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# Can salinity trigger cascade effects on streams? A mesocosm approach

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## HIGHLIGHTS

- We evaluate the cascade effect of increased salinity on the rivers' food web.
- We combined the use of biomarkers with community-level data in a stream mesocosm.
- Both predation and salt had an effect on the aquatic invertebrate community.
- The presence of predators had a clear cascade effect.
- Predators were significantly stressed by salt addition.

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## ABSTRACT

Human activities have greatly increased the salt concentration of the world's rivers, and this might be amplified by water scarcity in the future. While the lethal effects of salinity have been documented for a wide variety of stream invertebrates, the sub-lethal effects (i.e. changes in biological condition without mortality) are not deeply understood yet. One important sub-lethal effect that has yet to be investigated is changes in predation efficiency, which could trigger cascade effects associated to the abundance of herbivorous invertebrates that control algae biomass. In this study we combined the use of biomarkers with community-level data in a stream mesocosm to evaluate the potential cascade effect of increased salinity on the trophic food web. Both predation and salt treatments had an effect on the aquatic invertebrate abundance, richness and community composition. The presence of predators had a clear cascade effect, it reduced herbivorous invertebrate abundance and richness leading to higher chlorophyll *a* concentrations. The salt treatment significantly reduced taxa richness, but only in the gravel bed. The predators were significantly stressed by salt addition, as shown by the different analyzed biomarkers. Concordantly, in the presence of predators, Tanytarsini registered higher abundances and chlorophyll *a* showed a lower concentration when salt was added. However, none of these changes was significant. Therefore, although salt addition significantly stressed *Dina lineata*, our results suggest that a longer exposure time is needed to fully capture cascading effects (e.g. a decrease in chlorophyll *a* due to a relaxation of predation on herbivorous invertebrates). We suggest that the potential cascade effects of salinization need to be evaluated when addressing the impacts of water scarcity (as caused by climate change and increasing water demand) on river ecosystems, since flow reductions will lead to higher salt concentrations.



## 1. Introduction

Many rivers of the world have salt concentrations higher than those expected from rock weathering and other natural sources (Gaillardet et al., 1999; Kaushal et al., 2005). This salt increase has originated from a wide variety of human activities like agriculture, industrial processes and resource extraction (Cañedo Argüelles et al., 2013). In the future, the combination of an intensification of such human activities and lower water availability is likely to further increase the salt concentration of rivers throughout the world. Since freshwater organisms need to keep an osmotic balance with the river water, they are very sensitive to changes in salinity (Williams, 2001; James et al., 2003). When the river salinity increases the most sensitive species, those who are unable to cope with high salinity increases, tend to disappear (Busse et al., 1999; Ferreri et al., 2004; Horrigan et al., 2005; Kefford et al., 2006) thus

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having an important impact on the ecosystem functioning (Fritz et al., 2010; Schäfer et al., 2012).

Despite its potential effects, the impact of elevated salinity on rivers has only recently been recognized as an environmental issue and many aspects of it remain understudied. Aquatic invertebrates are often used as model systems because they cover wide environmental gradients, possess a great variety of biological traits, respond fast to environmental changes and are easy to manipulate (Bonada et al., 2006; Rosenberg and Resh, 1993). While the lethal effects of salinity concentrations beyond certain levels have been documented for a wide variety of stream invertebrates (e.g. Berenzina, 2002; Kefford et al., 2005), the sub-lethal effects (i.e. changes in biological condition without mortality) are not deeply understood yet. Aquatic invertebrates regulate their ionic balance at an energetic cost (Bradley, 2008; Jonusaite et al., 2011), which can be translated into less energy available for other biological functions and reduced fitness. Although not many studies are available, there are evidences that elevated salinities can reduce egg hatchling (Kefford et al., 2004; Paradise, 2009), larval recruitment (Blakeslee et al., 2013) and growth (Kefford and Nugegoda, 2005; Johnson et al., 2014), and increase time to emergence of larvae as flying adults (Hassell et al., 2006; Lob and Silver, 2012; Hale et al., 2014). One important sub-lethal effect that has yet to be investigated is changes in predation efficiency (i.e. how much prey a predator can consume in a given period of time). Seeking, killing and consuming prey are energy demanding activities that are likely to be affected by osmotic stress (i.e. energy spent in osmoregulation can't be allocated in other physiological processes). Changes in predation could trigger cascade effects, since invertebrate predators consume herbivorous invertebrates that control algae biomass (McIntosh and Townsend, 1996). Although, to our knowledge, no research on the possible cascade effects of increased salinity has been conducted yet, a study on solar evaporation ponds found that high salinities favored a corixid predator that fed on the algae-grazing *Artemia* leading to an increase in chlorophyll *a* (Herbst, 2006). At the same time, in shallow brackish lakes salinity is thought to be responsible for the loss of algae-grazing *Daphnia*, leading to a regime shift from clear to turbid at high nutrient concentrations (Jeppesen et al., 2007). Therefore, we hypothesize that salinity-induced cascade effects might be also happening in rivers.

