



Energetics as a lens to understanding aquatic insect's responses to changing temperature, dissolved oxygen and salinity regimes

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Assemblages of aquatic insects are structured by multiple biotic and abiotic conditions, including temperature, salinity and oxygen. Here we highlight recent developments in our understanding of how high temperatures, elevated salinities and low oxygen levels affect physiological processes, responses at the organismal level, and impacts on species interaction and community assembly. As aquatic insects may be exposed to multiple stressors, we review their sensitivity to interactive effects of multiple stressors. While each of these stressors may operate via different physiological mechanisms, they all influence the overall energy budget as well as the allocation of energy to competing functions such as homeostatic maintenance, growth, development and reproduction. As such, there is potential for interaction whereby one stressor may exacerbate the effect of another stressor. Integrating research on these stressors can provide a powerful approach for delineating the sensitivity of aquatic insects to multiple stressors and developing sound management practices.

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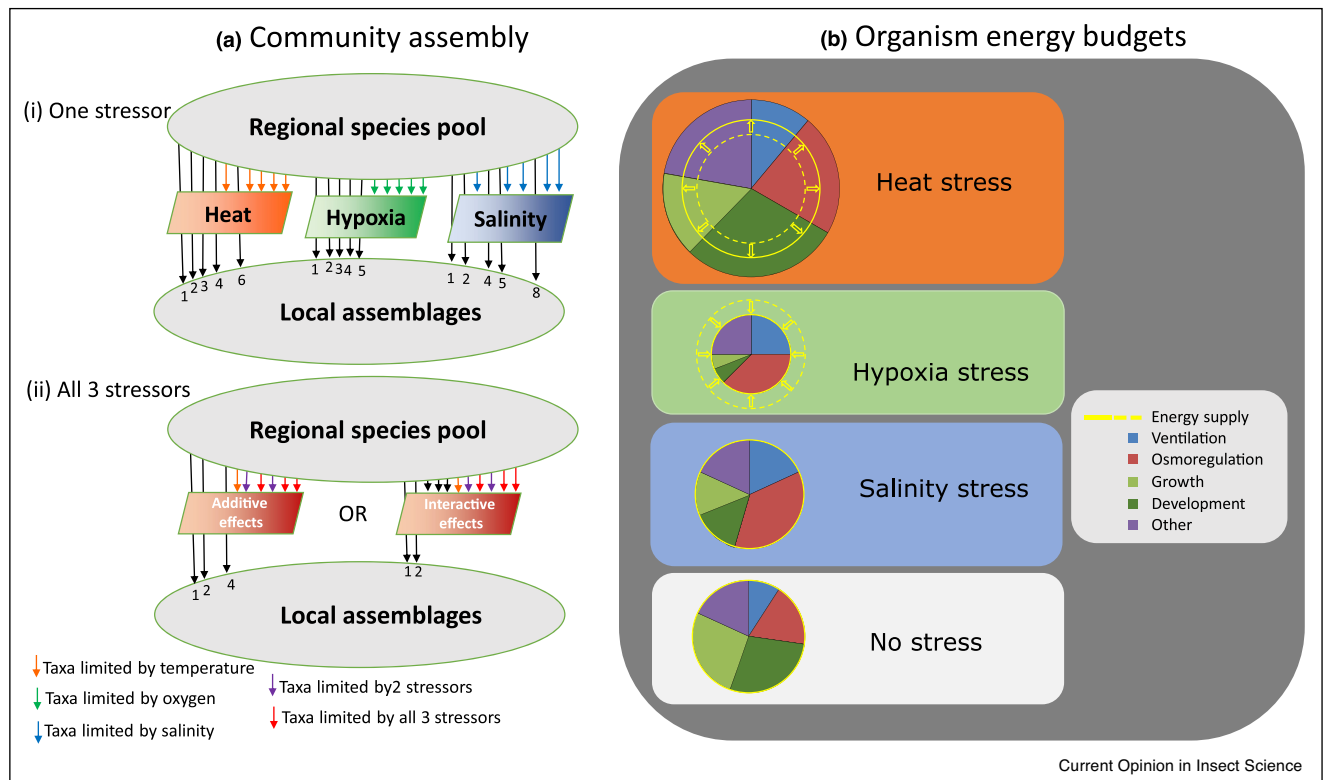
Temperature, salinity and dissolved oxygen as important niche axes

Assemblages of aquatic insects are structured by a multitude of biotic and abiotic conditions. High water temperature, elevated salinity and low levels of dissolved oxygen can act as stressors, directly impacting the physiology of aquatic insects and in turn their distributions (e.g. [1,2,3,4••]).

