RESEARCH ARTICLE

The cumulative impacts of anthropogenic stressors vary markedly along environmental gradients

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Abstract

Understanding the cumulative effects of multiple stressors on biodiversity is key to managing their impacts. Stressor interactions are often studied using an additive/antagonistic/synergistic typology, aimed at identifying situations where individual stressor effects are reduced or amplified when they act in combination. Here, we analysed variation in the family richness of stream macroinvertebrates in the groups Ephemeroptera, Plecoptera and Trichoptera (EPT) at 4658 sites spanning a 32° latitudinal range in eastern Australia in relation to two largely human-induced stressors, salinity and turbidity, and two environmental gradients, temperature and slope. The cumulative and interactive effect of salinity and turbidity on EPT family richness varied across the landscape and by habitat (edge or riffle) such that we observed additive, antagonistic and synergistic outcomes depending on the environmental context. Our findings highlight the importance of understanding the consistency of multiple stressor impacts, which will involve higher-order interactions between multiple stressors and environmental factors.

KEYWORDS
antagonism, consistency, cumulative effects, environmental context, multiple stress, stream invertebrates, stressor interactions, synergism

1 | INTRODUCTION

Biodiversity is increasingly subject to the cumulative effects of multiple stressors (Sánchez-Bayo & Wyckhuys, 2019; Vitousek, 1994). There is widespread recognition that understanding interactions among multiple stressors is key to identifying and managing their cumulative impacts on biodiversity (Halpern et al., 2015; Vörösmarty et al., 2010), particularly as climate change is adding additional stress to ecosystems (Brook et al., 2008; Mantyka-Pringle et al., 2012, 2014, 2015; Mora et al., 2007; Pounds et al., 2006). Understanding stressor interactions is key because it could help identify how often and under what circumstances to expect 'ecological surprises'

(Brook et al., 2008; Côté et al., 2016; Orr et al., 2020): situations where the combined effect of stressors differs from the sum of their individual or additive effects. For example, if stressors interact synergistically, their combined effects are amplified or magnified when they act together (Bliss, 1939; Breitburg et al., 1999; Folt et al., 1999; Hyslop, 1976; Orr et al., 2020; Wedemeyer, 1970), potentially accelerating biodiversity loss. Alternatively, stressors can interact antagonistically, meaning their combined effect is less than expected from their individual effects. In the extreme, antagonistic interactions can manifest as dominance (the singular effect of one stressor accounts for the cumulative effect of multiple stressors) or reversal (the cumulative effect is less than the singular effect of all stressors).

As interactions between multiple stressors determine if their effects on biodiversity are amplified or dampened, much attention has focused on classifying stressor interactions, especially using the additive/synergistic/antagonistic typology (Birk et al., 2020), evaluating the frequency of these interaction types (Côté et al., 2016; Crain et al., 2008; Heugens et al., 2001; Jackson et al., 2016; Piggott et al., 2015b), and trying to identify mechanisms that might explain how stressors interact in these different ways (De Laender, 2018; Franklin & Hoppeler, 2021; Hodgson et al., 2017; Jackson et al., 2021; Schäfer & Piggott, 2018; Verberk et al., 2020; Vinebrooke et al., 2004). Much research into multiple stressor impacts has used this typology, but less widely explored is the consistency of the interactions among multiple stressors. Simmons et al. (2021) introduce the idea of stressor consistency, which is the extent to which a stressor interaction stays the same under different circumstances. Simmons et al. (2021) consider 'consistency by scale': the extent to which the type and magnitude of the stressor interaction depends on the scale or level of biological organization at which the effects on biodiversity are measured, for example at the individual, population or community level. Equally, if not more relevant, is the extent to which a stressor interaction is consistent across space and time at any given scale. Specifically, if we determine the nature of two stressors' cumulative effect on a measure of biodiversity at one location and time, will we observe the same outcome at other locations and/or times? Clearly, consistency across space and time would assist in generalizing the findings from individual studies, allowing results obtained at one location and time to be applied elsewhere.

