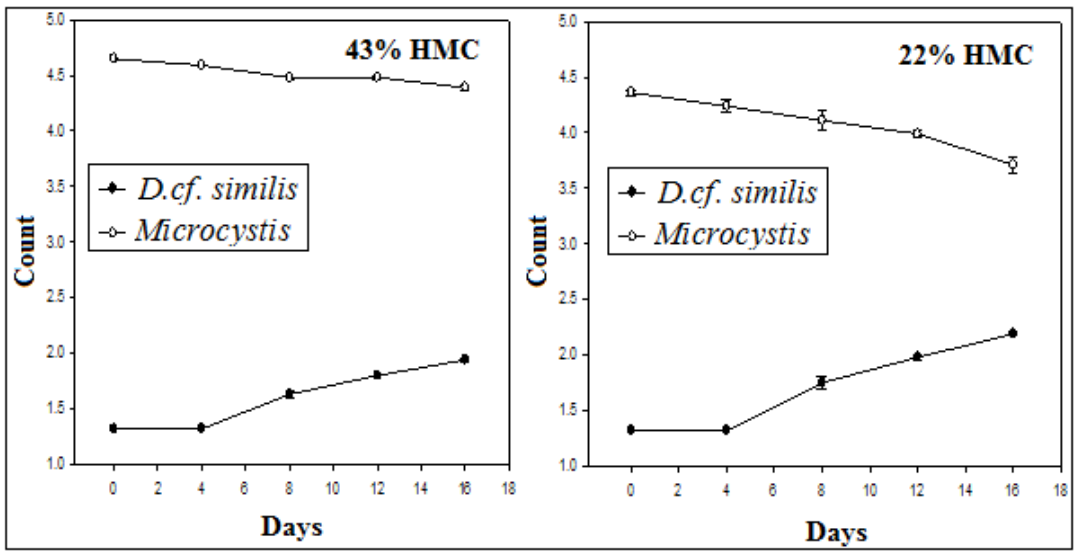

Figure 2. Interactions of *D.* cf. *similis* and *Mycrocystis* at different concentrations gradient of *Mycrocystis* (Log transformed count per liter).

In 43% HMC and 22% HMC treatments, *D.* cf. *similis* grew and reproduced, providing a clue for the ability of *D.* cf. *similis* to control *Microcystis*. Twenty eight percent and 51.3% of *D.* cf. *similis* were able to grow and reproduce in 43% HMC and 22% HMC, respectively. *Microcystis*

colonies significantly decreased compared to the first day of the experiment and the control (with no *D.* cf. *similis).* Only 2.28% of *Microcystis* remained at the end of the experiment in 22% HMC treatment. The response of *D.* cf. *similis* at lower concentration of *Microcystis*, 43% and 22% HMC treatment, is demonstrated in figure 2.

3.2. Population Growth *D.* cf. *similis*

The growth and reproduction of *D.* cf. *similis* was affected by the concentration of *Microcystis*. As the concentration of *Microcystis* increased, survival of *D.* cf. *similis* was decreased. In HMC treatment, *D.* cf. *similis* did not survive up to the end of the experiment, but at lower concentration gradients, 43% HMC and 22% HMC treatment, it managed to survive and increased in density. The *D.* cf. *similis* population showed high increase in the conrol group compared to those with *Microcystis*. Even though not the same as the control, in 43% HMC and 22% HMC treatments the population can grow and reproduce in the presence of *Microcystis* (Fig 3).

