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APG-Containing Product Reduces Water Quality and Food Availability to Primary Consumers in Freshwater Microcosms

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Introduction

As concern over degradation of freshwater resources rises, the use and production of eco-friendly, natural cleaning products is increasing (Balzer 2000). Production of natural surfactants (the key ingredients in eco-friendly household cleaners) such as alkyl polyglucoside (APG) reached an estimated production of 85,000 tons per acre worldwide by 1999 (Hill & LeHen-Ferrenbach 2009). APGs are nonionic surfactants made from glucose and fatty alcohols (Garcia et al. 1997) and have been shown to be readily biodegradable with little toxic effect on organisms, including bacteria (Garcia et al. 1997), algae (Lewis & Hamm 1986; Madsen et al. 1996), crustaceans (Madsen et al. 1996; Garcia et al. 1997), and fish (Madsen et al. 1996) in single-species laboratory studies. However, given that significant amounts of surfactants enter the environment through wastewater treatment plants or by direct release (Madsen et al. 1996), with runoff and adsorption being some of the likely routes (Krogh et al. 2002), evaluating the damage potential of APG to aquatic communities in the environment is essential.

Nonionic surfactants are generally considered to have low lethality to most aquatic organisms; however, sublethal effects on phytoplankton photosynthetic activity (Karpinska-Smulikowska 1984; Lewis & Hamm 1986) and zooplankton reproduction and mobility (Madsen et al. 1996) have been observed. Therefore, APG-containing products have the potential to cause trait-mediated indirect effects in aquatic systems, where changes in activity or behavior of one type of organism can influence the abundance of organisms at other trophic levels (Relyea & Hoverman 2006). For example, increased reproduction or decreased *Daphnia magna* mobility following exposure to APG (Madsen et al. 1996) can lead to changes not only in food availability to fish predators, but also in abundance of phytoplankton. Thus, determining the potential for APG-containing products to influence aquatic systems requires community-level understanding.

Another potential mechanism by which APG can cause harm in the environment is through oxygen depletion during biodegradation (Sutton & Cohen 2012). In a closed-bottle test, exposure to APG concentrations of 2 mg L^{-1} resulted in elevated microbial O_2 consumption, reducing oxygen concentration by > 60 % after 15 d, and by up to 80 % after 30 d (Garcia et al. 1997). Oxygen depletion in aquatic systems is problematic because dissolved oxygen (DO) is essential for aerobic metabolism (Connolly et al. 2004; Decker et al. 2004), with low concentrations inhibiting reproduction and growth of many aquatic species (Boyd & Watten 1989). For example, yellowfin tuna (*Thunnus albacares*) larval mortality occurred at dissolved oxygen levels < 2.2 mg L^{-1} (Wexler et al. 2011), and concentrations of 1 mg L^{-1} of oxygen resulted in complete mortality for other fish species, including smelt (*Retropinna retropinna*), rainbow trout (*Oncorhynchus mykiss*), and bully (*Gobiomorphus cotidianus*) after only 48 h (Dean & Richardson 1999). Weider and Lampert (1985) found the zooplankton *Daphnia pulex* to be able to adapt to oxygen depletion until reaching a "threshold" concentration of 1.0 mg L^{-1} when the zooplankton mortality increased. In a preliminary field study, an APG-containing cleaner was added to mesocosms in the field and resulted in reduced DO levels by 75-85 % (to < 2 mg L^{-1}) and a trend of decreased benthic macroinvertebrate abundance (Sutton & Cohen 2012). These studies suggest the idea that contaminants that reduce DO, even by only a few milligrams per liter, can have adverse effects on aquatic communities. However, the potential for DO

depletion from APG degradation to influence free-swimming zooplankton grazers in aquatic communities has not been examined.

The purpose of this study was to examine the effects of APG on water quality and a simple aquatic food chain by exposing microalga (*Chlorella spp*.) and zooplankton grazers (*Daphnia magna* and *Cypridopsis spp*.) to environmentally realistic APG concentrations. We hypothesized that of the presence of APG reduces water quality and plankton abundance. We predicted that, as APG concentrations increase, water quality and plankton abundance would decrease. Results from this study were used to evaluate whether field-testing for community-level effects should be required for environmentally relevant concentrations of APG.