Nevertheless, there are good reasons to anticipate inconsistencies in stressor interactions across space and/or time. Inconsistencies could arise if the nature of the interaction depends on other factors that vary spatially or temporally, for example along environmental gradients. Such inconsistency would imply higher-order interaction(s), whereby the form of a two-way stressor interaction changes in response to changes in other variables. Consequently, a stressor interaction classified as one type (e.g., additive, synergistic or antagonistic) at one location and time could plausibly switch to another type at a different location and/or time. Such inconsistency might limit the scope for understanding and predicting multiple stressor impacts by focusing on the additive/synergistic/antagonistic typology. Instead, it suggests a need to expand focus to evaluate the consistency of outcomes across space and time, which means considering higher-order interactions. Moreover, stressor interactions in statistical models can be non-linear (Duncan & Kefford, 2021), which may elude classification under the additive/synergistic/antagonistic typology. Hence, not only might the interaction between two stressors vary spatially and/or temporally, but interactions could take a wider range of forms than usually considered. Few multiple stressor studies have examined these issues because most are of limited spatial and/or temporal extent, and most consider two-way and linear interactions among multiple stressors.

Here we aim to use a large dataset spanning a wide range of environments (4658 sites across eastern Australia) to examine the nature

of the interaction between two globally important stressors in freshwater ecosystems (levels of suspended sediment measured as turbidity [Waters, 1995] and salinity [Cañedo-Argüelles et al., 2016]) on an invertebrate biodiversity index (the richness of families in the orders Ephemeroptera, Plecoptera and Trichoptera [EPT; Barbour et al., 1996, Eriksen et al., 2021]), and to quantify how interactions, and hence the cumulative effects of the two stressors, vary across two major environmental gradients in the region, mean temperature and terrain slope, and in two common habitats of flowing waters, riffle and edge. We accounted for statistical issues associated with estimating interactions, including the possibility that interactions are non-linear (Duncan & Kefford, 2021). We thus evaluate whether the form of the interaction between the two stressors is consistent such that the cumulative impact identified at one location is sufficient to understand the cumulative impact of the two stressors on freshwater biodiversity across the wider landscape.

2 | METHODS

2.1 | Field data collection

We analysed stream macroinvertebrate data and associated environmental variables collected at 4658 sites located throughout the Australian jurisdictions of Tasmania, Victoria, New South Wales and Queensland (Figure 1a). The sites spanned a wide latitudinal gradient from tropical to temperate (S11°–S43° or 3600 km) and elevation range 0–2020 m a.s.l. Each site comprised a section of stream in which one or both of two habitats (edge and riffle) were sampled for macroinvertebrates using standard methods (see Supplementary Text 1). For each habitat sampled at each site, we extracted data on the family richness of stream macroinvertebrates in the orders EPT.

We examined how the cumulative impacts of electrical conductivity (EC, μS/cm @ 25°C) and turbidity (NTU) on EPT family richness varied in relation to mean water temperature and terrain slope, factors that varied across the study region and potentially affect EPT family richness. EC is a proxy for salinity. In Australia, increased salinity in freshwater systems typically results in a consistent proportion of ions similar to that found in sea water (NLWRA, 2001; Sauer et al., 2016). Turbidity measures the clarity of water and, while high turbidity can be caused by dissolved organic matter and algae blooms, the major cause of high turbidity in Australian streams is suspended sediments (Harrison et al., 2011), with a typically moderate positive correlation between turbidity and suspended sediment concentration (Gippel, 1995). Levels of both salinity and suspended sediments can vary naturally with geology, climate and deposition of oceanic aerosols, but in eastern Australia the dominant driver of salinity and turbidity variation is human land-use modification often associated with agriculture (NLWRA, 2001, Sauer et al., 2016). More highly disturbed catchments tend to have elevated levels of EC and turbidity. We focus on the effects of salinity and suspended sediments (as measured by EC and turbidity, respectively) on EPT family richness because these

