

Review



Cite this article: Velasco J, Gutiérrez-Cánovas C, Botella-Cruz M, Sánchez-Fernández D, Arribas P, Carbonell JA, Millán A, Pallarés S. 2019 Effects of salinity changes on aquatic organisms in a multiple stressor context. *Phil. Trans. R. Soc. B* **374**: 20180011. <http://dx.doi.org/10.1098/rstb.2018.0011>

Accepted: 13 October 2018

One contribution of 23 to a theme issue ‘Salt in freshwaters: causes, ecological consequences and future prospects’.

Subject Areas:

ecology, environmental science, physiology

Keywords:

meta-analysis, salinization, dilution, additive effects, inland waters, transitional waters

Author for correspondence:

Josefa Velasco
e-mail: jvelasco@um.es

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4274648>.

Effects of salinity changes on aquatic organisms in a multiple stressor context

Josefa Velasco¹, Cayetano Gutiérrez-Cánovas², María Botella-Cruz¹, David Sánchez-Fernández^{1,3}, Paula Arribas⁴, José Antonio Carbonell⁵, Andrés Millán¹ and Susana Pallarés³

¹Department of Ecology and Hydrology, University of Murcia, Murcia, Spain

²Grup de Recerca Freshwater Ecology and Management (FEM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Diagonal 643, 08028 Barcelona, Catalonia, Spain

³Instituto de Ciencias Ambientales (ICAM), Universidad de Castilla-La Mancha, Toledo, Spain

⁴Island Ecology and Evolution Research Group, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), c/Astrofísico Francisco Sánchez 3, 38206 La Laguna, Islas Canarias, Spain

⁵Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

JV, 0000-0002-7457-0781

Under global change, the ion concentration of aquatic ecosystems is changing worldwide. Many freshwater ecosystems are being salinized by anthropogenic salt inputs, whereas many naturally saline ones are being diluted by agricultural drainages. This occurs concomitantly with changes in other stressors, which can result in additive, antagonistic or synergistic effects on organisms. We reviewed experimental studies that manipulated salinity and other abiotic stressors, on inland and transitional aquatic habitats, to (i) synthesize their main effects on organisms' performance, (ii) quantify the frequency of joint effect types across studies and (iii) determine the overall individual and joint effects and their variation among salinity–stressor pairs and organism groups using meta-analyses. Additive effects were slightly more frequent (54%) than non-additive ones (46%) across all the studies ($n = 105$ responses). However, antagonistic effects were dominant for the stressor pair salinity and toxicants (44%, $n = 43$), transitional habitats (48%, $n = 31$) and vertebrates (71%, $n = 21$). Meta-analyses showed detrimental additive joint effects of salinity and other stressors on organism performance and a greater individual impact of salinity than the other stressors. These results were consistent across stressor pairs and organism types. These findings suggest that strategies to mitigate multiple stressor impacts on aquatic ecosystems should prioritize restoring natural salinity concentrations.

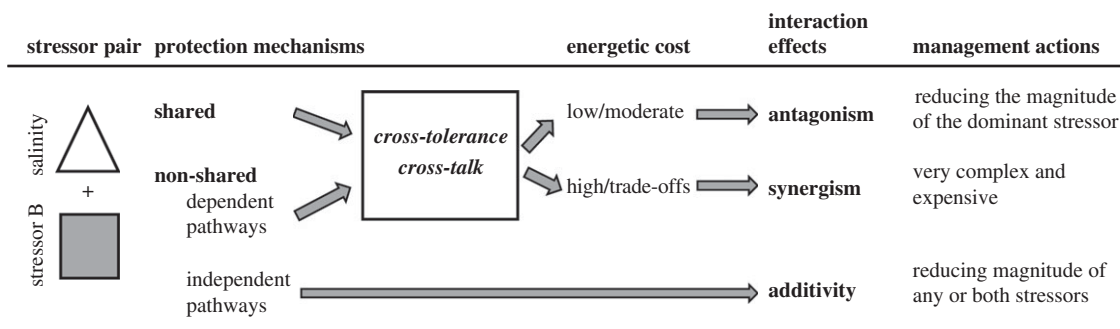
This article is part of the theme issue ‘Salt in freshwaters: causes, ecological consequences and future prospects’.

1. Introduction

In the face of global change, understanding and predicting the effects of multiple stressors is one of the most pressing challenges in conservation and applied ecology [1,2]. In particular, aquatic organisms are exposed to a growing number of stressors [3,4], such as freshwater salinization [5–7], water acidification [8,9] or eutrophication [10,11]. Given the heterogeneous nature and different mechanisms of actions of these stressors (e.g. physical versus chemical stressors), the co-occurrence of several of them can result in additive, synergistic or antagonistic effects on organism traits (e.g. survival, fecundity, metabolic and growth rates, etc.). Additive effects occur when joint stressor effects (i.e. cumulative effects *sensu* Crain *et al.* [12]) equal the sum of individual effects. Non-additive effects are reflected by a deviation from the additive response, which can be greater (synergism) or less (antagonism) than the sum of individual effects [13] and thus exacerbate or mitigate, respectively, the effects on organism performance

Box 1. Osmoregulation mechanisms to deal with salinity stress based on Bradley [27] and Rivera-Ingraham & Lignot [28].

organisms	osmoregulation mechanisms	physiological responses
osmoconformers only IIR mechanisms; most estuarine and marine species	isosmotic intracellular regulation (IIR) to adjust the intracellular osmotic pressure to meet that of the environment and minimize fluctuations in cell hydration	synthesis of compatible solutes, control of the permeability of cellular membranes
osmoregulators IIR and AER mechanisms	anisomotic extracellular osmoregulation (AER) to maintain body fluids volume, osmotic pressure and ionic composition under environmental salinity fluctuations, through changes in permeability and ion transport within different ion-transporting epithelia	
<ul style="list-style-type: none"> <i>hyper/iso osmoregulators</i> freshwater species 		active uptake of salts
<ul style="list-style-type: none"> <i>hyper/hypo osmoregulators</i> most inland saline, salt-marshes and some estuarine species 		reduction of extracellular water loss, increase of body water content, ion excretion

Box 2. Cross-tolerance/cross-talk framework to predict joint effects of stressor pairs depending on the overlap between protection mechanisms (based on Sinclair *et al.* [29]) and recommendations for management.