Methods

The effects of five concentrations of APG-containing product on water quality and abundance of grazers in a simple aquatic food chain were evaluated using a laboratory experiment conducted from June 11 to July 2, 2012 in the Department of Biology at Georgia Southern University, Statesboro, GA, USA. Five treatments were created using an all-purpose cleaner containing about 500 mg L^{-1} APG (Willing et al. 2004) to make concentrations of 0.0001, 0.001, 0.01, and 0.1 %, and 0 % serving as the control, with five-fold replication for a total of 25 microcosms. Microcosms were 1.5 L glass containers open to the atmosphere with 1 L of treatment solution, two species of arthropod grazers (six small *D. magna*, 10 *Cypridopsis spp*.), and the green microalga *Chlorella spp*. at a density of 2.25 X 10^4 cells ml^{-1}. The microcosms were then randomized by location and maintained on a 16:8 L:D cycle under daylight fluorescent bulbs at an irradiance of 80-100 μmol s^{-1} m^{-2} at a constant temperature of 22 °C.

Initially, and every 48 h for three weeks, electronic hand-held meters were used to measure DO (Oakton DO 110), pH (Oakton pHTestr 10), and conductivity (Eutech EC Testr low) at the same time of day. Number of live *D. magna* in each microcosm was also determined visually. *Chlorella spp.* is nonmotile, and the homogenization of the solution required to sample chlorophyll *a* concentration would require mixing and therefore aeration of the microcosms. Similarly, *Cypridopsis spp*. is a small, cryptic species and accurate counting would also require disruption of the water. Therefore, *Chlorella spp*. pigment concentration and *Cypridopsis spp*. density were measured only at the end of the experiment.

At the end of three weeks, after final water quality and zooplankton counts were conducted, *Chlorella spp*. abundance was determined using chlorophyll *a* concentration as a proxy. Solutions in each microcosm were homogenized by mixing, and a 100 ml subsample was vacuum filtered onto Whatman GF/F glass fiber filters. Filters containing *Chlorella spp*. were frozen at -20 °C for 48 h, followed by addition of 90 % acetone to extract the pigments from collected cells. After 24 h in the dark at -20 °C, solutions were measured for chlorophyll *a* concentration using a TD-700 fluorometer following the acidification method of Arar and Collins (1992).

Data were tested for normality and for homogeneity of variances, and only *Cypridopsis spp.* abundance met the assumptions for parametric tests. All other variables could not be

transformed and were analyzed nonparametrically. Therefore, differences in DO, pH, conductivity, and *D. magna* abundance across concentration treatments over the course of the experiment were analyzed using the nonparametric version of repeated measures ANOVA, Friedman's test. Differences in *Cypridopsis spp*. and *Chlorella spp*. abundance across treatments were evaluated only at the termination of the experiment and were therefore analyzed using one-way ANOVA and Kruskal-Wallis tests, respectively.

Results

Changes in water quality parameters (DO, conductivity, pH) occurred following the addition of APG. Over the course of three weeks, DO concentrations differed across APG concentration treatments (Friedman's test, $\chi^2 = 18.34$, $p < 0.01$). Marked declines were evident at three time points over the experiment, at the highest concentration of APG tested (Figure 1a). At 2 and 12 d, there was a 10 % (~ 1 mg L^{-1}) decline in DO concentration, and at 8 d, DO dropped by 20 % (~ 2 mg L^{-1}) in the 0.1 % APG treatment relative to all of the other concentrations. Conductivity declined across all APG treatments (Friedman's test, $\chi^2_4 = 18.34$, $p < 0.01$). However, the magnitude of the decrease was similar in all treatments except for the 0.1 % APG treatment, which decreased less than the other treatments (approximately 25 % vs. 18 % over three weeks) (Figure 1b). Although pH differed across treatments (Friedman's test, $\chi^2_{4,69} = 16.58$, $p < 0.01$), only the control exhibited a 12 % increase in pH on day 10 while pH in all APG treatments increased by 2 % by day 2 and remained stable for the remainder of the experiment (Figure 1c).

Plankton responses to APG addition were highly variable. *Chlorella spp*. chlorophyll concentrations were significantly reduced with increasing APG concentration, particularly at the 0.1 % concentration (Kruskal-Wallis, $\chi^2_{4,24} = 13.49$, $p = 0.0091$) (Figure 2a). In contrast, the number of *D. magna* remained similar across treatments (Friedman's test, $\chi^2_{4,69} = 0.86$, $p > 0.9$). However, after day 8 when broods were produced through parthenogenesis, an apparent pattern of reduced numbers of young in the 0.1 % APG concentration occurred until day 14 (Figure 2b). Individuals produced in the 0.1 % treatment also appeared smaller and paler relative to those in the lower concentration treatments. *Cypridopsis spp.* density did not differ among treatments (one-way ANOVA, $F_{4,24} = 1.03$, $p = 0.42$) (Figure 2c), and the organisms did not appear to reproduce; final abundance was less than or equal to initial abundance in all treatments.

