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The Effects of Sodium Lauryl Sulfate on the Abundance of Producers and Grazers in Aquatic Communities Using Freshwater Microcosms

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By
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Under the mentorship of Dr. Risa A. Cohen

Abstract

With increases in environmental awareness, industry has responded with products that reduce negative impacts on aquatic ecosystems. Sodium lauryl sulfate (SLS), a rapidly degrading chemical commonly found in cleaners labeled as 'environmentally friendly,' has been shown to have low toxicity in single species toxicity tests. However, that organisms have different sensitivities to SLS suggests a need for measuring effects at the community level. We exposed communities of microalgae (*Chlorella* sp.) and invertebrate grazers (a benthic snail, *Elimia* sp. and pelagic microcrustacean *Daphnia magna*) to 0, 0.5 or 1.5 mgL^{-1} SLS. Water quality and invertebrate abundance were measured every 48 hours for three weeks, and *Chlorella* sp. concentration was determined at the conclusion of the experiment. Water quality was influenced by SLS, but remained within acceptable ranges for survival of the organisms. *Chlorella* sp. abundance was unaffected by SLS, but number of *Daphnia magna* decreased in the presence of SLS. Our findings suggest that SLS does not affect water quality or grazer food supply, but may be toxic to *Daphnia magna* at low concentrations. Since *Daphnia magna* is an important food source for fish and other invertebrates, a decline in their populations may have implications for organisms at higher trophic levels.

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Introduction

That commonly used cleaning products have the potential to enter aquatic environments either directly through runoff to surface waters or indirectly through sewage treatment plants (Ying 2006) has led to increased use and production of cleaners that quickly biodegrade into simple, nontoxic compounds (Sandilands 1993). For example, the anionic surfactant sodium lauryl sulfate (SLS) is frequently found in household cleaning products (Singer and Tjeerdema 1993) because it is one of the most rapidly biodegradable surfactants, with 45-95 % degradation within 24 hours (Cserhati et al. 2002). However, biodegradation of surfactants can change water column temperature and dissolved oxygen (DO) since the process is exergonic and aerobic (Albers et al 1995; Jurado et al 2013), conductivity through release of ions (Chaturvedi and Kumar 2010), and pH due to production of alkaline degradation products (Yao 2013). Rapid biodegradation reduces the amount of time aquatic organisms are in contact with surfactants, therefore reducing direct toxicity (Kimerle and Swisher 1977). However, organisms have minimum requirements for water quality in order to survive, and degradation of surfactants may reduce water

quality below those minima. Therefore the presence of a low-toxicity, rapidly

degrading surfactant does not guarantee the safety of aquatic organisms (Benevente

and Cohen 2013).

Sodium lauryl sulfate negatively affects aquatic organisms including

phytoplankton, zooplankton and fish in single species toxicity tests, but the effects

differ depending on the type of organism, demonstrating a need for toxicity testing

at the level of the community (Cairns 1983). For example, Nyberg (1985) found that

SLS inhibited the growth of the freshwater algae *Nitzschia actinastroides* by 41% in

5 mgL^{-1} SLS. Lewis and Weber (1985) demonstrated an LC$_{50}$ of 10.3 mgL^{-1} SLS for

the microzooplankton *Daphnia magna*. Nunes et al. (2005) reported an LC$_{50}$ value of

15.1 mgL^{-1} SLS for the eastern mosquitofish (*Gambusia holbrooki*). Although

community tests of SLS have not been performed, Benevente and Cohen (2013)

found that the addition of APG, a nonionic surfactant with similar chemical

properties to SLS, to microcosms consisting of microalgae (*Chlorella* sp.) and

zooplankton grazers (*Daphnia magna*) decreased dissolved oxygen, conductivity,

and abundance of *Chlorella* sp., potentially reducing zooplankton food availability.

Furthermore, Kasai and Hanazato (1995) demonstrated that at 1 mgL^{-1}, the

herbicide, simetryn, completely suppressed phytoplankton photosynthetic rates in

outdoor experimental ponds, which decreased zooplankton density due to lack of

food supply and increased competition. That organisms at lower trophic levels, such

as phytoplankton, show increased susceptibility to SLS toxicity relative to organisms

at higher trophic levels, suggests the potential for loss of food resources, and

subsequent alterations in herbivory and competitive interactions.

