Science of the Total Environment 627 (2018) 1–10

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Multistressor effects on river biofilms under global change conditions

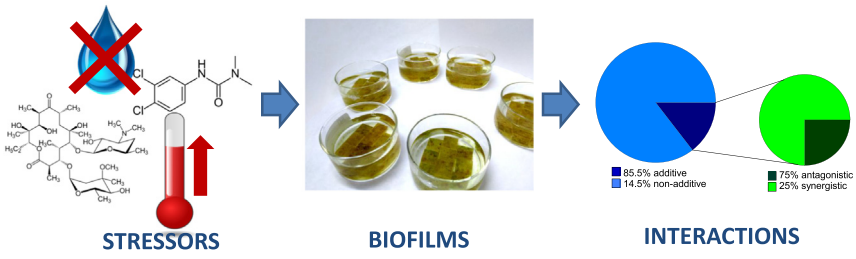
Ferran Romero [a,*], Sergi Sabater [a,b], Xisca Timoner [a], Vicenç Acuña [a]

[a] Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, 17003 Girona, (Spain)
[b] Institute of Aquatic Ecology, University of Girona, 17071 Girona, (Spain)

HIGHLIGHTS

- River ecosystems are often affected by a combination of chemical and physical stressors.
- A full factorial design revealed the short-term effects of four stressors on river biofilms.
- Responses to stressors combination were 85.5% additive and 14.5% non-additive.
- Non-additive responses were classified as antagonisms (75%) and synergisms (25%).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 November 2017
Received in revised form 16 January 2018
Accepted 16 January 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Biofilms
Multiple stressors
Microcosms
Water stress
Antagonism

ABSTRACT

Freshwater ecosystems are confronted with multiple chemical, biological and physical stressors. Co-occurring stressors commonly result in additive responses, but non-additive interactions may also occur, hindering our predicting capacity. Despite growing interest in multiple stressor research, the response of freshwater communities to co-occurring chemical and climate change-related physical stressors remains largely unexplored. Here, we used a microcosm approach to evaluate the effect of the combined action of chemical and physical stressors on river biofilms. Results showed that additive responses dominated, whereas 14.5% of all responses were non-additive (75% antagonisms and 25% synergisms). Among these non-additive interactions, physical stressors dominated over chemicals and drove the overall responses. Overall, the occurrence of these non-additive interactions, together with the dominance of the climate-change related physical stressors, might lead to unexpected responses as a result of climate change.

1. Introduction

Unravelling the mechanisms by which aquatic biota respond to global change is still an ongoing major challenge. Current predictions indicate that freshwater communities will face increased physical stress (higher water temperature and desiccation events) (IPCC, 2014), as well as chemical stressors of anthropogenic origin, such as pesticides and pharmaceutical products (Rodriguez-Mozaz et al., 2004; Kuzmanovic et al., 2015). These stressors co-occur in freshwater systems and cause unknown impacts on multiple levels of biological organization, from individual genotypes to communities (Segner and Sabater, 2014). The effects produced by these stressors can be additive, when the effect of the combined action of two or more stressors is equal to the sum of the individual effects, or non-additive (Folt et al., 1999). The latter is further

* Corresponding author at: Catalan Institute for Water Research (ICRA), Carrer Emili Grahit 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E- 17003 Girona, (Spain).
E-mail address: fromero@icra.cat (F. Romero).

https://doi.org/10.1016/j.scitotenv.2018.01.161
0048-9697/

depicted in antagonism or synergism, depending on the negative (antagonism) or positive (synergism) interaction that one stressor has on the other (Crain, 2008; Piggott, Townsend et al., 2015).

Recent analyses have emphasized that interactions in freshwater ecosystems may account for 40% to 69% of all ecological responses (Jackson et al., 2016; Schinegger et al., 2016) and that additive interactions may be as frequent as non-additive interactions (Nõges et al., 2016). A recent literature review suggested that the observed differences may depend on the ecosystem type and the organization level studied, from individual species to populations and whole ecosystems (Côté et al., 2016). Still, uncertainty persists over the combined impacts of multiple stressors from a climate change perspective (Christensen et al., 2006; Ormerod et al., 2010). Field-based approximations often lead to unclear results, due to the confounding effect of natural variability in freshwater ecosystems (Ponsatí et al., 2016). More solid cause-effect relationships can be established using experimental microcosms. These are simplifications of reality that allow for reduced natural variability and increased replication capacity. Although experimental designs using microcosms often use single-species approaches, more reliable results can be obtained if moving towards community-based analysis (Sabater et al., 2007). River biofilms incorporate species with different roles and functions, with autotrophs and heterotrophs co-existing in a highly complex entity. Because of its rapid response to perturbation and major role in nutrient cycling and ecosystem stability, they represent a good candidate to approach the impact of multiple stressors on rivers and streams (Sabater et al., 2007).