Observing cascade effects mediated by salinity is not simple, since salt toxicity might be acting simultaneously over all the components of the food web (from algae to predators). To overcome this limitation we used biomarkers to characterize the salinity-stress level of predators. A general pathway of toxicity induced by many chemical contaminants is related to their capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (ROS) to minimize oxidative damage to cellular components (Barata et al., 2005). Therefore, by analyzing the concentration of different antioxidant enzymes the stress level can be assessed. Another question to consider when studying salinity-induced cascade effects is the trade-off between realism and replicability. While field studies provide realistic conditions in which all the components of the ecosystem are taken into account, it is difficult to separate the effect of salinity from that of other environmental factors such as nutrient enrichment. Salinity is often used as a proxy for human disturbance in urban rivers (Paul and Meyer, 2001), since most wastewaters have high salt concentrations; therefore confounding effects of other stressors are very likely to emerge when studying salinization. On the other hand, laboratory assays offer the possibility to control all environmental conditions, but they are a poor representation of natural systems. Stream mesocosms offer a realistic alternative to field sampling and, at the same time, allow for examination of potential stressors on aquatic communities under controlled experimental conditions (Odum, 1984; Lamberti and Steinman, 1993).

In this study we combined the use of biomarkers with community-level data in a stream mesocosm to evaluate the potential cascade effect of increased salinity on the trophic food web. Our initial hypotheses were that:

1) The invertebrate predator (*Dina lineata*) would be significantly stressed by increased salinity.
2) Increased salinity would lead a reduction in the invertebrate total abundance and the number of taxa due to the loss of sensitive individuals.
3) Both increased salinity and predator presence would lead to changes in the invertebrate community composition. As being stressed by salinity, the effect of *D. lineata* on the invertebrate community composition would be affected by salinity.
4) The changes in the invertebrate community composition would propagate to lower trophic levels, as reflected by changes in chlorophyll *a* biomass.

## 2. Methods

The study was conducted in a 12-channel flow-through (i.e. no water re-circulation) artificial stream system (each channel with length: 2 m, width: 0.12 m, depth: 0.08 m) made from polyvinyl chloride (PVC) roof gutters. The water for the system was pumped from an irrigation channel that was fed with water of good quality (following European Union Water Framework Directive standards) from a section of the Llobregat River at Balsareny (sampling point L68 in Prat and Rieradevall, 2006), 60 km north of Barcelona, Spain. The pump provided a continuous supply of water of up to 20 L $s^{-1}$ to a 4000-L tank. A 2000 L tank contained saturated solution (250 g $L^{-1}$) of NaCl (common table salt, 96% purity, similar to the salts that enter the Llobregat River from adjacent salt mines) in river water that was continuously mixed using an industrial mixer. Both deposits fed 4 mixing tanks, each of them feeding three artificial stream channels. Flow in each of the streams was maintained at a constant rate of 0.33 L $s^{-1}$, similar to the hydraulic conditions present in nearby natural riffles. Baskets with 1-mm vinyl mesh were placed at the outlet of each stream to capture invertebrate drift, and they were emptied back into the artificial channels once a day during the experiment. Further information of the experimental facility and the location of the study site is provided in Grantham et al. (2012) and Cañedo Argüelles et al. (2012).

### 2.1. Pre-experiment colonization of the mesocosm

Stream invertebrates were collected the 25 of May of 2012 from a nearby (distance from the mesocosm ≈10 m) section of the Llobregat River using a 250 μm mesh-size hand-net. We selected a section with no presence of *D. lineata* in order to avoid collecting early instar *D. lineata* that could enter the treatments and alter the experimental design. Twelve adjacent riffles were kick-sampled for 1 min and the content of the net was gently placed into plastic trays with water. Each of the trays was emptied into one of the 12 artificial streams, providing a similar initial macroinvertebrate community resembling that of the river. The streambed of the channels was covered with gravel and 120 cobbles collected from the same stream section. The gravel and cobbles had been previously burnt (550 °C for 4 h) to remove attached organisms. Prior to treatment the water flowed trough the experimental channels for 17 days (from the 25/05/2012 to the 11/06/2012) to let the communities stabilize and allow for algal colonization. On day 18 the communities were subjected to 2 different treatments for 72 h: predator and salt addition. For the predation treatment 12 individuals of *D. lineata* (2 individuals per stream) of the same size were collected from the river and added to 6 of the streams (3 of which were also treated with salt). For the salt treatment salt-saturated solution was mixed with river water in 2 mixing tanks (Appendix 1) until reaching a conductivity of 7 mS $cm^{-1}$ (≈4.76 mg $L^{-1}$ NaCl). According to a previous experiment this conductivity maintained for 72 h should be enough to produce significant changes in the aquatic invertebrate community