Discussion

The addition of an APG-containing product influenced water quality in freshwater microcosms. As expected, the concentration of DO in microcosms treated with the APG product decreased. However, DO concentration decreased by 1-2 mg L^{-1} (10-20 %), and only at the highest concentration tested (0.1 %) in open containers in a temperature-controlled lab setting. Although much greater reductions of DO occurred in closed bottle tests treated with APG (Garcia et al. 1997), it is important to determine what effects environmentally relevant concentrations of APG might have on aquatic communities in field settings. Sutton and Cohen (2012) conducted an experiment in which 0.1 % APG addition caused a decline of 6-7 mg L^{-1} in DO in a pond exposed to ambient environmental conditions. Our findings suggest that APG

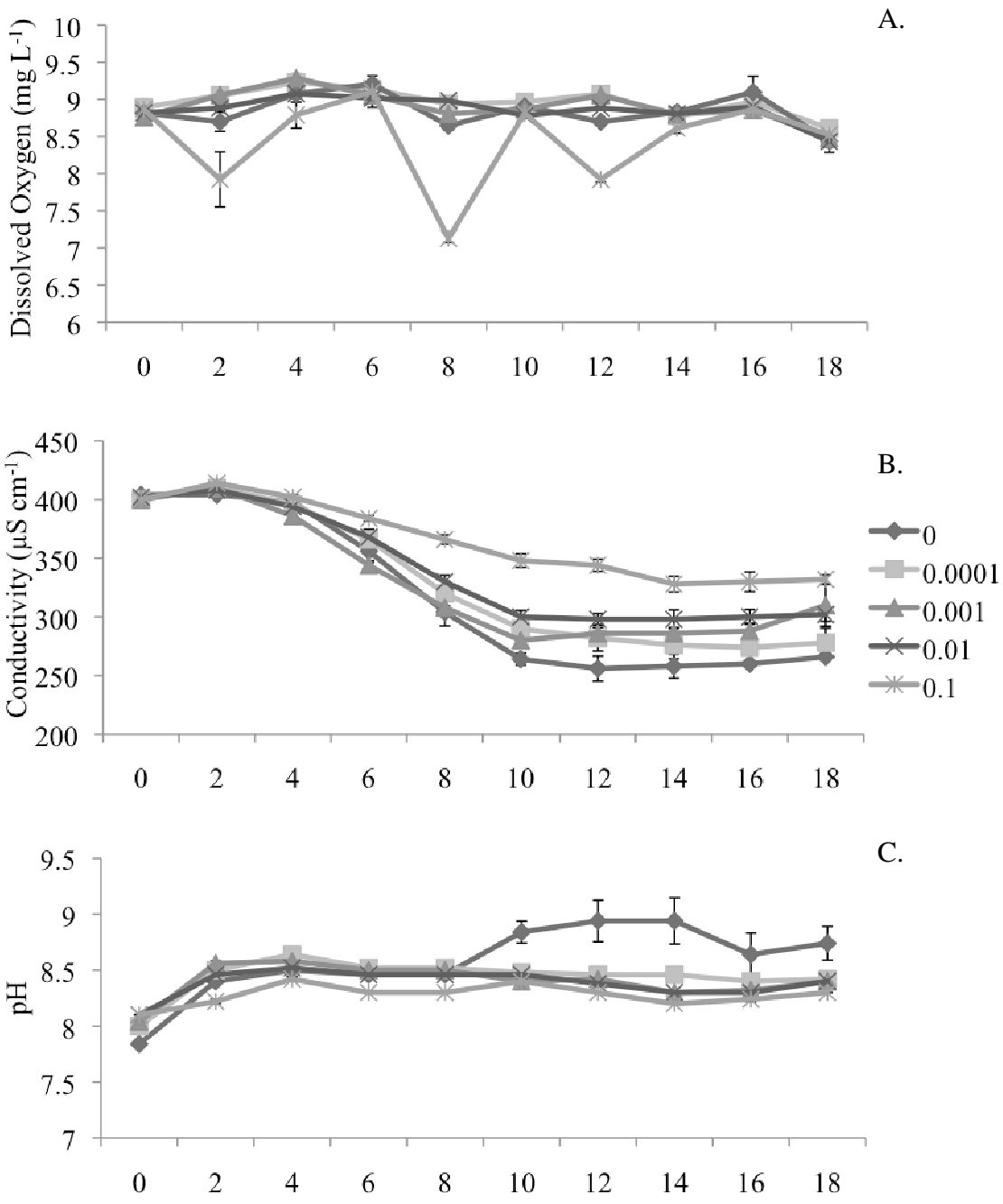

Figure 1. Average (a) DO concentration, (b) conductivity, and (c) pH in microcosms over 18 d of exposure to 0, 0.0001, 0.001, 0.01, or 0.1 % APG treatments (n = 5). Error bars are ± one standard error of the mean (SEM).

