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Effects of environmentally-relevant antibiotic mixtures on marine microalgal growth

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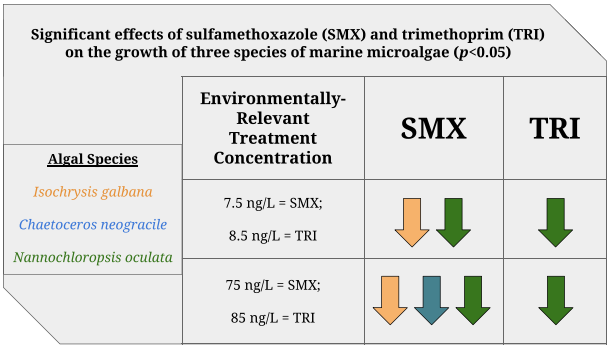
HIGHLIGHTS

- Long-term exposure of commonly prescribed antibiotics by marine microalgal species
- Sulfamethoxazole reduced growth in all three algal species in 3-week trials.
- Trimethoprim reduced growth in one marine microalgal species.
- Unicellular primary producers are sensitive to pharmaceutical contaminants.
- Bottom-up trophic effects have the potential to shift food webs.

GRAPHICAL ABSTRACT



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ABSTRACT

As of 2008, approximately 48% of Americans use prescription drugs within any given 30-day period. Many pharmaceutical compounds are not fully metabolized by the human body, nor fully removed by wastewater treatment systems, before release into the environment. As a result, a vast array of pharmaceuticals has been detected in marine and freshwater organisms, sediments, and waters, with unintended effects on non-target organisms, and limited studies of environmental effects. The antibiotics sulfamethoxazole (SMX), and trimethoprim (TRI), often prescribed together to treat bacterial infections, have been detected worldwide in marine and estuarine environments at concentrations up to 765–870 ng/L each. Little research has examined sub-lethal effects of antibiotic mixtures at environmentally-relevant concentrations on marine organisms. We examined the effects of mixtures of these two antibiotics on three marine microalgal species with wide geographic ranges: *Isochrysis galbana*, *Chaetoceros neogracile*, and *Nannochloropsis oculata*. In separate simulations using a temperature/light-controlled set-up, we measured the growth response for each species to environmentally-relevant levels of SMX and TRI. *N. oculata* growth was significantly reduced by mixture treatments of both drugs ($p < 0.05$), by TRI ($p < 0.001$), and by SMX ($p < 0.001$), whereas only aggregated SMX levels significantly reduced growth for the other two species ($p < 0.005$). The exposure time at which growth rates were affected varied across species, with significant reduction in growth focused in the latter half of the experimental period for *C. neogracile* and *N. oculata* (Days 15 and 6 respectively), and midway through the experimental period for *I. galbana* (by Day 3). This study finds that important marine primary producers respond to the presence of SMX and TRI in the water, offering an understanding of environmental consequences of anthropogenic pharmaceuticals contaminants, and specifically the suite of antibiotics, that are released into marine ecosystems at an ever-growing rate, and highlighting potential cascading effects through trophic levels.

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1. Introduction

Photosynthetic algae form the base of the food web in many aquatic ecosystems, and population level alterations to these photoautotrophic organisms can result in quickly-realized and severe bottom-up effects at higher trophic levels (Nie et al., 2013). Thus, microalgae are often used as indicators of pollution and water quality (McCormick and Cairns Jr., 1994).

Pharmaceuticals follow many documented pathways into the environment and may be significant stressors to organisms (Boxall, 2004). Pharmaceutical compounds are often not fully metabolized by the human body, nor are they fully removed by onsite and wastewater treatment plant systems, which release pharmaceutical-contaminated effluent water into the environment. In addition, improper disposal of expired or unneeded pharmaceuticals, industrial emissions, and agricultural uses contribute to environmental pollution (Boxall, 2004). As a result, diverse pharmaceuticals have been detected in marine and freshwater organisms, sediments, and waters, including drinking water, at concentrations of high ng/L with potential unintended effects on non-target organisms and food webs (Daughton and Ternes, 1999).

Aquatic studies of pharmaceutical effects on microalgae have focused on acute toxicity of freshwater microalgae, with most experimental tests completed within 96 h (Halling-Sørensen, 2000; Cleuvers, 2003; Eguchi et al., 2004; El-Bassat et al., 2012; González-Pleiter et al., 2013). There is a lack of chronic effects studies using environmentally-relevant drug concentrations. Yet several studies of pharmaceutical drugs applied to multiple freshwater organisms (such as algae, *Daphnia*, fish, and amphibians) identify freshwater microalgae as most affected (Isidori et al., 2005; El-Bassat et al., 2012). Thus, microalgae may be among the most sensitive aquatic organisms to antibiotics. In addition, recent studies on sensitivity of marine microalgal species to antibiotic pharmaceuticals have identified sublethal effects of the antibiotic tylosin on estuarine benthic microalgal, reducing biomass, primary productivity and diatom growth (Pinckney et al., 2013) and of the antibiotics tylosin, lincomycin and ciprofloxacin significantly reducing marine and estuarine microalgal species cell abundance (Swenson et al., 2012, Kline and Pinckney, 2016, Hagenbuch, 2013).