[14]. These changes at organism level are the primary and most sensitive responses to stress [15] but may ultimately alter community composition [16] and interfere with ecosystem processes and services, which sustain human welfare [17]. In recent years, several meta-analyses have synthesized the results of studies that have tested joint effects of multiple stressors in marine [12,18,19] and freshwater ecosystems [20] at different organizational levels, from organisms to communities, and have shown contrasting results. While an overall synergistic effect of multiple stressors has been found on marine systems, antagonistic joint effects dominate in freshwaters. However, none has specifically provided a comprehensive review of organism responses of inland aquatic species or populations to the combined effects of salinity changes with other global change stressors.

Human activities, like agriculture or salt mining, along with climatic aridification and rising sea levels, are increasing salt concentrations in inland freshwaters and coastal habitats [21], which produces severe negative economic and biological effects [6,22–24]. Contrarily, freshwater inputs, mainly caused by irrigated agriculture in arid landscapes, are diluting naturally saline rivers, estuaries and salt-marshes, with harmful effects [25]. At levels above or below the isosmotic point of organism internal fluids, salinity can disrupt metabolism and water balance [26]. Therefore, aquatic organisms have evolved different intra- and extracellular osmoregulation mechanisms

to control osmotic and dehydration stress in the face of salinity changes in the external environment [27,28] (box 1). However, organism osmoregulation capacities might be insufficient to deal with anthropogenic salinization and dilution and, most importantly, it is unknown whether the derived negative effects of these salinity changes can be amplified or mitigated in the presence of additional stressors.

The outcomes of multiple stressor interactions are context-dependent (type of ecosystem, trophic level, response level, response metrics, specific stressor pair, stress intensity and duration, etc.) [22,23,26]. At the physiological level, the joint effect of multiple environmental stressors ultimately depends on organism sensitivity to each stressor [2] and the overlap in the underlying mechanisms and molecular pathways used to combat their effects. Exposure to one stressor can enhance resistance to another if the same protective mechanism can cope with both stressors (cross-tolerance [29–31]) (box 2). Alternatively, different stressors may activate distinct mechanisms but dependent signalling regulatory pathways (cross-talk, e.g. [32]). In these cases, the resulting interaction effect depends on the energetic cost of the upregulated mechanisms. If such cost is low, antagonistic interactions would be expected. If there are energetic trade-offs between protective mechanisms, exposure to one stressor can compromise the response to the other and the general result could be a synergistic negative effect. Finally,

when independent mechanisms and pathways are activated, one stressor would have no effect on the response to the other, and an additive effect should be the most probable outcome. Some cross-tolerance and cross-talk responses involving salinity have been reported (e.g. [32–34]). Thus, this *cross-tolerance/cross-talk* framework, originally proposed for cold, desiccation and immune responses in overwintering insects [29], may be useful to yield broad-scale predictions of interactions among salinity and other stressors, which would require different management actions [1] (box 2).

Here, we review experimental studies that have explored the combined effects of changes in salinity and other key abiotic stressors associated with global change (e.g. temperature, pH, pollutants, etc.) at organism level on several physiological traits that determine the performance of aquatic organisms across inland (freshwater and saline) and transitional coastal ecosystems (estuaries and salt-marshes). Our aims were to (i) synthesize the main effects of salinity and other stressors at organism level and identify gaps in the salinity-multistressor literature, (ii) quantify the frequency of additive, synergistic and antagonistic joint effects and (iii) determine the overall individual and joint effects and their variation among salinity–stressor pairs and organism groups.

2. Methods

(a) Bibliographic search and screening

We focused on experimental studies that have assessed the effects of salinity and other stressors on organisms (autotrophs, invertebrates and vertebrates) of inland fresh and saline waters, along with estuarine and salt-marsh habitats (transitional habitats). We only considered experimental studies because they allow isolation of the effects of specific stressors from other confounding factors that cannot be controlled in the field. We searched the literature using Web of Science (last accessed in June 2018) with the following sequence of field tags and Boolean operators in advanced searches: (((TS= ((salin* OR *osmotic* OR conductivity) AND (temperature OR heat OR thermal OR hypoxia OR nutrient* OR radiation OR humidity OR drought OR dehydration OR desiccation OR *ionic* OR pollut* OR insecticide OR pesticide OR acidity OR 'pH' OR metals) AND (freshwater OR aquatic NOT marine) AND (physiolog*)))))). We refined the search by *stress** and no restriction was placed on publication year. From the resulting papers, we selected only those that had applied a full-factorial design, including a clearly defined control treatment (or a treatment deemed by the authors to be under non-stressful conditions for any of the stressors), treatments with one level or more of each single stressor and combined treatments of all the stressors. We also included studies from the cited literature of the selected papers that met these criteria but did not appear in the literature search. From this first filter by experimental design, we obtained two datasets.

Dataset 1 (electronic supplementary material S1) contained only those studies that statistically tested the interaction effect. These studies were used for an initial exploration of the individual and joint stressor effects reported and to identify knowledge gaps in the salinity-multistressor literature. We retrieved information for each experiment in each study to characterize stressor pairs salinity (stressor A), plus temperature, nutrients, metals, pesticides, CO₂, hypoxia, sulfate or pH (stressor B), organism (autotroph, invertebrate or vertebrate), habitat (inland freshwater, inland saline or transitional) and response type (survival and tolerance limits; fitness measurements,

including metabolic rates, growth and reproduction traits; molecular responses; physiological regulation, including osmotic capacity and metal uptake or accumulation measurements; and behaviour). We determined: (i) the significance of individual and joint effects by exploring the results of the statistical analyses performed in each study; (ii) the direction of such effects in individual performance terms compared with the control conditions (i.e. negative (worse performance) or positive (enhanced performance)) by looking at the *post hoc* tests and/or plots with errors. In multilevel experiments (i.e. those with more than two levels of each stressor), we focused on the highest level before the total mortality of individuals because the magnitude and direction of the joint effects could vary across the different levels of each stressor.

Dataset 2 (electronic supplementary material S2) was obtained by selecting exclusively those studies that report either raw data or mean, standard deviation and sample sizes for control, single and combined-stressor treatments. These data were used to quantify the frequency of joint effect types and for meta-analyses (see below). In this dataset, we simplified stressor B categories into temperature, desiccation, nutrients and toxicants (including metals, pesticides, pH and sulfates). We considered sulfates as a separate stressor from salinity because it could potentiate the negative osmotic effects of increasing salinity [35,36].