Temperature, salinity and dissolved oxygen therefore constitute important niche axes (*sensu* Hutchinson [5]). Here we review responses to these stressors at the level of individual organisms and species assemblages, focusing on their (potential) interactive effects (Figure 1). Interactive effects can arise because of coincident exposure (for example, warm waters tend to be more likely to deoxygenate) or dependent physiological sensitivity (warm water increases the respiration rate of aquatic insects, thus limiting their ability to cope with low dissolved oxygen). In addition, stressor induced changes in ecological interactions such as competition, predation and mutualisms can indirectly impact organisms that are not directly affected by a stressor. In the following paragraphs we briefly review how the three stressors directly impact aquatic insect physiology, life-history and geography, highlighting physiological and ecological interactions. We then highlight gaps in knowledge and outline directions for future research.

While water temperature, salinity and oxygen levels naturally vary in space and time, they are widely altered by anthropogenic influences (e.g. [6,7]). Yet, the scale of this variation differs. Variation in salinity, defined as the total concentration of dissolved inorganic ions (chiefly Na^+ , Ca^{2+} , Mg^{2+} , K^+ , Cl^- , SO_4^{2-} , HCO_3^- , CO_3^{2-}) and often determined from electrical conductivity, will be mainly manifested across space, varying naturally, depending on the mineralogy of rocks, sea spray, evapotranspiration, etc. in the catchment. Exposure of aquatic insects to elevated salinity arising from human activities occurs via effluents from industrial activities, various forms of mining and natural resource extraction, irrigation of arid lands, vegetational changes and the runoff of salts used in road de-icing. Spatial variation in water temperature across microhabitats will be generally small relative to those in terrestrial habitats (owing to the high heat conductivity of water) as will be temporal changes, especially in larger waters (owing to the high heat capacitance). In contrast, oxygen saturation levels can strongly vary between different microhabitats, especially in standing waters where equilibration with atmospheric PO_2 is slow. In running waters, water convection dissipates gradients in oxygen saturation, although dense leaf litter packs and the hyporheic zone may still present contrasting oxygen conditions. In small eutrophic waters, hypoxic events tend to grow more severe during the night when respiration is not counteracted by photosynthesis, whereas during daytime, insects can be exposed to hyperoxia [8•]. Also, low oxygen saturation is more likely in

Figure 1



Conceptual diagrams showing effects of temperature, dissolved oxygen and salinity as abiotic factors determining community assembly (a, left panel), via their effect on energy budgets (b, right panel). Individually each of the stressors can act as a filter for different species (numbered 1–10), although there is likely co-variation in tolerance. Insects can also be exposed to multiple stressors simultaneously, in which case two alternative scenarios are envisaged: i) in the case of additive effects, a species can persist if it manages to pass through all three filters individually, while ii) in the case of interactive effects, a species (e.g. species 4) that could theoretically cope with each of the three stressors in isolation is still unable to do so when exposed to multiple stressors. The number of species that pass through the filters, are given to illustrate the direction of the change. In panel b, schematic energy budgets are depicted in response to the different stressors. Energy supply is indicated by a yellow circle and the allocation of energy to various demands is illustrated by the pie charts. These pie charts are not drawn to scale (and will vary between species) but are rather intended as illustrating the direction of changes in energy allocation or changes in the overall energy budget. Each stressor changes supply and allocation to demand in different ways and can be compared to the standard (no stress). Under heat stress, animals increase both supply and demand, but increases in demand tend to outstrip those in supply (i.e. the yellow circle is smaller than the pie chart). Energy allocation changes include increased expenditures associated with ventilation, osmoregulation and general maintenance costs leaving a smaller fraction of energy for growth and development. In addition, the allocation of energy becomes biased more towards development rather than growth. Under hypoxia, energy supply is constrained, with more energy being spent on ventilation, and the reduction in energy reduces growth and delays development. Under salinity stress, supply is unaltered, but increased costs for osmoregulation sap the energy budget, leaving less energy for growth and development, although their relative allocation is unaffected and animals tend to mature at the same size.