Though antibiotics target bacteria, these drugs may cause detrimental effects to algae, as some algal organelles (such as chloroplasts and mitochondria) retain structural similarities to bacteria (Vannini et al., 2011; Guo et al., 2015). In addition, low levels of antibiotics over the long term may disrupt algal-bacterial interactions, leading to the suppression of population growth rates and potential effects on the availability of algae as a food source for higher trophic level organisms. Despite identified risk of some pharmaceuticals to algal species, remaining data gaps include pharmaceutical mixture effects, long-term effects of pharmaceuticals at environmentally-relevant concentrations on algal species (Guo et al., 2015), and effects of lower trophic level responses to pharmaceutical contaminants on higher trophic levels (Gaw et al., 2014).

Table 1
Antibiotic mixture treatment concentrations (high and low values are based off of worldwide occurrence data range averages). The 9 treatment levels (grey) are comprised of control (0), low, and high levels in every combination. For each treatment, $n = 9$. SMX = sulfamethoxazole, TRI = trimethoprim, with bold indicating SMX levels.

		SMX		
		0	**7.5 ng/L (Low)**	**75 ng/L (High)**
TRI	0	**0** – 0	**Low** – 0	**High** – 0
	8.5 ng/L (Low)	**0** – Low	**Low** – Low	**High** – Low
	85 ng/L (High)	**0** – High	**Low** – High	**High** – High

Pharmaceutical exposure studies on marine organisms are notably lacking (Gaw et al., 2014). We examined the growth response of three marine microalgal species to environmentally-relevant treatment levels of antibiotic mixtures of sulfamethoxazole (SMX) and trimethoprim (TRI). SMX and TRI are often prescribed together to treat a suite of bacterial infections. In a 5:1 ratio, they comprise the antibiotic drug Bactrim, a drug on the World Health Organization's List of Essential Medicines (WHO, 2015). Their mode of action is inhibiting synthesis of the bacterial enzyme tetrahydrofolic acid (Boothe, 2015), having synergistic

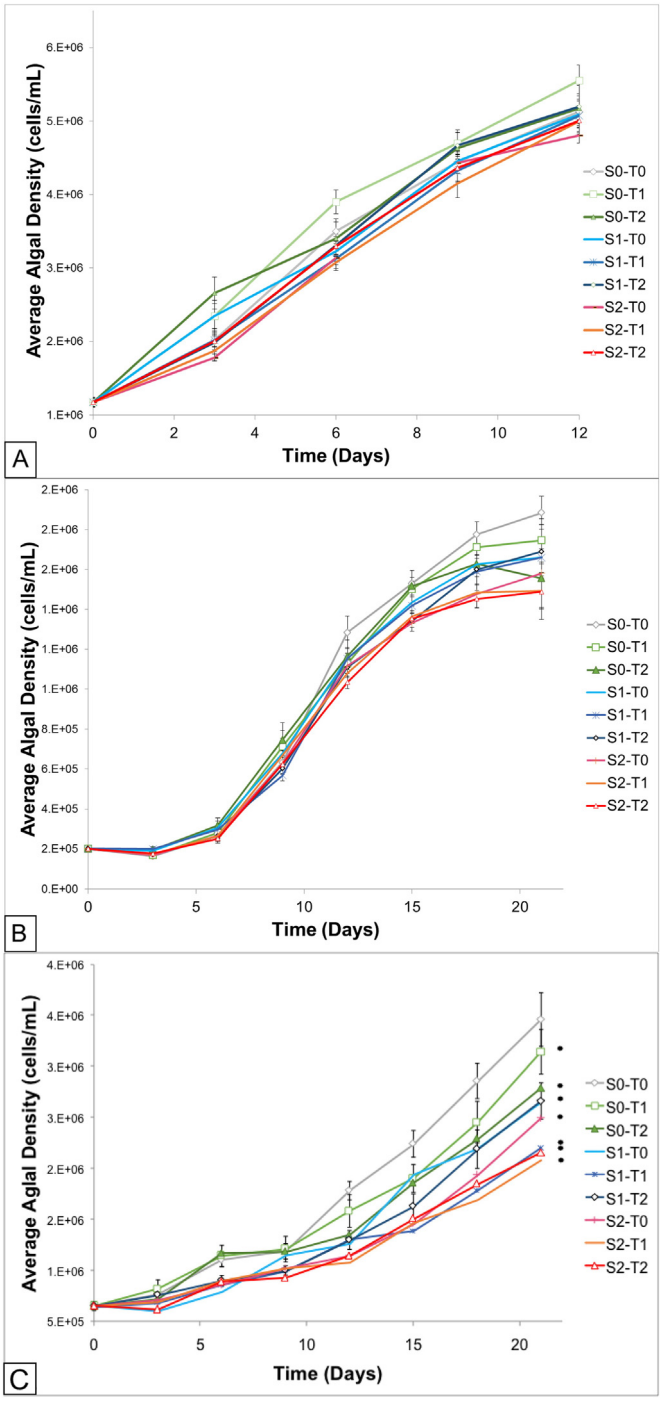

Fig. 1. Average algal cell densities (a proxy for growth rates during the growth phase), varied across treatment averages for A) *I. galbana*, B) *C. neogracile* and C) *N. oculata*. Data presented by antibiotic mixture treatment levels, and standardized by Day 0 concentrations. For each treatment, $n = 9$ (except for S2-T2 for A), where $n = 8$). Error bars indicate ±SE of the mean, and asterisks indicate significant differences from the control (S0-T0) treatment level, $p < 0.05$.