(a)
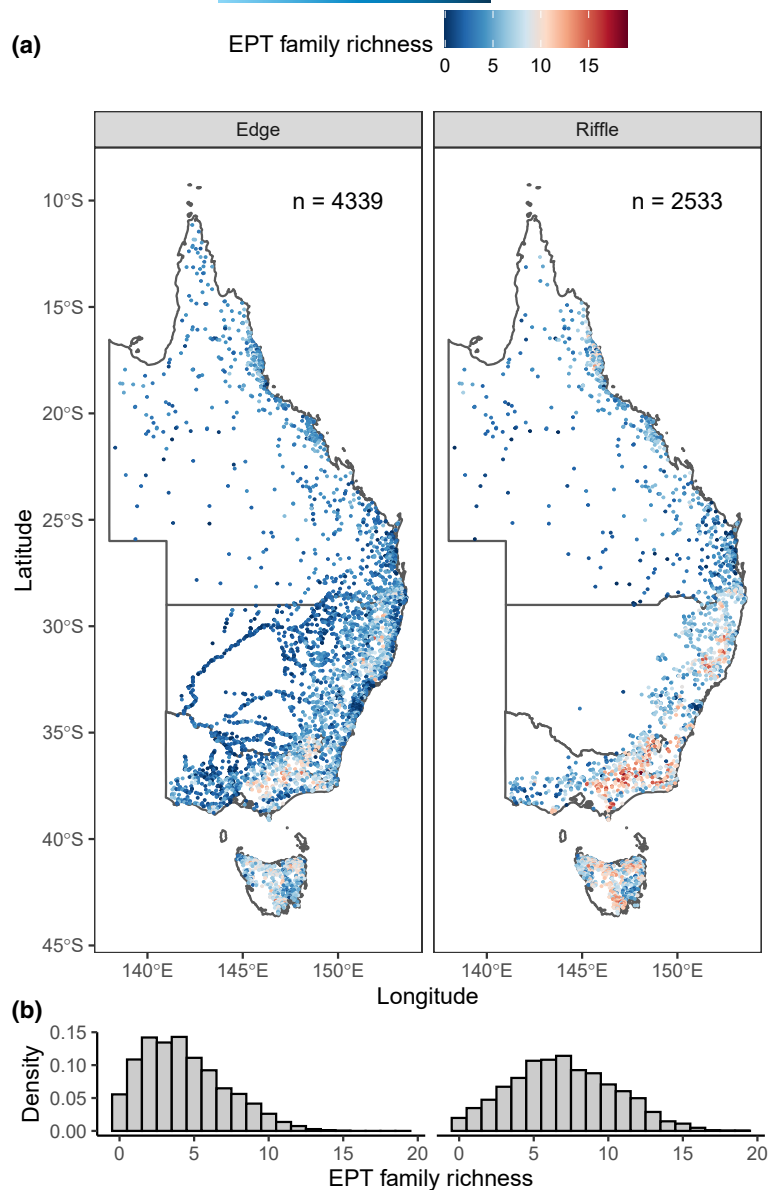
FIGURE 1 Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness (a) map of sites sampled in eastern Australia for macroinvertebrates by habitat with each site coloured by observed EPT family richness and (b) distribution of EPT family richness at sample sites by habitat.

two stressors have major impacts in Australian, and other, freshwater systems driven primarily by human activities.

Water temperature varied among sites primarily due to the wide latitude and elevation range across the study region. Temperature could be regarded as a stressor affecting EPT taxa because temperature directly affects many physiological processes (Schulte, 2015), including the uptake of major ions (Orr & Buchwalter, 2020) and individual performance (Colinet et al., 2015; Dowd et al., 2015). Across our study region, however, change in temperature will be correlated with changes in other factors including, for example, thermal (Janzen, 1967) and rainfall seasonality moving from tropical to temperate regions. While temperature per se could directly affect EPT family richness, we treat temperature as a surrogate for a range of factors that vary along latitude and elevation gradients in the study region. Little is known about how EPT family richness varies over these gradients at large spatial scales.

Terrain slope varied across the study region from flat coastal and inland plains to steep mountainous areas. Terrain slope is not a stressor per se but is a surrogate for factors that could directly affect EPT taxa. For example, stream power increases with slope steepness, affecting flow characteristics such as the instantaneous water velocity near the streambed, turbulence and sheer stress, which, in turn, influence the nature of stream bed substrates (Gordon et al., 2004; Knighton, 2014) and dissolved oxygen concentration (O'Connor & Dobbins, 1958). Because many EPT taxa favour fast-flowing water, we used an index of terrain slope to capture variation in these and related factors.