The aim of this study was to investigate the effects of SLS on water quality and organism abundance in freshwater community microcosms. I hypothesized that SLS affects the abundance of organisms in freshwater communities. I predicted that as SLS concentration increased, there would be a decrease in *Chlorella* sp. (and therefore food supply to grazers) since microalgae are more susceptible to surfactant toxicity than higher trophic levels. Further, I anticipated that grazer abundance would be influenced by decreases in water quality (particularly dissolved oxygen and pH), coupled with increased competition due to reduced abundance of *Chlorella* sp. I tested my hypothesis by exposing communities composed of the microscopic algae *Chlorella* sp., the benthic grazing snail *Elimia* sp., and the pelagic microzooplankton *Daphnia magna* to 0, 0.5, or 1.5 mgL^{-1} SLS and measuring water quality and the abundance of *Chlorella* sp. and invertebrate grazers. Findings from this study will be useful to begin to assess the mechanisms by which environmentally relevant concentrations of SLS may influence freshwater communities.

Materials and Methods

To test the hypothesis that SLS affects the abundance of organisms in freshwater communities, the alga *Chlorella* sp., the zooplankton *Daphnia magna*, and the snail *Elimia* sp. were exposed in the following combinations: 1) *Chlorella* sp. only, 2) *Chlorella* sp. + 5 *Daphnia magna*, 3) *Chlorella* sp. + 1 *Elimia* sp., and 4) *Chlorella* sp. + 5 *Daphnia magna* + 1 *Elimia* sp. to 0 (control), 0.5, or 1.5 mgL^{-1} SLS in a fully crossed factorial design with 4-fold replication (N = 48). Laboratory-reared

Chlorella sp. cultures and *Daphnia magna* neonates were used in the experiment. Five *D. magna* were used in treatments with *D. magna* present in order to allow for growth of the population without overcrowding in the 1 L of solution that was used. *Elimia* sp. were collected from the Ogeechee River at Rocky Ford, GA (32.65°N, 81.84°W) and slowly acclimated to laboratory conditions in an environmental chamber (Thermo Scientific Forma Model 3940) at the Georgia Southern University Department of Biology over a period of four weeks. Once acclimated, *Elimia* sp. were maintained in the laboratory for an additional 8 weeks prior to the start of the experiment. One *Elimia* sp. was used in treatments with *Elimia* sp. present in order to allow for competition among the grazers without overcrowding the microcosms.

Sodium lauryl sulfate treatments were established by dissolving crystallized SLS (Acros Organics) in natural spring water according to the methods of Kirk et al (2003). Each replicate experimental unit (microcosm) consisted of 950 mL of treatment solution and 50 mL of dense *Chlorella* sp. suspension ($\sim 3*10^6$ cells ml^{-1}) for a total volume of 1 L. Microcosms were randomized by location under daylight fluorescent lights (Sylvania Design 50 40W) at an irradiance of 80-100 μmol m^{-2} s^{-1} on a 16:8 L:D cycle (Benevente and Cohen 2013). The experiment was conducted from April 19, 2013—May 9, 2013. The 21-day duration was enough time for SLS to completely biodegrade, which has been seen to occur after 2 days in an acute test with *Ceriodaphnia* (Cowgill et al 1990), and allowed the *Daphnia magna* to produce at least one generation of offspring (Ebert 2005) after being exposed to the initial amount of SLS.

Water quality measurements (temperature, dissolved oxygen, conductivity, and pH) and abundance of *Daphnia magna* and *Elimia* sp. in each microcosm were recorded initially and every 48 hours for the duration of the experiment. Electronic hand-held meters were used to measure temperature and conductivity (Eutech EC Testr low), DO (Oakton DO 110), and pH (Oakton pHTestr 10) between 11:00 and 14:00 h on each sampling date. Numbers of live *Daphnia magna* and *Elimia* sp. in each jar were determined visually. *Daphnia magna* that died were not removed since molts and dead *Daphnia* could not be easily distinguished. In contrast, dead, decomposing *Elimia* sp. were removed to avoid decreasing water quality. Abundance of *Chlorella* sp. was determined by measuring chlorophyll *a* concentration, which required vigorous mixing of the solution in each microcosm. Since this sampling process aerated the microcosms and had the potential to alter measurements of DO, analysis of chlorophyll *a* concentration was performed only at the end of the experiment.