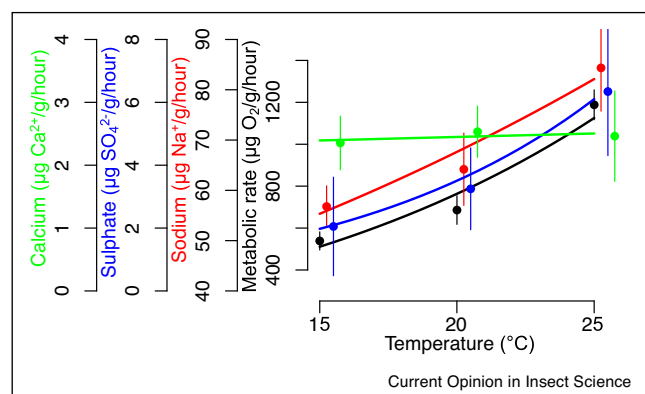
warmer waters where oxygen consumption rates are higher. Even under fully saturated conditions, oxygen availability may change as temperature or salinity changes (see [9]), although the effects of salinity on oxygen availability are modest given the range of salinity in inland waters (from a few mg/L to 100 s of g/L). Thus the spatial and temporal scale at which these three factors vary can be different, having repercussions for where and when insects are exposed to multiple stressors. Under some conditions, stressors are more likely to coincide, such as low flow (high temperatures, low oxygen saturation, and potentially increased salinity owing to less dilution of natural and anthropogenic salt sources), runoff

from agricultural lands (low oxygen, high salinity), and low rainfall (high temperatures, high salinity). A drier, hotter climate will also result in warmer, more hypoxic waters, greater evapotranspiration and thus increased salinity.

An energetic perspective grounded in ecophysiology

In this review we focus on stressful effects associated with high temperatures and salinity and low oxygen, although we acknowledge the opposite side of the spectrum may also be stressful. The osmolarity of insect hemolymph ranges from 250 to 400 mmol kg⁻¹ [10] which means that

Figure 2



Temperature has non-linear effects on metabolic rate and aqueous ion uptake rates in the mayfly *N. triangulifer*. While transport of Na^+ and SO_4^{2-} vary in magnitude, both increase in tandem with metabolic rate. In contrast, Ca^{2+} uptake did not vary with temperature (Adapted from [35]).

for all insects, freshwater constitutes a hypo-osmotic environment. Thus extremely dilute waters can also be physiologically stressful to freshwater insects as they have to maintain strong osmotic gradients between the external water and their internal fluids and/or they suffer deficiencies in essential elements such as Ca^{2+} , Mg^{2+} or Na^+ [11]. Likewise, there is evidence that long-term exposure to hyperoxia can also be stressful, especially for insects that normally live in oxygen poor habitats [12], likely because of increased ROS production (see [8]).

An energetics perspective can be used as a lens to understanding aquatic insect responses to each of these stressors (Figure 1b). At high temperatures, more energy is spent on protein synthesis and ion pumping to combat the increase in entropy at higher temperatures (e.g. Figure 2), which manifests itself as a faster inactivation and denaturation of proteins and dissipation of ion gradients. Salinity stress can also be considered as an energetic issue. Ion transporting cells (chloride cells or ionocytes) are rich in mitochondria, generating the ATP necessary to pump ions from relatively dilute water to ionically richer hemolymph. As ion uptake rates follow Michaelis–Menten type kinetics, uptake rates increase in tandem with the ionic concentrations of the water they live in (e.g. [13]). However, excessive ionic uptake must be balanced by increased ionic excretion rates via the insect renal system to maintain internal homeostasis [13]. To date, the strongest evidence for the energetic costs of increasing salinity comes from studies where developmental delays are associated with increasing salinity stress (e.g. a 2.6-fold decrease in growth rates [14] and up to a 1.5-fold increase in development time [15]). Conversely, slightly elevated salinity may actually enhance

growth or development rates in some freshwater insects (e.g. a 1.5-fold increase in growth rate [11]).

Owing to the 15-fold greater efficiency of aerobic metabolism to generate ATP compared to anaerobic metabolism, energy demand can be translated into demand for oxygen. Compared to in air, the availability of oxygen is much reduced in water [16] and aquatic insects display a diversity of adaptations to deal with the challenge of breathing under water (see [17]). Taken together, temperature, salinity (both ATP demanding), and oxygen availability (ATP supply) are linked by energetics. Understanding the interactions between them is important, and these have received much attention for temperature and oxygen (see below), whereas interactions between temperature and salinity, or all three of these stressors remain poorly studied.