2.2 | Data analysis

2.2.1 | Organizing the data

Sixty percent of sites were sampled more than once (up to 38 occasions) over the period of data collection (1990–2016). For each habitat (edge or riffle) at each site sampled more than once, we used

the means of EPT family richness, EC and turbidity, averaged over sampling occasions, in our analysis.

We used two methods to derive a comparative measure of temperature at each site. First, water temperature (°C) was measured directly at each site on each sampling occasion. To correct for seasonal variations in water temperature, we fitted a linear mixed-effect model with water temperature as the response variable, habitat (riffle or edge) as a fixed effect, and month of the year and site as random effects. The coefficients for the site random effects in this model estimate the degree to which sites deviate from an overall mean temperature (set to zero) having accounted statistically for habitat differences and monthly variation. We termed these relative temperature deviations 'scaled temperature'. Second, we used the WorldClim grid of global climate data (Fick & Hijmans, 2017) to extract the mean annual air temperature (°C) at all sites. Within habitats, our measure of scaled temperature was strongly positively correlated with WorldClim mean annual temperature (riffle: $r = .91$, edge: $r = .84$, Figure S1). We used scaled temperature in our analyses because this measure was derived from direct measurements at each site and hence captures temperature differences associated with variation in local site conditions, for example, shading by vegetation (Rutherford et al., 2004).

We calculated an index of terrain slope at each site using elevation data from the SRTM 90m digital elevation database using the getData function in the R package raster (Hijmans et al., 2021). We used the terrain function in raster to calculate the slope at each site (radians). We then calculated a slope index as: log(1/tan[slope]), that is, the logarithm of the ratio of horizontal distance travelled to vertical distance of terrain drop. Smaller values of this index imply steeper slopes. Scaled temperature and the slope index were moderately correlated ($r = .44$), in part because the flat sites of the inland plains tend to be warmer. Nevertheless, this moderate correlation implies that the two measures will capture some independent aspects of environmental variation that could influence EPT family richness. We log transformed values of EC and turbidity and then scaled all four explanatory variables to mean zero and standard deviation one for use in the analyses below.

2.2.2 | Fitting generalized additive models

We first examined univariate relationships between EPT family richness and each of the four explanatory variables within each habitat. We did this in part to assess the degree of non-linearity in these relationships, as failure to identify and model non-linear relationships can result in spurious or misleading interaction terms (Duncan & Kefford, 2021). To assess non-linearity, we fitted two generalized additive models (GAMs) to the data: one that specified a linear relationship and one that specified a smoothed term that allowed for a non-linear relationship. We then compared the fit of each model to the data using Akaike's information criterion (AIC), with smaller values indicating a better fitting model given the number of parameters estimated.

EPT family richness can take only zero or positive values. To accommodate this, we treated EPT richness as count data, rounding to the nearest whole number when richness was averaged over sampling occasions at a site. We then modelled variation in EPT richness as drawn from a negative binomial distribution to allow for overdispersion in the counts, specifying a log link function. To account for spatial dependence in the observations (nearby sites may not be independent if they have similar EPT family richness because of correlated but unmeasured geographically structured variables), we included smoothed terms for latitude and longitude in the model. GAM models were fitted using maximum likelihood as implemented in the R package mgcv (Wood, 2011).

For each habitat (riffle and edge), we examined how EPT family richness varied as a function of all four explanatory variables. We fitted models specifying smoothed terms for all main effects, two-, three- and four-way interactions, and latitude and longitude. We used a variable selection procedure via the option select = TRUE in the gam function in mgcv, which shrinks a variable's smoothing function towards zero when that variable is weakly related to the outcome (Marra & Wood, 2011). This meant we retained all main effect and interaction terms in the model, but terms for unimportant variables were shrunk towards zero and hence had little influence on the model predictions. As above, we modelled variation in EPT richness as drawn from a negative binomial distribution to allow for overdispersion in the counts and specified a log link function. All models were fit using maximum likelihood.