To measure the concentration of chlorophyll *a* in each microcosm the solution was first homogenized to resuspend the non-motile *Chlorella* sp. cells. In a darkened room, a 100 mL subsample of solution was taken from each microcosm, vacuum filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm) to trap algal cells, and filters were frozen at -20°C until analysis. Chlorophyll *a* from the cells on frozen filters was extracted in 8 mL of 90% acetone for 24 hours in the dark at -20°C. Chlorophyll *a* concentration was then determined using the EPA 445 acidification method (Arar and Collins 1997) on a Turner Designs Trilogy fluorometer.

Statistical Analysis

Data were tested for normality using the Shapiro-Wilk W test and homogeneity of variances using Levene's test, and only *Chlorella* sp. abundance met the assumptions for parametric tests following log transformation. *Chlorella* sp. abundance across treatments was only measured at the end of the experiment, thus the effects of SLS and organism treatment on chlorophyll *a* concentration were analyzed using two-way ANOVA. All other variables could not be transformed and were analyzed nonparametrically. Differences in temperature, DO, pH, conductivity, and *Daphnia magna* abundance across concentration treatments over the course of the experiment were tested using the nonparametric version of repeated measures ANOVA, Friedman's test. Abundance of *Elimia* sp. was too low to analyze statistically given that only one individual was used in each microcosm, therefore no statistical tests were performed on these data.

Results

SLS affected both water quality and the abundance of organisms in freshwater communities. Over the course of three weeks, temperature remained within one °C of the mean (23.5°C), but temperature was generally lowest in the control treatment and highest in the 1.5 mgL^{-1} SLS treatment (Table 1, Figure 1A). Although pH appeared to stay relatively constant within treatments for the duration of the experiment (Figure 1B), it was consistently higher in the treatments that received SLS compared to the control (Table 1, Table 2). Dissolved oxygen did not differ across treatments and remained at concentrations above 8 mg L^{-1} for the

duration of the experiment in all SLS and organism treatments (Table 2, Figure 1C). Conductivity generally decreased with time across all treatments (Table 1, Figure 1D), but was lowest in the low SLS concentration in all organism treatments except *Daphnia magna + Elimia* sp. (Table 2).

Chlorophyll *a* concentration of *Chlorella* sp. was not affected by SLS treatment (Two-way ANOVA, $F_{2,6}=0.0677$, p=0.9347), but was influenced by the identity of the species present (Two-way ANOVA, $F_{3,6}=14.9296$, p<0.0001), generally increasing with the presence of grazers (Figure 2). Number of *Daphnia magna* was reduced in the presence of SLS, regardless of concentration, particularly in the *Chlorella* sp. + *D. magna* treatment (Friedman's Test, χ^2 =7.68, 0.025<p<0.05). After day 11, *D. magna* abundance in both the 0.5 and 1.5 mgL^{-1} SLS concentration treatments declined to almost zero (Figure 3A). In contrast, *D. magna* abundance was higher in the 0.5 compared to the 1.5 mgL^{-1} SLS concentration in the presence of *Elimia* sp. (Friedman's Test, χ^2 =13.27, 0.001<p<0.005)(Figure 3B). In the 0.5 mgL^{-1} SLS treatment, number of *D. magna* in the *D. magna* + *Elimia* sp. treatment was greater than that in the *D. magna* alone treatment (Friedman's Test, χ^2 =4.84, 0.025<p<0.05) but was still approximately 50% lower than the control (Figure 3).