Temperature–oxygen interactions

There is evidence that hypoxia reduces the heat tolerance of aquatic insects, whereas their hypoxia tolerance is improved in colder water [18–21]. One explanation for such interactions between temperature and oxygen is that the increased oxygen demand at higher temperatures cannot be met by a corresponding increase in oxygen supply [22,23]. While studies have documented responses consistent with oxygen limitation (e.g. activation of HIF pathways, onset of anaerobic metabolism) at thermal extremes in aquatic insects (e.g. [20,24]), it is not clear if chronic effects of heat stress that manifest themselves at longer timescales are also mediated by oxygen limitation [21]. Prolonged exposure to stressful temperatures (under normoxia) did not result in upregulation of genes responsive to hypoxia in a mayfly species, whereas exposure to hypoxia did [24]. Factors other than physiological hypoxia contributed to temperature related fitness and survival deficits in this species [25]. Insects have a wide range of responses to low oxygen, ranging from increased gill ventilation to morphological changes in the tracheal system and metabolic reorganization [8]. Such compensatory changes may increase the capacity for oxygen uptake and mitigate the effects of low oxygen, high temperature, or both.

Prolonged exposure to warm water frequently results in insects growing to a smaller size [26,27], which can at least be proximally explained by warming temperatures having a disproportionately stronger effect on shortening development time than their stimulating effect on growth rates. Such size reductions have also been interpreted as a compensatory response to the increased demand of oxygen at high temperatures [23,28]. Assuming that larger individuals are more prone to hypoxia (something that has not been rigorously tested in aquatic insects) attaining maturity at a smaller size could be beneficial for maintaining aerobic scope. Consistent with this assumption, Piggot et al. [29] reported decreases in the mean body size

of aquatic invertebrate assemblages under warming, which was driven by the exclusion of larger size classes. While the effect of temperature on body size is magnified under low oxygen in terrestrial insects [30] and aquatic crustaceans [28], studies that manipulate both oxygen and temperature during the development of aquatic insects are lacking.

Field data on different mayfly species have provided evidence for interactive effects between oxygen conditions and temperature, with poor water oxygenation reducing mayfly occurrence, especially at warmer sites [3]. A comparison between these field data and experimental data on acute thermal stress indicated that the interaction between oxygen and temperature could be more influential under field conditions, which were less extreme and ecologically more relevant. Moreover, at stream sites where high flows mitigated oxygen stress, such combined effects of warming and oxygen stress were less apparent. The ability of aquatic insects to generate water flow can reduce the thickness of the boundary layer and thereby promote oxygen uptake. Water convection significantly augmented diffusive oxygen uptake in a river bug that employs plastron respiration (*Aphelocheirus aestivalis*), contributing to averting oxygen limitation at high temperatures [31**].

Other field studies also corroborate the idea that effects of warming are manifested via oxygen. For example, organic pollution manifests itself as oxygen deficits and Jourdan et al. [32] reported a loss of pollution sensitive taxa under warming in UK streams. Over a 13-year period of increased temperatures and reduced rainfall in Australia, Chessman [33] showed a loss of taxa with a preference not only for colder waters but also for faster flowing habitats. One important consequence of such interactive effects is that these negative trends in aquatic insects may be counteracted by improving water quality [34*] and restoring flows.

Temperature interactions with salinity

There are few laboratory studies on salinity-temperature interactions in freshwater insects. Recent work by Orr and Buchwalter [35*] demonstrated that $^{35}\text{SO}_4^{2-}$ and $^{22}\text{Na}^+$ uptake rates generally increased with increasing temperature in mayflies and a caddisfly. These temperature-induced increases in uptake rates closely matched increases in oxygen consumption rates, suggesting that increased metabolic rates affect the uptake of these ions, likely because insects will ventilate respiratory surfaces more extensively to meet oxygen demand (Figure 2). The alternative (temperature induces increased ion uptake which then affects oxygen consumption rates) is less likely as increasing salinity (and associated increased ion uptake rates) does not appear to consistently elevate metabolic rates. Thus, both high salinity and (temperature-induced) increases in ventilation will cause insects to

excessively take up ions which then must be excreted via the renal system. This is presumably an energetically expensive process and may explain why increasing temperature has been reported to heighten the detrimental effects of salts such as SO_4^{2-} [35*,36*] and how elevated salinities can become stressful even at concentrations lower than the osmolality of the hemolymph [37*,38]. However, uptake of $^{45}\text{Ca}^{2+}$ did not follow the same pattern and transport rates of this ion were consistently lower. It is possible that dietary acquisition of Ca^{2+} remains the primary mechanism of uptake, as gastrointestinal tract acquisition of ions would be the presumed ancestral state for these secondarily aquatic organisms.