Having fitted models to the data, we explored the outcomes by plotting predicted EPT family richness and its uncertainty for various combinations of values of the explanatory variables. For each explanatory variable, we specified low, intermediate and high values as having values of −1.6, 0 and 1.6, respectively, recalling that all explanatory variables were scaled to mean zero and standard deviation one. This range of low to high values encompassed the data region for which we had reasonable sample sizes (Figure S2) so that model predictions would be reliable. For the fitted univariate models, we quantified the marginal impact of each explanatory variable by calculating the proportional reduction in EPT family richness associated with shifting from a low to a high value of a variable, with the proportional reduction calculated as: 1 − predicted EPT family richness at the high stressor value/predicted EPT family richness at the low stressor value.

2.2.3 | Interpreting the GAMs

For each of the nine combinations of low, intermediate and high values for temperature and slope, we plotted the relationship between EPT family richness and turbidity for each of low, intermediate and high values of EC. This allowed us to examine how the combined effect of turbidity and EC on EPT family richness varied across the different combinations of low to high values of temperature and slope. Specifically, we were interested in the consistency of the relationship between the two stressors (turbidity and EC) and EPT family

richness. We assessed the consistency of the relationship by examining the interaction terms in the fitted GAMs. If the relationship was consistent, we would expect no strong three- or four-way interaction terms in the models—these higher-order interaction terms should be unimportant and hence shrunk towards zero.

If there were strong higher-order interactions, we could visualize the nature of the interactions using the plots described above. To guide this, and to frame our findings in terms of the additive/synergistic/antagonistic typology, we used a rough heuristic to classify the cumulative effects of turbidity and EC on EPT family richness as additive, synergistic or antagonistic for each combination of low to high temperature and slope values. If the cumulative effect of turbidity and EC was additive, we would expect lines plotting the relationship between EPT family richness and turbidity for different values of EC to parallel each other, even if the relationship between EPT family richness and turbidity was non-linear. To assess departure from additivity, we calculated the difference in predicted EPT family richness when EC was high and when EC was low, for each of a range of turbidity values from low to high. If the lines for high and low EC were parallel, this difference would be constant across the range of turbidity values. We then calculated the ratio of the maximum to minimum difference, with larger values (>1) implying greater departure from parallel and hence greater departure from additivity of effects. We arbitrarily chose a ratio cut-off value of two to identify relationships that clearly departed from additivity (i.e., ratio > 2), a value that aligned with our visual impression of when relationships appeared non-additive. Interactions identified as non-additive were then classified as synergistic or antagonistic from the form of the interaction.

The above approach allows us to evaluate the consistency of the interaction between turbidity and EC in relation to temperature and slope. We evaluated a second way in which the cumulative effect of the two stressors on biodiversity could be regarded as consistent by calculating the proportional reduction in EPT family richness associated with shifting from sites where both stressors had a low value to sites where both stressors had a high value. The proportional reduction was calculated as: 1 – predicted EPT family richness when both stressors had high values/predicted EPT family richness when both stressors had low values. If this proportion varies under different circumstances, it implies that the cumulative impact of the two stressors varies: combined, the magnitude of their impact is greater under some conditions relative to others. The cumulative impact of two stressors could be additive under all circumstances but the magnitude of their combined impact could vary considerably as stressor levels increase. This type of inconsistency could also be regarded as generating 'ecological surprises' that arise from higher-order interactions because the same two stressors could have relatively little cumulative impact on biodiversity at some locations, but substantial impact at others. We used this approach to quantify the cumulative effects of EC and turbidity given different combinations of temperature and slope. To understand what drives variation in these cumulative effects, we also calculated the marginal effect of both EC and turbidity at each temperature and slope combination as

the predicted proportional reduction in EPT family richness in going from low to high values of EC (turbidity) averaged across the range of low to high turbidity (EC) values.

3 | RESULTS

### 3.1 | Singular effects of the four variables

EPT family richness ranged from zero to 19 families per habitat per site with, on average, higher richness in riffle relative to edge habitats (Figure 1b). EPT family richness declined with increasing values of all four explanatory variables. For slope, richness declined linearly, but relationships with the other three explanatory variables (temperature, turbidity and EC) were non-linear as indicated by AIC values (Figure 2). Each variable alone explained between 0.39 and 0.46 of the total deviance in each habitat.