(b) Joint effect types and meta-analyses

From dataset 2 (electronic supplementary material S2), we calculated the individual, main and joint effect sizes for each experiment and study using Hedge's *d* according to factorial meta-analysis methods, where a significant interaction effect signifies deviation from the null model of additivity [37] (see meta-analysis details in electronic supplementary material S3). Individual effects reflect the response to one stressor alone in relation to the control. Main effects represent the individual stressor effect plus its contribution to the interaction effect, calculated in the presence and absence of the other stressor. To ensure a positive relationship of the response variables with performance, we inverted the sign of main and individual effect sizes from experiments that measured response variables negatively related with performance (e.g. mortality response was transformed into survival response by changing the effect size sign). To make a quantitative assessment of interaction type frequencies, interaction effects were classified using effect sizes according to Crain *et al.* [12], i.e. additive interactions were those whose 95% confidence interval (CI) include zero value. Synergistic interactions were those in which both individual effect sizes were negative, or one was negative and the other positive, and the interaction effect size was significantly lower than zero. Antagonism occurred when the interaction effect size was bigger than zero and at least one individual effect size was negative. Because studies with two positive individual effects had interaction terms opposite for the majority of studies with negative individual effects, the interaction effect sizes for these studies were inverted [12].

We used a random-effects model meta-analysis to determine the weighted mean effect sizes of the main and joint effects of stressors for the studies included in dataset 2 (electronic supplementary material S2) using the *metafor* R package [38]. Different meta-analyses were performed with the data subsets that allowed consistent analyses by considering a relatively balanced distribution of studies and effect sizes across moderator categories or groups (organism type, habitat). The selected categorical moderators were treated as fixed effects to assess the mean interaction effects at each level of all the categories (where $n \geq 10$) (see electronic supplementary material S4 for more details). Firstly, we conducted an overall meta-analysis across all the

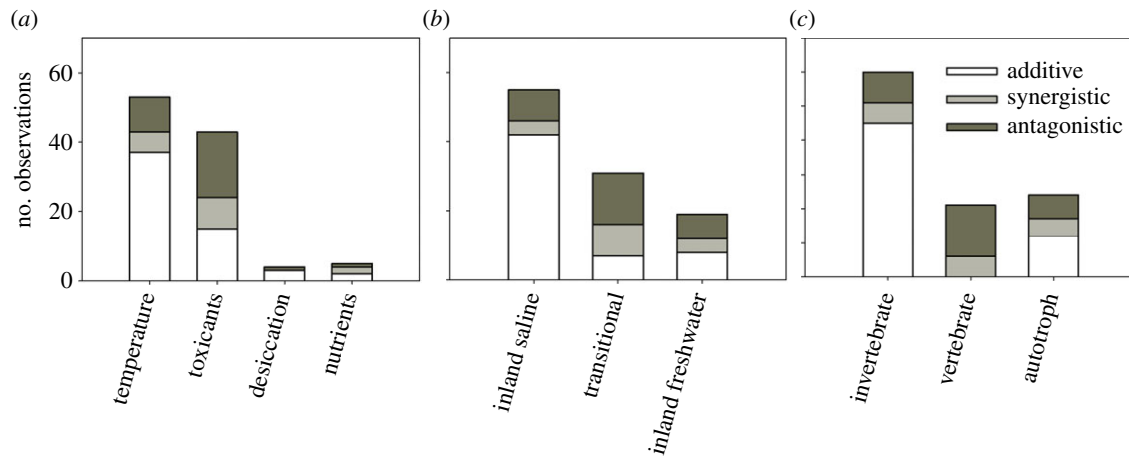


Figure 1. Frequency distribution of the interaction types across (a) stressor pairs, (b) habitat and (c) organism groups, estimated from effect size calculations and categorized following the classification of Crain *et al.* [12] (see electronic supplementary material S2). (Online version in colour.)

studies that tested salinity increase effects ($n = 85$) and other stressors using organism type as the moderator (autotroph, $n = 21$; invertebrate, $n = 43$; vertebrate, $n = 21$). We also conducted meta-regressions to analyse how overall effect sizes varied with (i) publication year ($n = 85$), (ii) the magnitude of salinity change (treatment – control) ($n = 83$) and (iii) the magnitude of temperature change ($n = 38$). A second meta-analysis was done for autotrophs ($n = 21$) using habitat as the moderator (inland freshwater, $n = 10$, versus transitional, $n = 11$). Finally, for inland saline water invertebrates, three subgroup meta-analyses were run for the following stressor pairs: salinity increase \times temperature increase ($n = 29$), salinity decrease \times temperature increase ($n = 16$) and salinity increase \times toxicant increase ($n = 9$). We assessed publication bias in all the meta-analyses by using funnel plots and Rosenthal's fail-safe number [39].

The codes of the functions used to run all these analyses, which were performed with R v. 3.3.2 [40], are available in electronic supplementary material S5.

3. Results and discussion

Of 2157 screened articles, only 64 studies met our experimental design criteria. Of these, 45 papers tested interaction effects and were reviewed, including a total of 208 study cases from 46 distinct species (dataset 1, electronic supplementary material S1). We obtained quantitative data from 24 papers to determine the frequency of interaction types and conduct the meta-analyses, in which the main effect sizes of stressors and their interaction were estimated for 105 study cases of 28 species (dataset 2, electronic supplementary material S2).

(a) Research contributions and gaps in the salinity-multistressor literature

Although many experimental studies have tested the effects of salinity and other stressors in combination, only a small proportion of them have employed full-factorial experimental designs and appropriate analytical approaches to identify non-additive joint effects. In dataset 1 (electronic supplementary material S1), the statistical analyses most frequently used to test interaction effects were ANOVA-type models, which assume a simple addition model as the null model [2]. Most studies tested more than two levels of each stressor, but only two of the 45 reviewed studies [41,42] used five or

more treatment levels, essential to establish a reliable stressor–effect relationship from a factorial experiment [2].

Multiple stressor studies are clearly biased toward certain stressor pairs, habitats and organisms. The most frequently studied stressors in combination with salinity were temperature (34% of study cases) and metals (24%). However, other relevant stressors have received less attention (e.g. nutrients and desiccation stress). The most represented habitats were transitional ones (55%), followed by inland saline (26%) and freshwater ecosystems (18%). The number of observations made on vertebrate and invertebrate organisms was similar (36 and 34%, respectively), while autotrophs were less represented (30%). Molecular responses (e.g. activity or gene expression of ion transporter and antioxidant enzymes), and survival and tolerance limits, as well as fitness measurements (e.g. growth, reproduction and metabolic rates), were the most frequently studied traits (see electronic supplementary material S1).