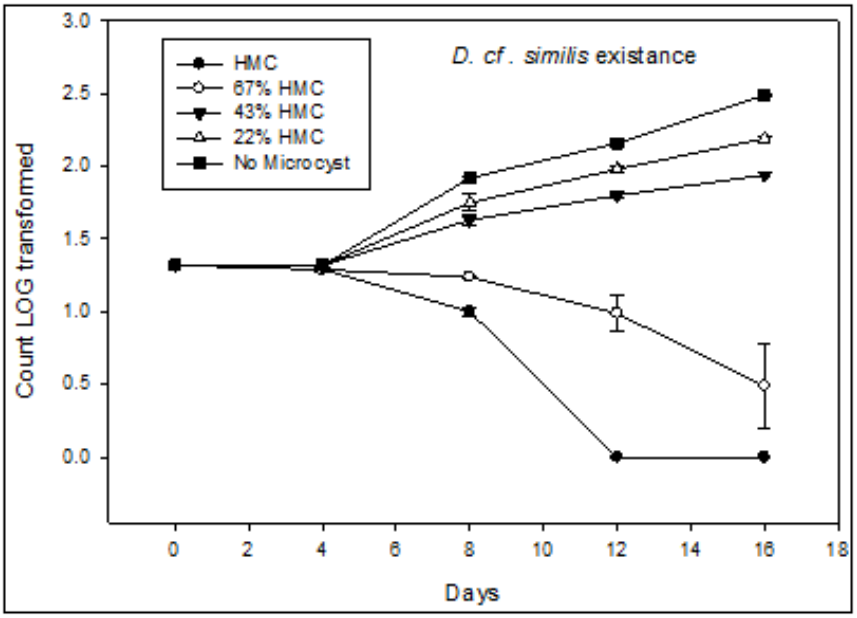

Figure 3. The survival (existence) of *Dcf. Simils* (log transformed individual per liter) along the concentration gradient of *Microcystis.*

4. DISCUSSION

At lower (23,000 colonies per liter) and medium (44,600 colonies per liter) concentration of *Microcystis*, *D.* cf. *similis* were able to graze and control the *Microcystis* population. This can be

justified by the fact that cyanobacteria (*Microcystis*) are among the complementary food items of *Daphnia* (Kurmayer, 2001). There are also other supporting evidences that indicate ingestion of *Microcystis* by *Daphnia* species (Rohlack et al., 2001). This indicates that *D.* cf. *similis* was able to feed on *Microcystis* and result in decrease in the density of *Microcystis* in lower and medium treatments. This is in agreement with the finding of Paterson et al. (2002) who provide considerable evidence that *Daphnia* can have large negative effects on cyanobacterial abundance despite the relative grazing-resistance. The twenty *D.* cf. *similis* that were immersed at lower (22% of the original HMC) and medium (43% HMC) concentration of *Microcystis* managed to survive, grow, reproduce and create negative impact on the *Microcystis* population growth. This finding is in agreement with the work of Chen et al. (2007), who observed a reduction of *Microcystis* population as a result of *Daphnia* stocking.

The controlling effect of *D.* cf. *similis* on *Microcystis* is controversial (Lurlingr, 2003; Sarnelle et al., 2010). However, the result of the present study also clearly showed two important conditions. This condition was also reported by Sarnelle (2007) who conducted enclosure experiment using *Daphnia pulicaria* and *Microcystis* at different initial conditions.

The growth and reproduction of *D.* cf. *similis* in the treatments was not the same as that of the control which had no *Microcystis*. This is due to the fact that *Microcystis* are not good in nutrition because they lack essential fatty acids or lipids, and the poor nutritional value may have effects on *Daphnia* growth and reproduction (Von Elert et al., 2003). Probably this may be the reason for low density of *D.* cf. *similis* in 22% HMC and 43% HMC even though they can survive and reproduce in the presence of *Microcystis*. Significant decrease in *Microcystis* colony in 43% and 22% HMC treatment was primarily due to *D.* cf. *similis*. Tadesse *et al.* (2009) also found negative association between *Daphnia carinata* and cyanobacteria and reported a drop in the relative abundance of cyanobacteria along with an increase in the densities of *D. carinata*.

The result of this study clearly showed how a gradient of *Microcystis* concentration affects the ability of *Daphnia* to suppress *Microcystis.* This is in agreement with the majority of studies that reported the effect of *Microcystis* on *Daphnia* (Lurlingr, 2003; Ghadouni et al., 2004). This is in agreement with Reinikainen et al. (1994) who reported higher mortality of *Daphnia* when *Microcystis* is abundant or when it is the only food source. Besides, the colonial forms of Microcystis cells have digestibility, toxicity and low fatty acid composition (Gliwicz and lampert, 1990; Sarnelle et al., 2010; Muller, 1995). The other possible explanation could also be

due to the increased secondary metabolites produced by *Microcystis*, such as poly unsaturated fatty acid or protease inhibitors might result for the death of *Daphnia* (Watanabe et al., 1988; DeMott et al., 1991). It may be also true in case of this study because high mortality of *D.* cf. *similis* was observed at higher concentration of *Microcystis*. Some studies reported that the ability of *Daphnia* to tolerate or resist the toxic effect of *Microcystis* (Sarnelle and Wilson, 2005). However, to tolerate the toxic effect of *Microcystis*, *Daphnia* need long time exposure to toxic, however, our experiment was performed in short period of time and the HMC treatment contained extremely higher concentrations of *Microcystis* which might be the reason for the death of *D.* cf. *similis* in this treatment.