3.2 | Cumulative effects of the four variables

For each habitat, a GAM that included all four explanatory variables and their interactions explained about half of the total deviance in EPT family richness (riffle deviance explained = 0.51, edge deviance explained = 0.49), and plots of observed versus predicted values indicated a reasonable fit of the models to the data with no clear bias (Figure S3). Parameter estimates for the fitted models indicated there were statistically significant three-way interactions in both habitats that involved all four explanatory variables (Table 1). These interactions imply that the form of the relationship between turbidity and EC was inconsistent and varied depending on temperature and slope. For the models fitted to each habitat, the chi-square and *p*-values indicate the strength of evidence against the null hypothesis of no effect for each variable. On this basis, while there was evidence for significant three-way interactions, EPT family richness in both habitats was most strongly associated with the main effects of the four explanatory variables and with the interaction between EC and temperature (Table 1).

In a GAM, the effective degrees of freedom (EDF) associated with each parameter measures the degree of non-linearity in a relationship, with values of one indicating a linear relationship. As a rule of thumb, EDF values >1 but ≤2 indicate a weakly non-linear relationship and values >2 a highly non-linear relationship (Hunsicker et al., 2016; note that some parameters had EDF values <1 caused by the variable selection procedure we used: parameters with EDF <1 had been shrunk towards zero). The variables most strongly linked to EPT family richness (the four main effects and the EC-temperature interaction) had, except for slope, highly non-linear relationships (Table 1). Several significant three-way interactions were also highly non-linear.

In the univariate models, a shift from low to high values was associated with a similar proportional reduction in predicted EPT family richness for each explanatory variable (mean = 0.45, range: 0.39–0.49, Figure 2). The cumulative effect of a shift in both EC and turbidity
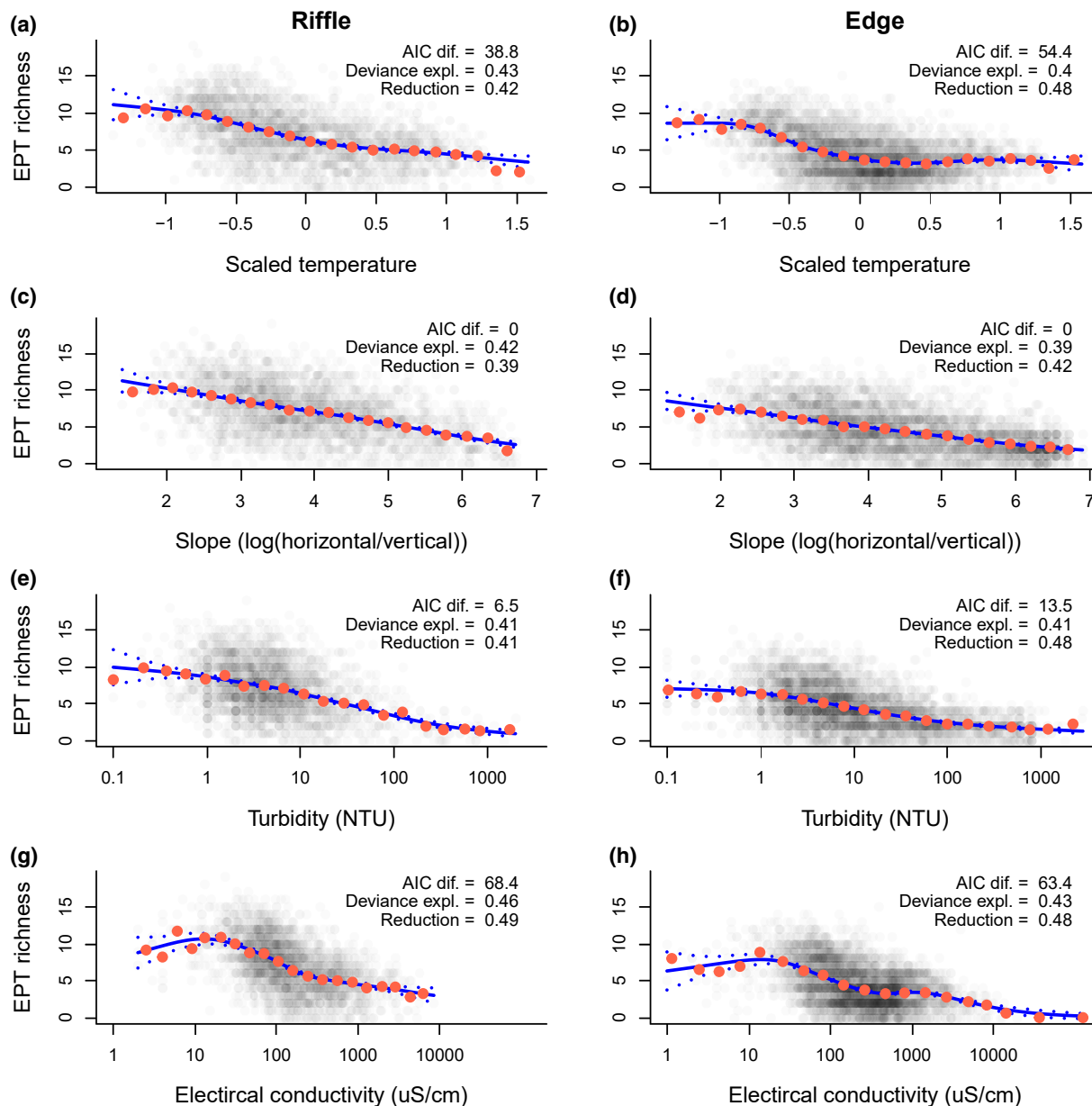
FIGURE 2 Univariate relationships between Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness and the four variables, (a, b) scale temperature (derived from water temperature °C), (c, d) slope, (e, f) turbidity (NTU) and (g, h) electrical conductivity (EC, µS/cm @ 25°C), for each habitat type, (a, c, e, g) riffle and (b, d, f, h) edge, with fitted generalized additive models (GAMs). Grey circles are the raw data, red circles are the mean value for EPT family richness for equal-sized bins of the variable shown on the x-axis. Solid blue lines are the fitted GAM (mean estimate) and dotted blue lines are 95% confidence intervals around the mean estimate. In each panel, AIC dif. is the difference in AIC between a model specifying a linear relationship and one allowing a smooth non-linear relationship. A value of zero indicates no difference in AIC between the two models, values greater than zero indicate that the non-linear model provides a better fit to the data. Deviance expl. is the proportion of the total deviance explained by the fitted non-linear model.