Studies on salinity-hypoxia interactions in freshwater insects are especially rare. Under normoxia, rates of oxygen consumption were not affected by salinity in aquatic beetles [39], but increased with salinity in corixid bugs, especially at the higher temperature [40]. It is possible that aquatic insects experience osmoregulatory stress at high temperatures as has been suggested for aquatic crustaceans (e.g. [41]), driving a greater demand for ATP and hence oxygen, but too few taxa have been studied to date to make such a generalization. Also, a greater energy requirement for osmoregulation may be resolved by reallocating energy rather than increasing oxygen uptake (Figure 1). In this respect, it would be useful to examine whether ion transport rates change in response to hypoxia.

The cost of osmoregulation is reflected in delayed development when insects are exposed to elevated salinity [11,15**,42] and here the effects of salinity differ from those of temperature. Warming results in faster growth rates over a markedly reduced timeframe, resulting in adults being smaller and less fecund. Thus, warming increases energy supply, and energy is allocated preferentially to development (e.g. Figure 1; [27**]). Salinity challenges appear to incur an energetic cost, delaying growth and development. However, the remaining energy is not allocated differently between growth and development as animals grow to more or less the same adult size with the exception of individuals exposed to overtly toxic SO_4^{2-} concentrations [15**]. Future studies should examine the longer-term interactions of temperature and salinity from both a physiological and life history perspective.

Because insect groups differ strongly in their sensitivity to salinity, significant shifts in community structure are observed in salinized waters. Mayflies are especially sensitive with about 50% of mayflies appearing to suffer significant mortality at external osmolality less than the osmolality of their hemolymph ([38]; Table 1). Other aquatic insects, e.g. Coleoptera, Hemiptera and some Diptera by contrast can even thrive in hypersaline lakes (Table 1). However, species inhabiting saline waters are

Table 1

Overview of sensitivity to each of the three stressors for major groups of insects. 3 indicates most sensitive to the stressor indicated, 1 most tolerant. Respiration modes distinguished: cA: convection of air into an open tracheal network; diffusion of dissolved oxygen into a closed tracheal system with (dW+) or without tracheal gills (dW-); dWA: diffusion of dissolved oxygen into an air store (plastron/physical gill) from which convection of air takes place into an open tracheal network; cW: convection of water to aid diffusion of dissolved oxygen into a closed tracheal system, either facilitated by flow through a case (some Trichoptera) or a branchial chamber (Odonata: Anisoptera).

(main) mode of respiration	major groups of aquatic insects	Sensitivity to stressors: heat, salt, hypoxia;	
		standing waters	running waters
dW-/dW+	Plecoptera	● ● ●	● ● ●
dW+	Ephemeroptera	● ● ●	● ● ●
dW+	Zygoptera (odonata)	● ● ●	● ● ●
dW+	Megaloptera	● ● ●	● ● ●
dW-/dW+	some Diptera and Trichoptera	● ● ●	● ● ●
dW+	Coleoptera (most larvae)	● ● ●	● ● ●
dWA	some Coleoptera and Hemiptera	● ● ●	● ● ●
cW	some Trichoptera	● ● ●	● ● ●
cW	Anisoptera (odonata)	● ● ●	● ● ●
cA	Some diptera	● ● ●	● ● ●
cA	adult Coleoptera and Hemiptera	● ● ●	● ● ●

generalists in their fundamental niches: they can tolerate a wide variety of salinities but avoid competition and predation by inhabiting saline waters [43]. A mesocosm study similarly found a large role for biological interactions mediating taxon responses to salinity [44]. These findings are also consistent with salinity stress representing an energetic burden but to different degrees for different organisms, which are more clearly manifested during species interactions.