In addition, the toxic effect of *Microcystis,* colony size also affects the survival probability of *D.* cf. *similis*. Even though the size of *Daphnia* is enough to feed on colony (Rohrlack et al., 2001), at HMC and 67% HMC treatments, there were attachment of many colonies into one large colony and the movement of *D.* cf. *similis* was highly affected. All *D.* cf. *similis* remain in the bottom side of the jar and the long chain of *Microcystis* 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 bottom of the jars. This effect of *Microcystis* was also observed in the work of Debenardi and Guissani (1990), who found the morphological effect of *Microcystis* on *Daphnia*. Concentration gradients of *Microcystis* had great effect on the collection of available food (Gliwicz and Lampert, 1990). As the concentration of *Microcystis* increase their mechanical interference with the collection of available food sources also increase. Therefore, at higher concentration of *Microcystis, Daphnia* may not be able to filter the available food and this may lead to starvation then death followed. In general HMC and 67% HMC treatments produce stressed environment and all this are the probable reason for the death of *D.* cf. *similis* before the experiment ended. Due to all these reasons *D.*cf. *similis* were unable to reproduce and grow. This study indicated that *Microcystis* had lethal effects upon *Daphnia* if the concentration of *Microcystis* was very high. However, although it was not possible to distinguish whether the *Microcystis* was toxic or non-toxic in this study, previous study in Tigray reservoirs has shown that they could be potentially toxic (Tsehaye, 2009).

Generally the result showed a negative association between *D.* cf. *similis* and *Microcystis* across the treatment and found that *Microcystis* concentration gradient had strong effect on the interaction of *D.* cf. *similis* and *Microcystis*. Depending on the concentration gradients of

Microcystis, D. cf. *similis* has shown that it can suppress blooms of *Microcystis* in low concentrations (at 43% and 22% HMC), but not in higher concentration (at HMC and 67% HMC treatments). Up to now there is a great debate in literature on the interaction between *Daphnia* and *Microcystis*. This debate is due to different factors like, environmental conditions, use of different experimental procedure and the use of different species in different researcher. However, the result of this study indicates the possibility that *Daphnia* can suppress *Microcystis* population if the concentration is in between 23,000 – 44,600 colonies per liter and a single *D.* cf. *similis* can reduce 886 colonies within two weeks.

Finally, the necessity of applying *Daphnia* at early phase of algal growth before blooming is recommended to control *Microcystis* bloom. Furthermore study focusing on more sites where more than one species of *Daphnia*, with toxic and non-toxic *Microcystis* is recommended.

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6. REFERENCE

Anderson, D. M. 2012. Harmful Algal Blooms. http://www.whoi.edu/redtide/impacts /freshwater/ cyanobacteria.

Boon, P.I., Bunn, S.E & Green, J.D. 1994. Consumption of cyanobacteria by freshwater zooplankton: implications for the success of top-down control of cyanobacteria blooms in Australia. *Australian Journal of Marine & Freshwater Research,* **45**: 875–887.

Chen, H., Xie, P & Qin, B. 2007. Different competitive outcomes among four species of cladocerans under different alga combinations of colonial *Microcystis* spp. and green alga *Scenedesmus obliquus. Hydrobiology*, **581**: 209-215.

Codd, G.A., Morrison, L.F & Metcalf, J.S. 2005. Cyanobacterial toxins: risk management for health protection. *Toxicology and Applied Pharmacology,* **203**: 264-272.

Debenardi, R & Guissani, G. 1990. Are blue-green algae a suitable food for zooplankton? An overview. *Hydrobiology,* **200**: 29-41.