from low to high values tended to generate a greater proportional reduction in richness (ranging from 0.45 to 0.7) although this varied by habitat, temperature and slope (Figures 3 and 4). The cumulative effect varied strongly with temperature: a shift in both EC and turbidity from low to high values was associated with a greater reduction in EPT family richness at cool relative to warm sites in both riffle and edge habitat (Figures 3 and 4). This outcome was due primarily to the strong EC–temperature interaction (Table 1): the marginal effect of a shift

from low to high values of EC was associated with a proportionally larger richness reduction (0.46–0.57) at cool sites, relative to a smaller reduction (0.04–0.31) at warm sites (Figures 3 and 4). In contrast, the marginal effect of a shift from low to high values of turbidity was similar across the temperature gradient (proportional richness reduction at cool and warm sites 0.2–0.46 and 0.23–0.44, respectively). Overall, turbidity had a larger marginal effect in edge (mean = 0.42) relative to riffle habitat (mean = 0.28).

Term	Riffle			Edge		
	EDF	Chi sq	p	EDF	Chi sq	p
s(temperature)	3.5	31.4	<.001	2.5	52.1	<0.001
s(slope)	1	34.5	<.001	1	38	<0.001
s(turbidity)	2.7	40.6	<.001	3.4	197.7	<0.001
s(EC)	3.6	153.3	<.001	5.4	146.5	<0.001
ti(temperature, slope)	0.7	1.2	.14	2.4	12.8	<0.001
ti(temperature, turbidity)	0.2	0.2	0.262	0	0	0.883
ti(temperature, EC)	2.9	26.7	<0.001	4	24.9	<0.001
ti(slope, turbidity)	0.5	1.2	0.11	2.8	5.6	0.041
ti(slope, EC)	0.8	4.5	0.014	3	6.1	0.05
ti(turbidity, EC)	1.2	2.9	0.051	1.9	9.4	0.002
ti(temperature, slope, turbidity)	5.2	9.2	0.035	0	0	0.427
ti(temperature, turbidity, EC)	3.8	5.8	0.089	4.6	9.5	0.018
ti(slope, turbidity, EC)	0.7	2.3	0.048	1.4	4.8	0.011
ti(temperature, slope, turbidity, EC)	0	0	0.739	0.8	1.8	0.083
s(longitude, latitude)	23.4	238.3	<0.001	25.5	305	<0.001