The individual effect of salinity decreased organism performance in most of the observations (43%, e.g. decreased survival and growth, increased osmolyte concentration in body fluids, changed metabolic rates, etc.) and was positive in only 20% of the responses, most frequently increasing survival or tolerance to heat or cold stress. Similarly, the individual effects of the other stressors (named stressor B, see the Methods section) resulted in worse performance in most cases, but enhanced it in 30% of the cases. Approximately 50% of the studies reported significant non-additive effects of combined stressors, among which most decreased organism performance, mainly survival.

(b) Frequency of additive, antagonistic and synergistic effects

The classification of joint effect types based on effect size estimates (see electronic supplementary material S2) yielded a higher frequency of additive (54%) than antagonistic (30%) and synergistic effects (16%). These patterns varied across stressor pairs, habitat or organism categories. Additive effects were more frequent in the stressor pair salinity \times temperature, inland saline habitats and invertebrates. However, antagonistic effects dominated for the combination of salinity with toxicants, and in both transitional habitats and vertebrates (figure 1).

These results can be discussed within the cross-tolerance/cross-talk framework earlier described (box 2). In the case of

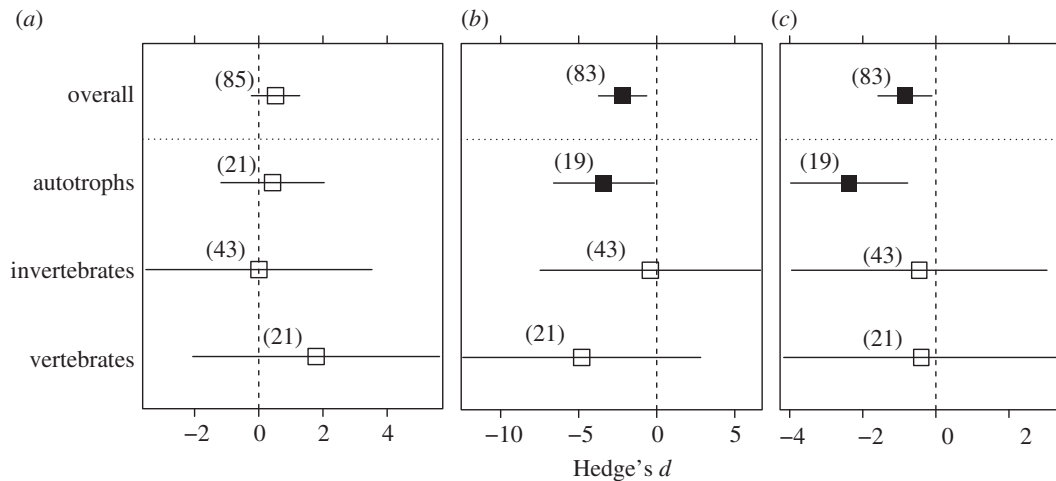


Figure 2. Mean effect sizes (Hedge's $d \pm 95\%$ confidence intervals), overall and by organism groups of (a) joint effect, (b) salinity individual main effect and (c) stressors B individual main effect. The number of observations (n) of each analysis is indicated in parentheses. Filled black squares indicate significant effects ($p < 0.05$).

high temperature and salinity, different physiological mechanisms are activated (i.e. heat shock and osmoregulatory responses, respectively), so they are more likely to interact in an additive manner, as we generally observed. For example, Garreta-Lara *et al.* [43] found a strong influence of salinity on the metabolomic profile of the invertebrate *Daphnia magna*, but no significant interaction with temperature. Though less frequent, some synergistic and antagonistic responses between salinity and temperature were also found (e.g. [44] in dataset 2, electronic supplementary material S2; see also [45] in this issue), which suggests that the mechanistic relationship between heat and osmotic stress is still not well understood.

Unlike high temperature, common homeostatic and excretory mechanisms are primarily used against the stress induced by salinity and metals, although each particular metal elicits other specific responses once it has accumulated in the organism [46]. Partially shared mechanisms and common regulatory pathways could explain the higher frequency of antagonisms found in the salinity and toxicant stressor pair (most corresponded to osmoconformer estuarine, anadromous and catadromous fish), a pattern that has also been observed in marine ecosystems [12]. For example, De Polo *et al.* [47] identified enzyme carbonic anhydrase (CA2) as the mechanistic link at the molecular level involved in the antagonistic effects of copper and osmotic stress on ion homeostasis in the estuarine fish *Cyprinodon variegatus*. In estuarine and marine invertebrates, increased salinity generally protects against the negative effects of metals [48], which can be partly explained by competitive interactions with major cations for sensitive ion transport sites [49]. These competitive interactions diminish under low salinity conditions because of lower concentrations of free ions, which facilitates metal uptake [50]. In freshwaters, poor osmoregulator species, for which these cross-protective effects of salinity likely play a smaller role, may be much more vulnerable to water pollution by metals than are saline species.

Antagonistic interactions between salinity and pesticides are also typical. In this case, neuroendocrine responses may be involved as cholinesterase inhibition is the main mode of pesticide action [51]. For example, hypersaline acclimation reduces mortality in subsequent exposure to chlorpyrifos in the euryhaline anadromous fish *Salmo trutta*, and it

has been suggested that this protective effect could be associated with reduced neuronal signalling under hypersaline conditions [52].

A poorly explored stressor pair with shared protective physiological mechanisms is desiccation and salinity. Both stressors disrupt water and ionic balance and thus cross-tolerance might be expected (box 2). Indeed, antagonistic responses to these stressors are common in plants [53] and have been found in some aquatic insects [34,54], in which the pre-activation of osmoregulatory mechanisms during salinity exposure seems to contribute to minimize water loss during a subsequent desiccation exposure.

Interestingly, in most antagonistic interaction cases, the individual effects of stressors were both negative, which means that although the negative impact is mitigated in the presence of both stressors, they still produce a reduction in organism performance (e.g. the upper thermal limit of saline water beetles decreases after acclimation at stressful salinities and temperatures, but less than under each stress alone [55]). Opposing individual effects leading to antagonistic interactions typically occur with nutrients, whose positive effects can overcompensate for the negative effect of salinity (e.g. [56]), as happens with toxicants [12]. We also found, among the few cases of synergistic interactions, opposing individual effects (electronic supplementary material S2), mostly between salinity and toxicants. For example, in cyanobacteria, the activity of the antioxidant enzyme peroxidase increased in the presence of Cu or Cu + NaCl, but not of NaCl alone [57].

