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## An integrated perspective on salinization of freshwater ecosystems

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Salinization of freshwater ecosystems due to seawater intrusion and/or man-driven activities (e.g. salt use as de-icers) has gained much attention in the last years as it may lead to the loss of important services aside the evident lessen of biodiversity.

As an easier way to deliver information on the potential ecotoxicological effects of increased osmotic stress in freshwaters, many studies used sodium chloride (NaCl) as a surrogate salt. Despite, other ions are present in salt mixtures and, it has been suggested that ion-specific guidelines must be developed in order to construct more effective and environmentally protective frameworks. Yet, ecotoxicity data available for other salts is quite poor, outdated, and neglects the effects that may be caused to important ecological groups. A broader range of ecological groups and up-to-date information is urgently needed. Thus, this work aimed at: i) delivering new data on the ecotoxicity effects of other major salts sharing a common anionic form with NaCl ( $MgCl_2$ , KCl, and  $CaCl_2$ ); ii) deriving hazard concentrations that protect 5% of population ( $HC_5$ ) for each salt in order to compare and provide future working and discussion material intended to be integrated in the so long wanted ion-specific guidelines; iii) evaluating the suitability of other(s) salts as a substitute for NaCl, and that might constitute a more conservative approach for the protection of freshwater ecosystems; and, iv) within the perspective of climate-change associated sea level rise, induced salinization to compare the aforementioned  $HC_5$  values with that obtained for natural seawater (NSW).

To our knowledge, we provide here median effective concentrations for  $MgCl_2$ , KCl, and  $CaCl_2$  not reported before for two freshwater species: *Brachionus calyciflorus* (a filter-feeder) and *Hydra viridissima* (cnidarian). Furthermore, the following  $HC_5$  (in mg  $Cl^-/L$ ; and respective confidence limits at 95% and  $R^2$  - curve fitness) were obtained: 0.56 (0.38-0.83;  $R^2=0.92$ ) for NaCl; 0.12 (0.02-0.88;  $R^2=0.77$ ) for  $MgCl_2$ ; 0.26 (0.19-0.34;  $R^2=0.95$ ) for KCl; and 0.53 (0.32-0.82;  $R^2=0.90$ )  $CaCl_2$ . These values indicate firstly that the lack of data points is reflected in the spread of the confidence limits and the lowest adjustment of the curve to the model (e.g.,  $MgCl_2$ ) but also that the integration of different species is of great relevance due to the broad inter-species variability; secondly, that ecotoxicity induced by KCl is lower than that induced by NaCl and so, KCl might be in the future proposed as a surrogate for NaCl, although ecotoxicity data must be largely expanded so that solid conclusions can be withdrawn. Finally, the comparison of the  $HC_5$  here derived with those derived in previous works for NSW (1.17 and 6.64 mg  $Cl^-/L$  at sublethal and lethal levels) suggested that along with NaCl, also KCl might be used as a surrogate for NSW (with the cautions above mentioned).

This work aside from providing new data liable to be included in guidelines for the protection of freshwater systems, shows that this topic continues to require investment from laboratory (and field) research, but that this knowledge must be shared with regulatory agents and stakeholders, aiming accurate and targeted management actions.