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# Multiple stressors in freshwater ecosystems: antibiotic mixtures and temperatures

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## Abstract

Interactions among different anthropogenic stressors are at the core of unexpected ecological impacts on ecosystems as they can provoke complex effects that lessen or amplify the direct effect of each stressor. One of the most important stressors in freshwaters are pharmaceuticals, including antibiotics. Antibiotics have been used in large quantities for some decades but it is only in recent years that they have been investigated to assess the environmental risk they might pose. We performed two different laboratory-based experiments to explore the effects of antibiotic mixtures and temperature together. Firstly we used the bacterium *Pseudomonas fluorescens* and in a second experiment, we used a natural bacterial community coming from a leaf litter biofilm. We exposed them to a mixture of ciprofloxacin, ofloxacin and sulfamethoxazole at low concentrations (under 100  $\mu\text{g/L}$ ) and under different temperatures treatments. We measured the bacterial fitness of the final bacterial population - exposed to antibiotics for 7 days – by assessing its growth under the different temperature and antibiotic treatments, and by comparing it to the growth of the ancestral bacterial strain or community -which was never exposed to antibiotics. While *P. fluorescens* growth was negatively affected by the presence of antibiotics – in particular ciprofloxacin and ofloxacin alone- the natural bacterial community growth was positively affected. Metabolic alterations caused by the resistance mechanisms can increase the growth of the bacterium in the presence of the antibiotics, even sometimes causing dependence to the antibiotics. In both experiments, all antibiotic combinations induced bacterial resistance after 7 days, especially at lower temperatures. Because of the constant release of antibiotics in the environment, we do not expect antibiotic resistance in stream biofilms to be reduced in the near future and we need to follow up with experiments looking at the combined effects of stressors in short and long-term scenarios.

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