Discussion

I hypothesized that SLS affects both water quality and the abundance of organisms in freshwater communities. In particular, C*hlorella* sp. concentration was expected to decrease with increasing concentration of SLS. I found that *Chlorella* sp. concentration was not affected by the SLS concentrations tested. It is possible that

toxicity to *Chlorella* sp. may require a higher concentration of SLS than the 1.5 mgL^{-1} used in this study. For example, a concentration of 5 mgL^{-1} SLS reduced the growth of the freshwater algae *Nitzschia actonastroides* in a 5 day toxicity test(Nyberg 1985). In addition, the freshwater alga *Raphidocelis subcapitata* was shown to have an IC50 value of 36.58 mgL^{-1} SLS in 72-hour toxicity tests (Liwarska-Bizukojc et al 2005). I also expected the presence of grazers in this study to reduce concentration of *Chlorella* sp. The observed increase in chlorophyll *a* concentration in the presence of grazers relative to controls was somewhat surprising. However, grazer excretion of nitrogenous and phosphorus waste products from *Elimia* sp. in particular could have provided nutrients to *Chlorella* sp., resulting in an increase in abundance that exceeded losses from grazing. For example, algal biomass increased by the addition of snails and crayfish in artificial pools (McCormick 1990).

My prediction that sodium lauryl sulfate would affect water quality in freshwater microcosms was supported, but none of the measured parameters deviated from acceptable ranges for the organisms present. Temperature was higher with greater concentrations of SLS, likely due to the exothermic nature of microbial breakdown of surfactants (Albers et al 1995). However, the small (1°C) temperature increase from 23 to 24°C should not have affected *Daphnia magna* or *Elimia* sp. survival given that *Daphnia magna* can withstand ranges of 13-25°C (Lagerspetz 200) and *Elimia* sp. survive between 14-26°C (Hanley and Ultsch 1999). The pH was higher with the addition of SLS, consistent with SLS being a basic substance (Yao 2013), although some products of SLS degradation are acidic (Chaturvedi and Kumar 2010). Overall, the range of pH observed among the

microcosms was 7.65-9.0, so the increase in pH from addition of SLS should not have interfered with *D. magna* (6.5-9.5 pH; Ebert 2005) or *Elimia* sp. (7.6-9.0 pH; Smith 1980). Dissolved oxygen was not influenced by SLS. We expected DO to decrease with the addition of SLS since microbial breakdown of anionic surfactants requires oxygen (Jurado et al 2013). Our findings suggest there was not enough microbial breakdown of SLS to influence DO levels, possibly due to the low concentrations of SLS used. The microcosms were also open to the atmosphere, so some oxygen could have escaped through gas exchange. As expected, conductivity decreased over time in all treatments, including the control possibly due to bacterial use of some of the ions formed during degradation, for nutrition (Chaturvedi and Kumar 2010).

That SLS neither affected food availability (as chlorophyll *a* concentration) nor decreased water quality below acceptable ranges suggests that the observed decline in *D. magna* was due to toxicity of SLS. This result was somewhat unexpected given that Lewis and Weber (1985) revealed *D. magna* sensitivity to SLS concentrations of 10.3 mgL^{-1} SLS, although this result was for acute 48 h exposure rather than the chronic exposures used in the present study. Our study revealed that *D. magna* numbers started declining in the SLS treatments on day 9, consistent with the idea that effects from chronic exposure to SLS occur at lower concentrations than effects from acute tests. I also predicted that the presence of *Elimia* sp. would be detrimental to *D. magna* survival due to competition for food resources. While I found no evidence of competition between *D. magna* and *Elimia* sp. for food, the presence of *Elimia* sp. reduced the negative effects of SLS on *D. magna*, suggesting

that the presence of *Elimia* sp. decreases the toxicity of SLS to *D. magna*. Martin and

Marriott (1981) found that when phosphatidylcholine was incorporated into an

aqueous media, the toxicity of SLS was reduced in goldfish. The presence of *Elimia*

sp. may have had a similar mitigating effect on the toxicity of *D. magna* in this study.

The findings from this study suggest that SLS affects the abundance of

organisms in freshwater communities. However, the mechanism does not appear to

be through a reduction in water quality or competition for food resources. Instead,

SLS is likely toxic to *D. magna* at much lower concentrations than previously

thought, and the presence of *Elimia* sp. appears to mitigate effects at SLS

concentrations of 0.5 mgL^{-1}. If microcrustaceans such as *D. magna* are particularly

susceptible to SLS, reductions in their abundance could have serious implications

for organisms in higher trophic levels including fishes, insects, and other

invertebrates that utilize *D. magna* as a food resource.