Despite interactions between ion uptake rates and temperature (Figure 2) and their effects on energy budgets (Figure 1b), Velasco et al. [45] reported in their meta-analysis of existing studies that additive effects between temperature and salinity were most common and in cases where there were non-additive effects, antagonistic effects were more common than synergistic effects. The commonness of additive effect could be because the effects of salinity dominate the effect of temperature (see [46]) at the levels of temperature and salinity considered by most existing literature.

Respiratory mode as a major determinant of sensitivity to salt, heat and hypoxia

A logical extension of the energetics perspective applied to the three stressors reviewed here is that vulnerability across major groups of insects may strongly co-vary with their mode of respiration (Table 1). Sufficient oxygen must be present to convert nutritional resources to ATP, and aquatic insects differ in their mode of respiration, ranging from a closed tracheal system with cutaneous gas exchange to an open tracheal system with aerial gas exchange (see Table 1). An insect's vulnerability to the

combined effects of warming and hypoxia has indeed been related to a species' capacities for regulating oxygen uptake, with those employing convective flow of water or having access to atmospheric oxygen being less vulnerable than those relying on plastrons or cutaneous respiration [21,47]. Animals can increase heat tolerance when having previously experienced warmer or hypoxic conditions. While such phenotypic plasticity (or acclimation) is usually not sufficient to fully compensate for increases in warming, the degree of plasticity varies between species. Interestingly, in a group of water beetles that can breathe underwater via setal gills, a mode of respiration akin to plastron breathing, there are relationships between the density of gills, their habitat and their plasticity in heat tolerance [48], with species relying more on extracting oxygen from the water rather than from air displaying limited plasticity.

The mode of respiration will also affect an aquatic insect's ability to generate the energy necessary for coping with salinity stress. Air-breathing aquatic insects, e.g. adult beetles and hemipterans, are not only free from reliance on dissolved oxygen, but may also maintain a less water and ion-permeable cuticle and thus have a lower exposure to salinity stress [49]. Conversely, in gilled taxa, such as mayflies, ionocytes are located on the gills, likely contributing to their high susceptibility to stressor combinations: increasing respiration (e.g. by increasing water convection across gill surfaces) or morphological enlargements of gill surfaces will increase capacity for oxygen uptake, but at the same time may result in enhanced uptake of ions. In damselflies, gill autotomy (amputation of the gill as an antipredator mechanism) reduces surface area and hence

capacity for oxygen uptake, which reduced their heat tolerance but improved their tolerance to toxicants [50]. Still, Kefford et al. [11] observed little difference in the length of gills of the final aquatic instar of damselfly that developed at different salinities, suggesting that plasticity in gill length is low or that other functions of the gills such as locomotion override the expected gill reductions. Adult insects that rely on plastron breathing, while relying on dissolved oxygen, have been found to be far less susceptible to toxicants [51], as the surrounding water and hence the toxicants do not touch the integument.

In conclusion, combined effects of salinity, temperature and oxygen on freshwater insects may be highly related to fundamental traits such as mode of respiration.

Future research directions

The influence of any abiotic stressor is determined by exposure frequency, duration and magnitude coupled with species-specific physiological traits. There is ample opportunity for these three stressors to co-occur in freshwaters, and therefore aquatic insects will often be exposed to a combination of these stressors. In addition, although the pathways by which each stressor may elicit responses will differ for salinity stress [38], extreme heat [21] and hypoxia [8^{*}], they share energetic consequences. This could result in correlations between sensitivities across species and traits, such as gill respiration which could render a species sensitive to one stressor may predispose it to also be sensitive to another stressor, see also Table 1). Partly, such correlated sensitivities will be a function of their mode of respiration, but they could also relate to other aspects of their metabolic niche. While temperature will affect the whole energy budget, the effects of salinity and hypoxia are more specific, representing an increase in energetic costs or a decrease in energetic supply (Figure 1). Key areas for future research include comparative studies linking respiration and salinity, temperature and hypoxia tolerance across a range of species exhibiting different modes of respiration, addressing questions such as: How much energy do aquatic organism spend on ion regulation? How are these costs prioritized in the face of hypoxia and how do they change with temperature? To what extent can physiological mechanisms involved in dealing with salinity, heat and hypoxia be generalized across insect groups? And what are the consequences of (multiple) stressors for their life history outcomes (size at maturity, development time, fecundity) which will ultimately govern their fitness?

Declarations of interest

None.

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