(c) Overall individual and joint stressor effects: meta-analyses

The meta-analysis conducted on all the salinity increase studies and organism groups revealed an overall additive joint effect ($d = 0.527 \pm 0.379$, $p = 0.164$, $n = 85$; figure 2a; electronic supplementary material S4). When this dataset was moderated by organisms, joint effects were also additive for autotrophs ($d = 0.464 \pm 0.793$, $p = 0.559$, $n = 21$), invertebrates ($d = 0.005 \pm 0.952$, $p = 0.630$, $n = 43$) and vertebrates ($d = 1.777 \pm 1.117$, $p = 0.240$, $n = 21$). The individual mean effect sizes of salinity increase ($d = -2.223 \pm 0.779$, $p = 0.004$, $n = 83$) and stressor B ($d = -0.907 \pm 0.378$, $p = 0.017$,

$n = 83$) were significantly negative (figure 2*b,c*). Remarkably, the mean effect size of salinity increase was more than two times higher than the effect size of stressor B. In addition, overall salinity effect size became more negative with time of study publication ($d = -0.326 \pm 0.152$, $p = 0.032$, $n = 83$). When analysed with organism taken as a moderator, we found significant negative effects of salinity ($d = -3.499 \pm 1.593$, $p = 0.028$, $n = 21$) and stressor B ($d = -2.553 \pm 0.796$, $p = 0.001$, $n = 21$) on autotrophs (figure 2*b,c*).

In the autotrophs subgroup meta-analysis, the joint effects of salinity and stressor B were also additive in both freshwater ($d = 1.053 \pm 1.402$, $p = 0.452$, $n = 10$) and transitional habitats ($d = 0.104 \pm 1.949$, $p = 0.626$, $n = 11$). In the meta-analyses performed with the subset of studies on invertebrates occurring in inland saline waters, we found additive overall joint effects for increasing salinity–temperature ($d = 0.396 \pm 0.324$, $p = 0.223$, $n = 19$, figure 3*a*), decreasing salinity–temperature ($d = -0.257 \pm 0.215$, $p = 0.231$, $n = 29$, figure 3*b*) and increasing salinity–toxicants stressor pairs ($d = -0.068 \pm 0.669$, $p = 0.919$, $n = 9$, figure 3*c*). Salinity increase did not have a significant main individual effect but the main effect of salinity decrease was negative ($d = -0.652 \pm 0.23$, $p = 0.005$). Such negative effect of salinity decrease contrasts with the general pattern of high survival of saline insects in freshwater–low salinity conditions found in a more extensive review of this topic [58]. Temperature had no significant effect, while the individual main effect of toxicants was significantly negative ($d = -0.670 \pm 0.323$, $p = 0.038$). The significant results found in the meta-analyses were generally robust against publication bias according to the symmetry observed in funnel plots (see electronic supplementary material S3) and Rosenthal fail-safe numbers greater than critical thresholds (see electronic supplementary material S4).

The overall and relative magnitude of stressors may play a critical role in determining their interactive effects (e.g. [15]). However, our meta-regressions showed no significant relationships between the absolute salinity or temperature changes and the joint or individual effect sizes (see electronic supplementary material S4). Joint effects of multiple stressors also depend on the timing at which they act [14,31]. When stressors operate sequentially, additive effects are more likely to occur because homeostasis can be re-established in the time between exposure to the first and second stressor. By contrast, interactive effects are more frequent when the two stressors act simultaneously or very close in time. In our study, this effect was controlled because the vast majority of the experimental designs included simultaneous exposure to both stressors.

Overall, our findings revealed no interactive effects (i.e. additive effects) of salinity changes in combination with other stressors, which contrasts with the overall synergistic effects reported for marine systems [12] and the overall antagonistic effect of multiple stressor pairs found in freshwaters [20]. Nonetheless, our results should be cautiously compared with other meta-analysis studies, for several reasons. First, responses at different organizational level are highly heterogeneous [12]. Second, Crain *et al.* [12] and Jackson *et al.* [20] did not focus specifically on salinity (it was pooled with other chemical stressors in [20]) but explored instead a wide range of stressor pairs. One possible explanation for the dominance of additive effects and the higher frequency of antagonisms than synergisms in our study is that salinity may frequently act as a dominant stressor, so that the other

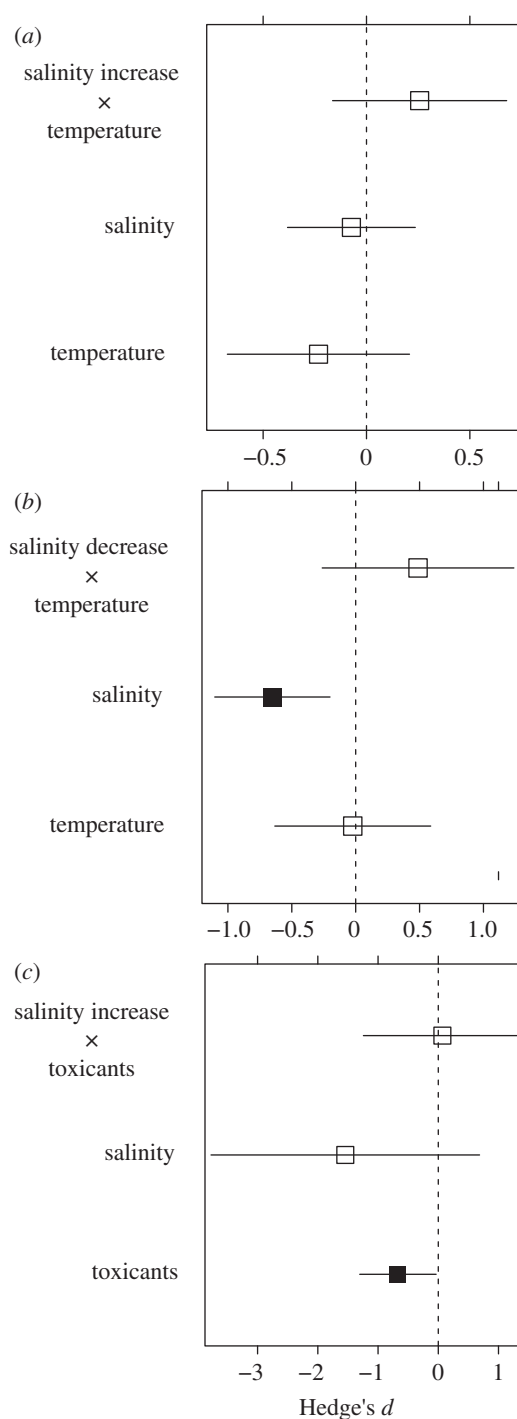


Figure 3. Mean effect sizes (Hedge's $d \pm 95\%$ confidence intervals) on joint and individual main effects on inland saline invertebrates for (a) salinity increase and temperature, (b) salinity decrease and temperature, and (c) salinity increase and toxicants. Filled black squares indicate significant effects ($p < 0.05$).

stressors have little additional effect [2,15]. Indeed, the overall individual effect of salinity increase was generally higher than those of the other stressors analysed, such as temperature. Szöcs *et al.* [59] also found a greater effect of salinity than pesticides on macroinvertebrate communities, and no significant interaction effect between these stressors. These results have important implications for management of aquatic ecosystems. Mitigation strategies aimed at reducing the magnitude of salinity changes could reduce significantly the impact on organisms and substantially improve the health of populations and communities, as other authors have previously suggested [12,60]. In any case, the notable

variability in the importance of the interaction types among stressor pairs in different aquatic systems suggests that responses are highly context-dependent and, therefore, a general framework for predicting interactions and guiding management could be difficult to establish [1].