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Table 1. Ranges of water quality measurements for all organism treatments combined (n=16) for each SLS treatment over the course of the three-week experiment.

	Temperature	Dissolved Oxygen	pH
Zero Concentration	22.73-23.7	8.32-10.79	7.65-8.78
Low Concentration	23.18-23.75	8.45-10.68	8.05-8.98
High Concentration	23.4-24.05	8.31-11.15	8.1-9.0

Table 2. Friedman's test results showing differences across SLS treatments for each of the different organism treatments.

	Temperature		DO		pH		Conductivity	
	χ^2	p-value	χ^2	p-value	χ^2	p-value	χ^2	p-value
Chlorella sp.	20.2	p<0.001	1.27	p<0.9	20.2	p<0.001	19.4	p<0.001
D. magna	21	p<0.001	3.45	p<0.9	21	p<0.001	10.4	p<0.01
Elimia sp.	20.2	p<0.001	1.27	p<0.9	20.2	p<0.001	17.6	p<0.001
D. magna + Elimia sp.	18.1	p<0.001	5.04	p<0.1	18.1	p<0.001	3.8	p<0.5

Figure 1. Average A. temperature, B. pH, C. dissolved oxygen and D. conductivity across all organism treatments over 3 weeks of exposure to one of three SLS treatments (n=16). Error bars are ± one standard deviation (SD).