DeMott, W.R., Zhang, Q.X & Carmichael, W.W. 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia. Limnology and Oceanography*, **36**: 1346-1357.

Fernando, C.H. 2002. A guide to tropical freshwater zooplankton (identification, ecology and impact of fisheries). Back hay publisher, Netherlands, pp 99-122.

Ghadouani, A., Pinel-Alloul, B & Plath, K. 2004. Effects of Microcystis aeruginosa and purified microcystin-LR on the feeding behavior of Daphnia pulicaria. *Limnology and Oceanography*, **49**: 267–280.

Gliwicz, Z.M & Lampert, W. 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. *Ecology*, **71:** 691-702.

Gustafsson, S., Rengefors, K & Hansson, L. 2005. Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. *Ecology*, **86**: 2561.

John, D.M., Whitton, B.A & Brook, A.J. 2002. The fresh water algal flora of the British islas: an identification guide to fresh water and terrestrial algae. Cambridge University Press, United States of America, pp 25-591.

Kurmayer, R. 2001. Competitive ability of Daphnia under dominance of non-toxic filamentous cyanobacteria. *Hydrobiology*, **442**: 279–289.

Lurling, M. 2003. *Daphnia* growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga Scenedesmus obliquus. *Limnology and Oceanography*, **48**: 2214–2220.

Muller Navarra, J.D. 1995. Biochemical versus mineral limitation in *Daphnia. Limnology and Oceanography*, **40**: 1209-1214.

Paterson, M.J., Findlay, D.L., Salki, A.G., Hendzel, L.L & Hesslein, R.H. 2002. The effects of Daphnia on nutrient stoichiometry and filamentous cyanobacteria: A mesocosm experiment in a eutrophic lake. *Fresh Water Biology*, **47**: 1217–1233.

Reinikainen, M., Ketolajvl, H & Walls, M.1994. Effects of the concentrations of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex. Limnology and Oceanography*, **39**: 424-432.

Rohrlack, T., Dittmann, E., Börner, T & Christoffersen, K. 2001. Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp. *Applied and Environmental Microbiology*, **67**: 3523-3529.

Sarnelle, O. 2007. Initial conditions mediate the interaction between *Daphnia* and bloom-forming cyanobacteria. *Limnology Oceanography*, **52**: 2120–2127.

Sarnelle, O & Wilson, A.E. 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. *Limnology and Oceanography*, **50**: 1565.

Sarnelle, O., Gustafsson, S & Hansson, L. 2010. Effects of cyanobacteria on fitness components of the herbivore. *Daphnia Journal of Plankton Research,* **32**: 471-477.

Tadesse, D., Tsehaye, A., De Meester, L., Afework, M., Abrha, G., Risch, S., Pals, A., Vander Gucht, K., Vyverman, W., Neyssen, J., Dechars, J & Declark, S. 2008. Limnological and ecological characteristics of tropical highland reservoirs in Tigray, Northern Ethiopia. *Hydrobiology*, **610**:193-208.

Tadesse, D., Tsehaye, A., Rousseaux, S., Teklit, G., Abrha, G., Mekonnen, T., Neyssen, J., Declark, S., Vander Gucht, K., Vyverman, W., De Meester, L & Dechars, J. 2009. Impact of the fish *Garra* on the ecology of reservoirs and the occurrence of *Microcystis* blooms in semi-arid tropical highlands: an experimental assessment using enclosures. *Freshwater Biology,* **54**: 1605–1615.

Tsehaye, A. 2009. Phytoplankton community structure and cyanobacterial blooms in the semi-arid highlands of Tigray, Ethiopia. PhD Thesis.

Tsehaye, A., Tadesse, D., Declark, S., Neyssen, J., Vander Gucht, K., Risch, S., Rousseaux, S., De wit, J., Afework, M., Nigussie, H., Abrha, G., Poesen, J., Dechars, J., Vyverman, W & De Meester, L. 2007. Ecological Atlas of reservoir in Tigray, Northern Ethiopia. *Tigray Livelihood Papers*, No. 4, VLIR, Mekelle University IUC Program and Zala-Daget Project, pp 27- 32.

Von Elert, E., Martin Creuzburg, D & Le Coz, J.R. 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). *Proceedings of the Royal Society of London Biological Sciences,* **270**:1209-1214.