Our comprehensive review included taxa with different habitats, life-history traits, stressor sensitivities and evolutionary histories (i.e. colonization from marine or terrestrial environments, transitions from fresh to saline waters, etc.), as well as a variety of strategies to cope with salinity stress. For example, while most marine and transitional water organisms are osmoconformers, the majority of organisms in saline inland waters are osmoregulators, such as aquatic insects of terrestrial origin [61–63], and can cope with wide salinity fluctuations by hyper-regulation capacity in freshwater and hypo-regulation capacity in saline waters [62] (box 1). Thus, it remains to be investigated how these different osmoregulatory strategies and their associated energetic costs determine the type of interactions with other stressors.

4. Concluding remarks and future perspectives

The multiple stressor studies reviewed herein focus primarily on the combined effects of increasing salinity and increasing temperature or toxicants (metals and pesticides), while other important stressor combinations have received very little attention (e.g. desiccation or nutrients). The number of multiple stressor experimental studies conducted in inland waters is still limited compared with those on transitional and marine systems. Thus, more research efforts are needed in freshwater and saline inland waters, which are particularly vulnerable to multiple global change pressures [1,64].

Additive effects of salinity and other stressors were prevalent, but antagonistic interactions were relatively frequent in some organism groups (vertebrates), habitats (transitional waters) or stressor pairs (salinity \times toxicants). Salinity has a stronger negative individual effect on organismal performance traits than other stressors, which highlights the need to increase management efforts for this single stressor (box 2).

From this review, some considerations for future research arise. First, we need to improve our understanding of the mechanisms and pathways by which a single stressor modulates the physiological responses to other stressors. Second, to analyse multi-stressor effects, models more complex than additive ones should be applied if stressor–effect relationships and the correlation between organism’s sensitivity to each stressor are known [2,65]. Third, to better understand and predict the effects of ongoing salinization and dilution processes in aquatic ecosystems, it is crucial to explore the role of the origin and evolution of the osmoregulation strategies of aquatic organisms in determining the type of interactions that arise between salinity and other stressors.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors’ contributions. J.V. conceived, designed and coordinated the study and drafted part of the manuscript; S.P. participated in the study design, carried out the literature review, data selection and analyses, created figure 1 and electronic supplementary material S1 and S2 and drafted part of the manuscript; C.G.-C. carried out the meta-analyses, created figures 2 and 3, and electronic supplementary material S3, S4 and S5, and drafted part of the manuscript; M.B.-C. carried out the literature review, data selection and analyses, and created electronic supplementary material S1 and S2, and the reference lists; D.S.-F., P.A., J.A.C. and A.M. contributed with experimental data. All the authors discussed the results and the manuscript revision and gave final approval for publication.

Competing interests. We have no competing interests.

Funding. Some physiological studies of the interaction of pair–stressors salinity–temperature, salinity–ionic composition and salinity–desiccation on the performance of aquatic saline insects were done by the Aquatic Ecology Research Group (University of Murcia, Spain) as part of the I + D + i projects CGL2010-15378 (J.V.) and CGL2013-48950-C2-2-P (J.V. and A.M.) (Spanish Ministry of Economy and Competitiveness) co-funded by FEDER funds. C.G.-C. and P.A. are supported by ‘Juan de la Cierva-Formación’ research contracts (MINECO, FJCI-2015-25785 and FJCI-2014-20581, respectively), D.S.-F. by a post-doctoral contract funded by the Universidad de Castilla-La Mancha and the European Social Fund (ESF) and M.B.-C. by a PhD grant from the Universidad de Murcia.

Acknowledgements. We thank Miguel Cañedo-Argüelles for inviting us to contribute to this monographic volume, and Cristina Coccia for providing experimental data. We are also grateful to three anonymous referees who carried out constructive reviews of this work.

References

- Côté IM, Darling ES, Brown CJ. 2016 Interactions among ecosystem stressors and their importance in conservation. *Proc. R. Soc. B* **283**, 1–9. (doi:10.1098/rspb.2015.2592)
- Schäfer RB, Piggott JJ. 2018 Advancing understanding and prediction in multiple stressor research through a mechanistic basis for null models. *Glob. Chang. Biol.* **24**, 1817–1826. (doi:10.1111/gcb.14073)
- Sala OE *et al.* 2000 Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774. (doi:10.1126/science.287.5459.1770)
- Grizzetti B, Pistocchi A, Liqueste C, Udias A, Bouraoui F, Van De Bund W. 2017 Human pressures and ecological status of European rivers. *Sci. Rep.* **7**, 1–11. (doi:10.1038/s41598-017-00324-3)
- Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz CJ. 2013 Salinisation of rivers: an urgent ecological issue. *Environ. Pollut.* **173**, 157–167. (doi:10.1016/j.envpol.2012.10.011)
- Cañedo-Argüelles M *et al.* 2016 Saving freshwater from salts. *Science* **351**, 914–916. (doi:10.1126/science.aad3488)
- Abellán P, Sánchez-Fernández D, Picazo F, Millán A, Lobo JM, Ribera I. 2013 Preserving the evolutionary history of freshwater biota in Iberian National Parks. *Biol. Conserv.* **162**, 116–126. (doi:10.1016/j.biocon.2013.04.001)
- Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP. 2013 Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Chang. Biol.* **19**, 1884–1896. (doi:10.1111/gcb.12179)
- Muniz IP. 1990 Freshwater acidification: its effects on species and communities of freshwater microbes, plants and animals. *Proc. R. Soc. Edinb. B* **97**, 227–254. (doi:10.1017/S026972700005364)
- Smith V. 2003 Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environ. Sci. Pollut. Res.* **10**, 126–139. (doi:10.1065/espr2002.12.142)
- Smith VH, Schindler DW. 2009 Eutrophication science: where do we go from here? *Trends Ecol. Evol.* **24**, 201–207. (doi:10.1016/j.tree.2008.11.009)
- Crain CM, Kroeker K, Halpern BS. 2008 Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol. Lett.* **11**, 1304–1315. (doi:10.1111/j.1461-0248.2008.01253.x)
- Sperfeld E, Raubenheimer D, Wacker A. 2016 Bridging factorial and gradient concepts of resource co-limitation: towards a general framework applied to consumers. *Ecol. Lett.* **19**, 201–215. (doi:10.1111/ele.12554)