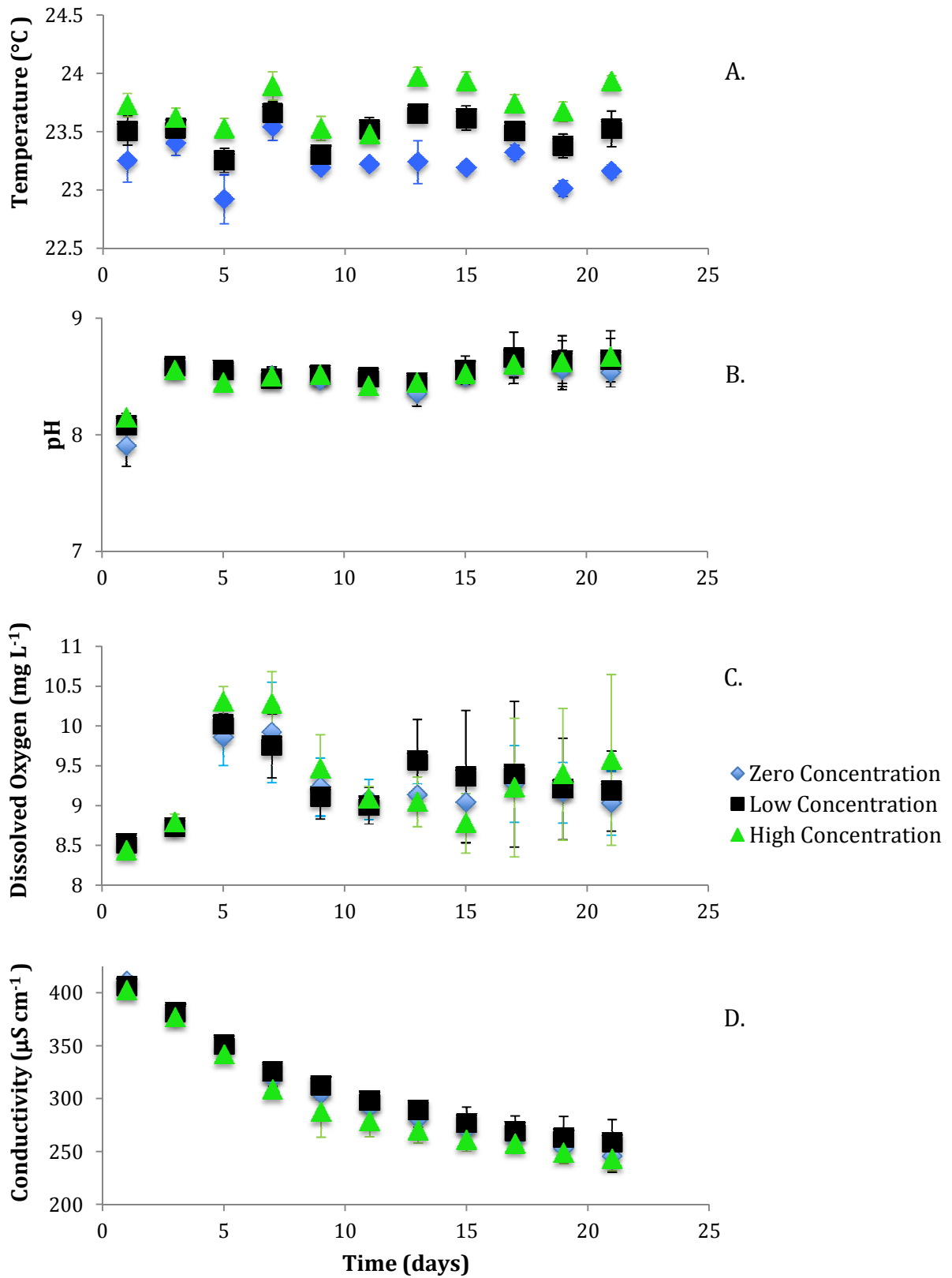Figure 2. Mean final concentrations of chlorophyll *a* across SLS and organism treatments (n=4). Error bars are ± one SD.
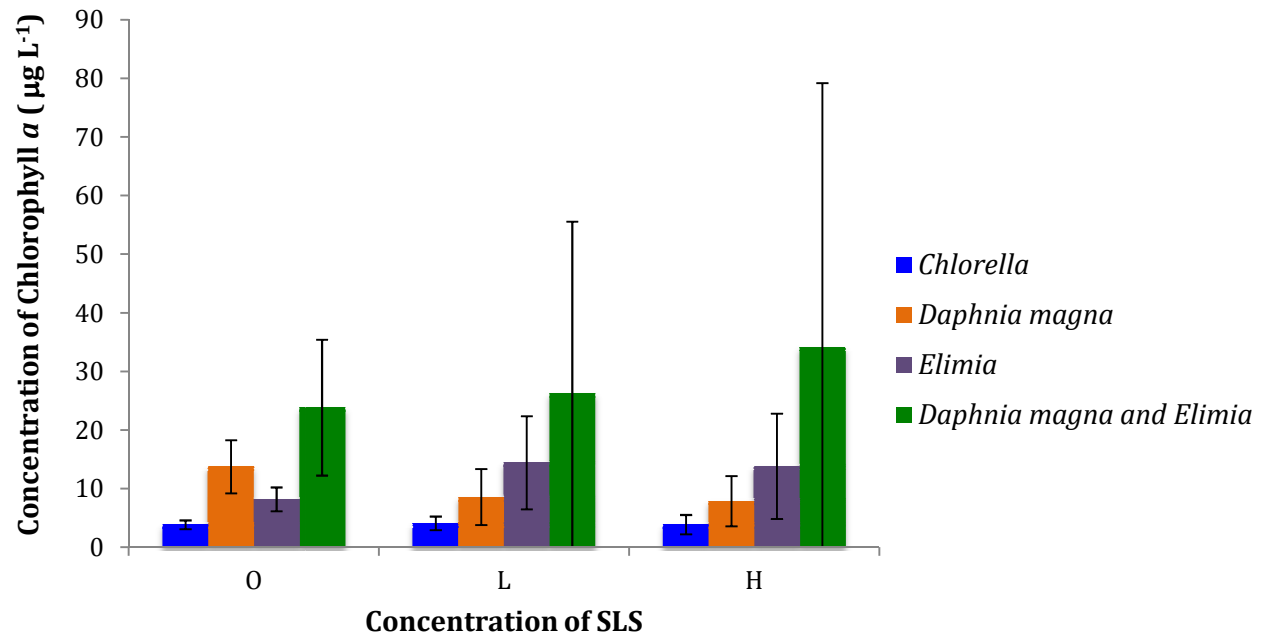
Figure 3: Average number of *Daphnia magna* in the A. absence or B. presence of *Elimia* sp. during exposure to different concentrations of SLS for 3 weeks (n=4). Error bars are ± one SD.