Here, we experimentally manipulated two physical stressors (water temperature and desiccation) and two chemical stressors (an herbicide and an antibiotic) in a full factorial model using river biofilms as a model community. Physical stressors were applied following scenarios of future climate change (IPCC, 2014), whereas chemical stressors were applied following realistic worst-case current scenarios (Hirsch et al., 1999; Rabiet et al., 2010). Among chemical stressors, diuron and erythromycin were selected because of their toxicity and occurrence in the environment. Diuron is a phenylurea herbicide widely used to control broadleaf in vineyard areas and flower gardens. Its mode of action is through the blockage of the chloroplast electron transport chain at the photosystem II (*PSII*) level, ultimately leading to the inhibition of photosynthesis (Moreland, 1980). Its concentration in the environment ranges from <1 μg L^{-1} to 10 μg L^{-1} during flood events (Rabiet et al., 2010). Erythromycin is a macrolide considered a wide-spectrum antibiotic against gram-positive and some gram-negative bacteria. The mode of action of erythromycin is through binding to the 23S rRNA molecule in the 50S subunit of the bacterial ribosome, which then blocks the elongation in growing peptide chains, thus inhibiting protein synthesis (Prescott et al., 2000). Erythromycin is commonly found in freshwater ecosystems and, although its concentration is on average low, it may be present at >5 μg L^{-1} at sewage treatment plant effluents (Hirsch et al., 1999).

This study aimed to evaluate the combined impact of 4 stressors on river biofilms. A main question was to determine whether these stressors lead to additive or non-additive responses. We hypothesized that: (i) chemical stressors will have targeted effects on specific biofilm components, consistent with their specific mode of action (ii) physical stressors will mostly have generalized effects, producing non-specific alteration in the selected response variables (iii) desiccation will affect the overall performance of biofilm algae and bacteria, making them sensitive to chemical stress (iv) higher water temperature will oppose the negative effect of chemical stressors by enhancing biofilm metabolism and (v) when occurring, non-additive responses will mostly be antagonistic, given the adaptation of river biofilms to high natural variability (Jackson et al., 2016), therefore showing an inherent capacity to adapt to multiple stressor effects.

2. Material and methods

2.1. Experimental design

We used glass microcosms in an experimental design that followed a full factorial replicated ($n = 4$) design with four factors and two levels per factor (2^4): erythromycin (E), diuron (D), desiccation (W) and water temperature (T) (Fig. 1). Both chemical stressors (E and D) were applied at nominal concentrations of 10 μg L^{-1}. These represent environmentally realistic concentrations though in the higher rank (Hirsch et al., 1999; Rabiet et al., 2010). Temperature was increased by 7 °C according to the predictions of short-term climatic extreme events (IPCC, 2014). Desiccation was applied by letting biofilms air-dry for 4 h. This caused a 70% decrease in photosynthetic efficiency, equivalent to that occurring under field-conditions after 5–6 days of complete desiccation (Acuña et al., 2015; Timoner et al., 2012).

Biofilms were exposed to the stressors over 40 h (impact period), and immediately allowed to recover for 40 h (recovery period) by removing all stressors. Biofilm response to multiple stressors was assessed at the end of the impact and the recovery periods using a variety of structural and functionally-related variables. Basal fluorescence (F_0) and ash-free dry weight (AFDW) were used as a surrogate of algal and total biofilm biomass, respectively (Sabater et al., 2007). Photosynthetic efficiency (Y_{eff}), photosynthetic capacity (Y_{max}) and non-photochemical quenching (NPQ) were used as photosynthetic descriptors of the primary producers in the biofilm. Y_{eff} and Y_{max} respectively indicate the effective and optimal photosynthetic activity, whereas NPQ indicates the algal capacity to dissipate light excess during stress conditions (Ponsatí et al., 2016). Leucine aminopeptidase activity (LAPA) relates to the biofilm capacity to transform dissolved organic nitrogen into inorganic compounds (Ylla et al., 2014). Community respiration (CR) accounts for the capacity of the biofilm community to oxidize organic compounds (Corcoll et al., 2015). Finally, the abundance of three gene transcripts was assessed by quantitative PCR (Smith and Osborn, 2009). The abundance of 16S rRNA and 18S rRNA gene transcripts was used to determine the status of bacterial and eukaryotic communities, respectively. The abundance of *psbA* gene transcript was used as a surrogate of the activity of the autotrophic compartment, as it codes for the D1 protein, which is the main component of the photosystem II (*PSII*) (Kim Tiam et al., 2012).

2.2. Experimental conditions

Each microcosm consisted on an independent glass crystallizer (diameter = 7 cm, height = 4 cm) filled with 100 mL of water and 10 colonized glass slides. Biofilms were grown in artificial channels (see details at Corcoll et al., 2015) for 4 weeks using an inoculum from a non-impacted reference water body, the Llémena river (Sant Esteve

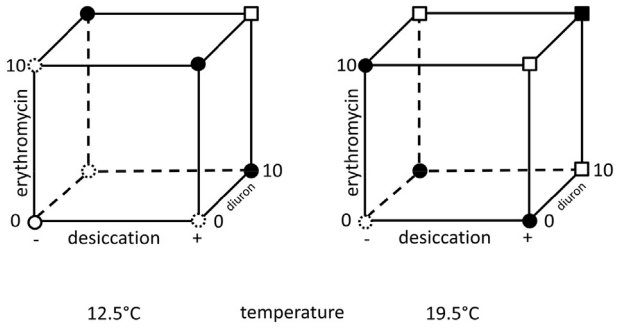


Fig. 1. Experimental design used in this study: full-factorial design with 4 factors and 2 levels per factor. The solid white circle represents the control case. Main effects are represented by dotted circles, 2-way interactions by solid black circles, 3-way interactions by solid squares and the 4-way interaction is represented by a solid black square. Concentrations are indicated in μg L^{-1}.