14. Gunderson AR, Armstrong EJ, Stillman JH. 2016 Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Ann. Rev. Mar. Sci.* **8**, 357–378. (doi:10.1146/annurev-marine-122414-033953)
15. Folt C, Chen C. 1999 Synergism and antagonism among multiple stressors. *Limnol. Oceanogr.* **44**, 864–877. (doi:10.4319/lo.1999.44.3)
16. Gutiérrez-Cánovas C *et al.* 2019 Do all roads lead to Rome? Exploring community trajectories in response to anthropogenic salinization and dilution of rivers. *Phil. Trans. R. Soc. B* **374**, 20180009. (doi:10.1098/rstb.2018.0009)
17. Berger E, Frör O, Schäfer RB. 2019 Salinity impacts on river ecosystem processes: a critical mini-review. *Phil. Trans. R. Soc. B* **374**, 20180010. (doi:10.1098/rstb.2018.0010)
18. Harvey BP, Gwynn-Jones D, Moore PJ. 2013 Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.* **3**, 1016–1030. (doi:10.1002/ece3.516)
19. Przeslawski R, Byrne M, Mellin C. 2015 A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Glob. Chang. Biol.* **21**, 2122–2140. (doi:10.1111/gcb.12833)
20. Jackson MC, Loewen CJG, Vinebrooke RD, Chimimba CT. 2016 Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Glob. Chang. Biol.* **22**, 180–189. (doi:10.1111/gcb.13028)
21. Venâncio C, Castro BB, Ribeiro R, Antunes SC, Abrantes N, Soares AMVM, Lopes I. 2019 Sensitivity of freshwater species under single and multigenerational exposure to seawater intrusion. *Phil. Trans. R. Soc. B* **374**, 20180252. (doi:10.1098/rstb.2018.0252)
22. Karim MF, Mimura N. 2008 Impacts of climate change and sea-level rise on cyclonic storm surge floods in Bangladesh. *Glob. Environ. Chang.* **18**, 490–500. (doi:10.1016/j.gloenvcha.2008.05.002)
23. Bhuiyan MJAN, Dutta D. 2012 Assessing impacts of sea level rise on river salinity in the Gorai River network, Bangladesh. *Estuar. Coast. Shelf Sci.* **96**, 219–227. (doi:10.1016/j.ecss.2011.11.005)
24. Nielsen DL, Brock MA. 2009 Modified water regime and salinity as a consequence of climate change: prospects for wetlands of southern Australia. *Clim. Change* **95**, 523–533. (doi:10.1007/s10584-009-9564-8)
25. Millán A, Velasco J, Gutiérrez-Cánovas C, Arribas P, Picazo F, Sánchez-Fernández D, Abellán P. 2011 Mediterranean saline streams in southeast Spain: what do we know? *J. Arid Environ.* **75**, 1352–1359. (doi:10.1016/j.jaridenv.2010.12.010)
26. Kefford BJ, Papas PJ, Crowther D, Nuggeoda D. 2002 Are salts toxicants? *Australas. J. Ecotoxicol.* **8**, 63–68.
27. Bradley TJ. 2009 *Animal osmoregulation*. New York: Oxford University Press.
28. Rivera-Ingraham GA, Lignot J-H. 2017 Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. *J. Exp. Biol.* **220**, 1749–1760. (doi:10.1242/jeb.135624)
29. Sinclair BJ, Ferguson LV, Salehipour-Shirazi G, Macmillan HA. 2013 Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr. Comp. Biol.* **53**, 545–556. (doi:10.1093/icb/ict004)
30. MacMillan HA, Walsh JP, Sinclair BJ. 2009 The effects of selection for cold tolerance on cross-tolerance to other environmental stressors in *Drosophila melanogaster*. *Insect Sci.* **16**, 263–276. (doi:10.1111/j.1744-7917.2009.01251.x)
31. Todgham AE, Stillman JH. 2013 Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. *Integr. Comp. Biol.* **53**, 539–544. (doi:10.1093/icb/ict086)
32. Uyhelji HA, Cheng C, Besansky NJ. 2016 Transcriptomic differences between euryhaline and stenohaline malaria vector sibling species in response to salinity stress. *Mol. Ecol.* **25**, 2210–2225. (doi:10.1111/mec.13609)
33. Davies SA, Overend G, Sebastian S, Cundall M, Cabrero P, Dow JAT, Terhazs S. 2012 Immune and stress response ‘cross-talk’ in the *Drosophila* Malpighian tubule. *J. Insect Physiol.* **58**, 488–497. (doi:10.1016/j.jinsphys.2012.01.008)
34. Pallarés S, Botella-Cruz M, Arribas P, Millán A, Velasco J. 2017 Aquatic insects in a multistress environment: cross-tolerance to salinity and desiccation. *J. Exp. Biol.* **220**, 1277–1286. (doi:10.1242/jeb.152108)
35. Zaliniak L, Kefford BJ, Nuggeoda D. 2006 Is all salinity the same? I. The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates. *Mar. Freshwater Res.* **57**, 75–82. (doi:10.1071/MF05103)
36. Buchwalter D, Scheibener S, Chou H, Soucek D, Elphick J. 2019 Are sulfate effects in the mayfly *Neocloeon triangulifer* driven by the cost of ion regulation? *Phil. Trans. R. Soc. B* **374**, 20180013. (doi:10.1098/rstb.2018.0013)
37. Gurevitch J, Morrison JA, Hedges LV. 2000 The interaction between competition and predation: a meta-analysis of field experiments. *Am. Nat.* **155**, 435–453. (doi:10.1086/303337)
38. Viechtbauer W. 2010 Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.* **36**, 1–48. (doi:10.18637/jss.v036.i03)
39. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. 2009 *Introduction to meta-analysis*. Chichester: John Wiley & Sons.
40. R Core Team. 2016 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <https://www.r-project.org>.
41. Heilmayer O, Digialleonardo J, Qian L, Roesijadi G. 2008 Stress tolerance of a subtropical *Crassostrea virginica* population to the combined effects of temperature and salinity. *Estuar. Coast. Shelf Sci.* **79**, 179–185. (doi:10.1016/j.ecss.2008.03.022)
42. Santos B, Ribeiro R, Domingues I, Pereira R, Soares AMVM, Lopes I. 2013 Salinity and copper interactive effects on Perez’s frog *Pelophylax perezii*. *Environ. Toxicol. Chem.* **32**, 1864–1872. (doi:10.1002/etc.2257)
43. Garreta-Lara E, Campos B, Barata C, Lacorte S, Tauler R. 2018 Combined effects of salinity, temperature and hypoxia on *Daphnia magna* metabolism. *Sci. Total Environ.* **610**, 602–612. (doi:10.1016/j.scitotenv.2017.05.190)
44. Hopkins GR, French SS, Brodie ED. 2017 Interacting stressors and the potential for adaptation in a changing world?: responses of populations and individuals. *R. Soc. open sci.* **4**, 161057. (doi:10.1098/rsos.161057)
45. Jackson JK, Funk DH. 2019 Temperature affects acute mayfly responses to elevated salinity: implications for toxicity of road de-icing salts. *Phil. Trans. R. Soc. B* **374**, 20180081. (doi:10.1098/rstb.2018.0081)
46. Furness P, Rainbow S. 1990 *Heavy metals in the marine environment*. Boca Raton, FL: CRC Press.
47. De Polo A, Margiotta-Casaluci L, Lockyer AE, Scrimshaw MD. 2014 A new role for carbonic anhydrase 2 in the response of fish to copper and osmotic stress: implications for multi-stressor studies. *PLoS ONE* **9**, e107707. (doi:10.1371/journal.pone.0107707)
48. Heugens EHW, Hendriks AJ, Dekker T, Straalen NM, Admiraal W. 2001 A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Crit. Rev. Toxicol.* **31**, 247–284. (doi:10.1080/20014091111695)
49. Baysoy E, Atlı G, Gürlür CÖ, Dogan Z, Eroglu A, Kocalar K, Canlı M. 2012 The effects of increased freshwater salinity in the bioavailability of metals (Cr, Pb) and effects on antioxidant systems of *Oreochromis niloticus*. *Ecotoxicol. Environ. Saf.* **84**, 249–253. (doi:10.1016/j.ecoenv.2012.07.017)
50. McLusky DS, Bryant V, Campbell R. 1986 The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* **24**, 481–520.
51. Thompson CM, Richardson RJ. 2003 Anticholinesterase insecticides. In *Pesticide toxicology and international regulation* (eds TC Mars, B Ballantine), pp. 89–127. Chichester, UK: John Wiley & Sons, Ltd.
52. Amiri BM, Xu EG, Kupscio A, Giroux H, Moseinzadeh M, Schlenk D. 2018 The effect of chlorpyrifos on salinity acclimation of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **195**, 97–102. (doi:10.1016/j.aquatox.2017.12.011)
53. Tuteja N. 2007 Mechanisms of high salinity tolerance in plants. *Methods Enzymol.* **428**, 419–438. (doi:10.1016/S0076-6879(07)28024-3)
54. Elnitsky MA, Benoit JB, Lopez-Martinez G, Denlinger DL, Lee RE. 2009 Osmoregulation and salinity tolerance in the Antarctic midge, *Belgica antarctica*: seawater exposure confers enhanced tolerance to freezing and dehydration. *J. Exp. Biol.* **212**, 2864–2871. (doi:10.1242/jeb.034173)
55. Arribas P, Velasco J, Abellán P, Sánchez-Fernández D, Andújar C, Calosi P, Millán A, Ribera I, Bilton DT.