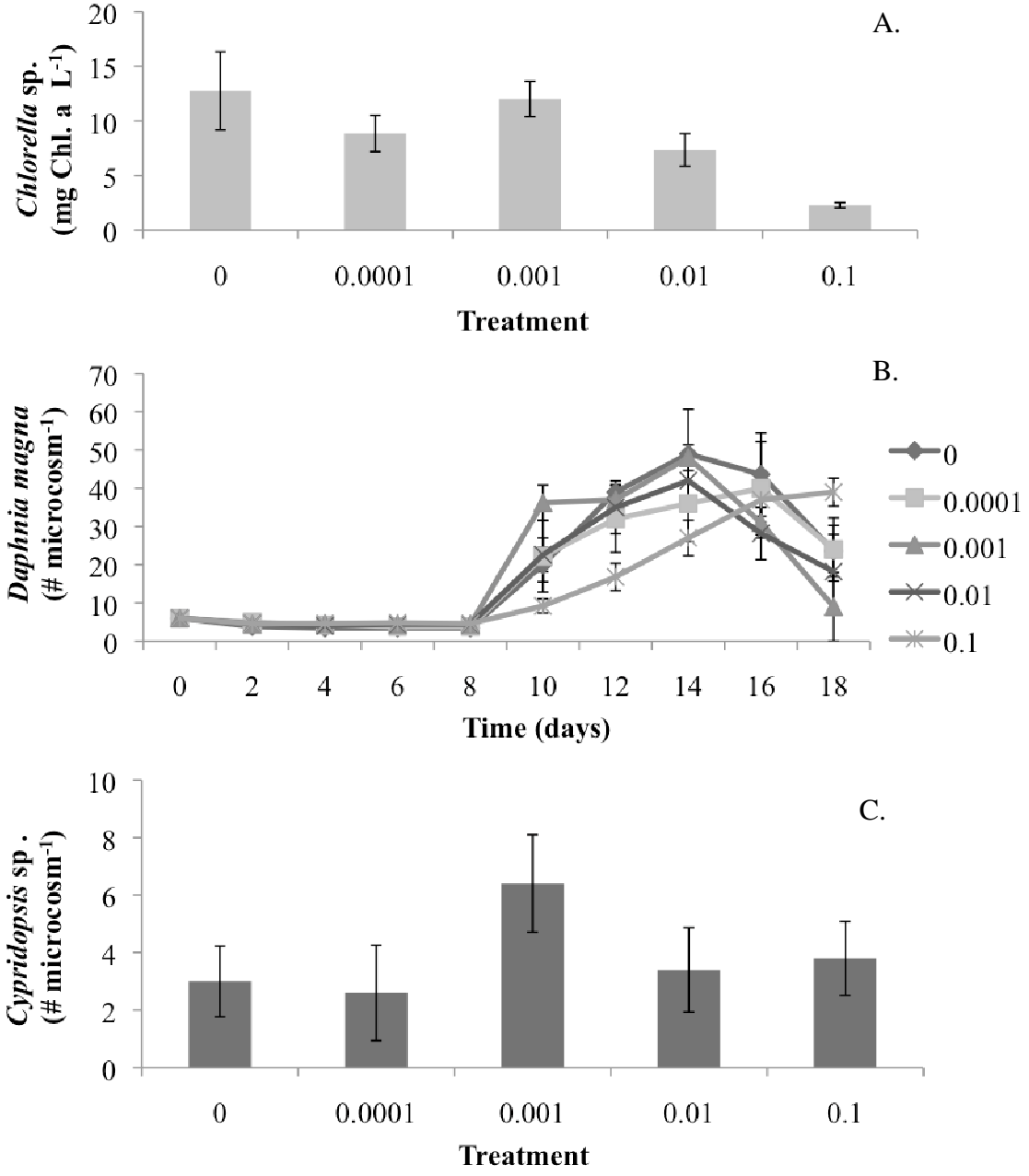

Figure 2. Average (a) chlorophyll *a* concentration of *Chlorella spp*., (b) abundance of *D. magna*, and (c) abundance of *Cypridopsis spp*. after 18 d of exposure to 0, 0.0001, 0.001, 0.01, or 0.1 % APG treatments (n = 5). Error bars are ± one SEM.