- 2012 Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *J. Biogeogr.* **39**, 984–994. (doi:10.1111/j.1365-2699.2011.02641.x)
56. Kamer K, Fong P. 2001 Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*. *Mar. Ecol. Prog. Ser.* **218**, 87–93. (doi:10.3354/meps218087)
57. Karimi R, Norastehnia A, Abbaspour H, Saedisar S NA. 2012 Toxicity assessment of *Anabaena* sp. following exposure to copper oxide nanoparticles and sodium chloride. *Appl. Ecol. Environ. Res.* **15**, 1–15. (doi:10.15666/aeer/1504_20452059)
58. Arribas P, Gutiérrez-Cánovas C, Botella-Cruz M, Cañedo-Argüelles M, Antonio Carbonell J, Millán A, Pallarés S, Velasco J, Sánchez-Fernández D. 2019 Insect communities in saline waters consist of realized but not fundamental niche specialists. *Phil. Trans. R. Soc. B* **374**, 20180008. (doi:10.1098/rstb.2018.0008)
59. Szöcs E, Kefford BJ, Schäfer RB. 2012 Is there an interaction of the effects of salinity and pesticides on the community structure of macroinvertebrates? *Sci. Total Environ.* **437**, 121–126. (doi:10.1016/j.scitotenv.2012.07.066)
60. Kath J, Thomson JR, Thompson RM, Kefford BJ, Dyer FJ, Mac Nally R. 2018 Interactions among stressors may be weak: implications for management of freshwater macroinvertebrate communities. *Divers. Distrib.* **24**, 939–950. (doi:10.1111/ddi.12737)
61. Herbst DB. 2001 Gradients of salinity stress, environmental stability and water chemistry as a template for defining habitat types and physiological strategies in inland salt waters. *Hydrobiologia* **466**, 209–219. (doi:10.1023/A:1014508026349)
62. Pallarés S, Arribas P, Bilton DT, Millán A, Velasco J. 2015 The comparative osmoregulatory ability of two water beetle genera whose species span the fresh-hypersaline gradient in inland waters (Coleoptera: Dytiscidae, Hydrophilidae). *PLoS ONE* **10**, e0124299. (doi:10.1371/journal.pone.0124299)
63. Pallarés S, Arribas P, Bilton DT, Millán A, Velasco J, Ribera I. 2017 The chicken or the egg? Adaptation to desiccation and salinity tolerance in a lineage of water beetles. *Mol. Ecol.* **26**, 5614–5628. (doi:10.1111/mec.14334)
64. Dudgeon D *et al.* 2006 Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev. Camb. Phil. Soc.* **81**, 163–182. (doi:10.1017/S1464793105006950)
65. Liess M, Foit K, Knillmann S, Schäfer RB, Liess HD. 2016 Predicting the synergy of multiple stress effects. *Sci. Rep.* **6**, 1–8. (doi:10.1038/srep32965)