alone is sufficient to cause a decline in DO under controlled conditions, supporting the conclusion of Sutton and Cohen (2012) that APG effects on DO have the potential to be amplified by synergistic interactions with environmental factors. Furthermore, in an environment with already low DO conditions (e.g. 4-5 mg L^{-1}), a 2 mg L^{-1} decline can have a large impact on organism reproduction, growth, and mortality (Nebeker et al. 2009). The conductivity of the water increased with increasing concentrations of APG, which was not surprising given that

higher levels of conductivity often correlate with increased concentrations of surfactants (Dukhin & Goetz 2005), and the addition of the APG product alone has been shown to increase ion concentrations in the water (Sutton & Cohen 2012). However, conductivity also decreased over time in all treatments, likely due to APG biodegradation within the microcosms as surfactant molecules were degraded into water and carbon dioxide (Zhang et al. 2011). It is also possible that *Chlorella spp*. utilized some of the ions produced during APG degradation as nutrients (Sutton & Cohen 2012), especially since conductivity declined in the control treatment as well. The increase of nutrients within the environment due to APG-containing products may result in a shift of species present, leading to a possibility of algal blooms and a decrease in water quality (Codd 2000). Finally, that the pH of the water was higher in the control group than in any APG treatment could have been a result of elevated *Chlorella spp*. photosynthetic rates, which can deplete H^+ concentrations as dissolved CO_2 is taken up from the medium, making the environment more basic (Dodson 2005). Freshwater environments most often have a pH of 6-9, with *D. magna* being able to withstand pH ranges of 4.5-10.13 (Ghazy et al. 2011), indicating the pH levels found within the APG treatments likely had no adverse effects.

The reduction in *Chlorella spp.* abundance that occurred in treatments exposed to higher concentrations of APG was likely due to direct toxicity of the APG-containing product rather than elevated *D. magna* grazing rates. Green algae have been shown to be sensitive to APG, with inhibition of growth at concentrations as low as 0.001 % (Karpinska-Smulikowoka 1984; Lewis & Hamm 1986). Furthermore, the *Chlorella spp*. cells in the highest APG treatments tested appeared paler green than those in lower concentration treatments, and the *D. magna* in the 0.1 % treatment were noticeably smaller and paler in color than those found in the lower concentrations. One reason for the apparent physical differences could be that food, (i.e. *Chlorella spp*.) was relatively scarce, and declines not only in phytoplankton availability (Fleeger et al. 2003) but also nutritional quality (Sterner et al. 1993) can lead to decreased abundance of aquatic grazers. Therefore, it is important to investigate food web interactions in aquatic communities to understand potential consequences surfactants have on nutrient content within these environments.

We expected decreases in DO as a result of APG degradation to have an impact on zooplankton abundance. Environments with DO levels of 3.0 mg L^{-1} can decrease *Daphnia* filtering and respiration rates (Heisey & Porter 1977) and reduce juvenile growth rate and maturation size at DO levels of 1.3–2.3 mg O_2 L^{-1} (Hanazato 1995). Results from this study showed that there was no effect of APG concentration on *D. magna*, which was not surprising given that even with a 2.0 mg L^{-1} decrease, DO concentrations never dropped below 7.0 mg L^{-1} in any treatment. *Cyrpidopsis spp*. also appeared to be unaffected, although the final number after three weeks was lower than the initial density, suggesting that this short-lived grazer (life span of 21-28 d (Mills & Wyatt 1974) likely did not reproduce during the experiment. However, in an environment with naturally occurring low DO levels, the presence of APG in the water could reduce DO to critical levels for aquatic organisms.

Our findings that APG-containing products reduce water column DO and microalgal abundance suggest that the effects of APG in aquatic systems should be evaluated on the community level, especially given the potential for food limitation of zooplankton and higher trophic levels. In particular, investigation of the effects of surfactants under field conditions is

needed due to the presence of factors that can have synergistic interactions with APG such as temperature and other environmental pollutants (Lewis & Hamm 1986). Additional studies of APG-containing products within field settings are essential to fully understand the potential for disturbance to aquatic communities and to develop water quality policy regarding safe amounts of “natural” cleaning products that can enter aquatic systems.

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