TABLE 1 Results of fitting generalized additive models (GAMs) to the data for riffle (*n* = 2533 sites) and edge (*n* = 4339 sites) habitats. The response variable was Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness at each site. The column Term shows the terms included in the model with s(var1) indicating a smoothed relationship was fitted for var1, and ti(var1, var2) indicating a smoothed interaction was fitted for var1 and var2. Effective degrees of freedom (EDF) is the effective degrees of freedom, Chi sq is the chi-square value associated with each term and *p* is an approximate *p*-value indicating the significance of each term in the model. s(longitude, latitude) is a smoothed term accounting for spatial dependence in the response variable.

3.3 | Classifying cumulative effects in the additive/antagonistic/synergistic typology

Other significant two- and three-way interactions in both the riffle and edge habitat models (Table 1) implied that the form of the two-way interaction between turbidity and EC varied by temperature and slope. In riffle habitats, about half (5 of 9) of the relationships between EC and turbidity were additive. Of the remainder, sites with shallow slopes interactions were synergistic regardless of temperature, while interactions at warm and steep sites were antagonistic (Figure 3). In edge habitats, the interactions were mostly (7 of 9) antagonistic, although cool and steep to intermediate slopes were additive (Figure 4).

4 | DISCUSSION

Our key finding is that the nature of the cumulative effects of the two stressors, EC and turbidity, differed between habitats (riffle and edge) and varied across the landscape as a function of differences in temperature and, to a lesser extent, slope. While all four explanatory variables were strongly and independently associated with variation in EPT family richness (Figure 2), the cumulative effects of EC and turbidity varied as a function of habitat, temperature and slope because of (1) a strong two-way interaction between EC and temperature (Table 1), which resulted in high EC being linked to a greater reduction in EPT family richness at cool relative to warm sites (Figures 3 and 4); (2) a stronger effect of turbidity in edge relative to riffle habitats and (3) significant, though weaker, two- and three-way interactions in both habitats that resulted in additive, antagonistic and synergistic effects of EC and turbidity depending on the temperature and slope combination.

Overall, higher levels of the two anthropogenic stressors, EC and turbidity, were associated with large reductions in EPT family richness (Figure 2), an outcome in broad agreement with other research on EPT in our study region (Kefford et al., 2011; Pettigrove, 1990) and elsewhere (Akamagwuna et al., 2019; Conroy et al., 2016). While our findings are correlative, they are consistent with outcomes obtained from experimental studies on EPT responses to altered salinity (Bray et al., 2019; Clements & Kotalik, 2016; Johnson et al., 2015) and turbidity/sedimentation (Piggott et al., 2012, 2015a).

Our finding of higher EPT family richness in riffle relative to edge habitats also agrees with previous work (Gerth & Herlihy, 2006; Hewlett, 2000; Metzeling et al., 2006), although the different effects of stressors in these habitats are less well understood. The stronger effect of turbidity on EPT family richness in edge relative to riffle habitats is potentially the result of slower water velocity and a higher rate of sediment settlement in edge habitats, which could result in greater impacts on stream invertebrates where sediment can bury habitat (Waters, 1995) and eggs (Kefford et al., 2010), relative to faster flowing riffle habitat.

We are unsure why elevated EC was associated with a greater reduction in EPT family richness at cool relative to warm sites. This outcome runs counter to single-species short-term laboratory experiments (Cañedo Argüelles et al., 2013; Dunlop et al., 2008; Jackson & Funk, 2019; Moulding et al., 2022), although outcomes may differ over the long term (Moulding et al., 2022; Orr & Buchwalter, 2020; Verberk et al., 2020). Much of the variation in scaled water temperature among sites was associated with changes in latitude (Figure S4). Consequently, the EC by temperature interaction could be associated with other latitudinal trends such as seasonal variability (Janzen, 1967). In eastern Australia, for example, greater rainfall seasonality in the warmer north could result in greater natural seasonality in stream salinity and thus a greater evolved ability to resist
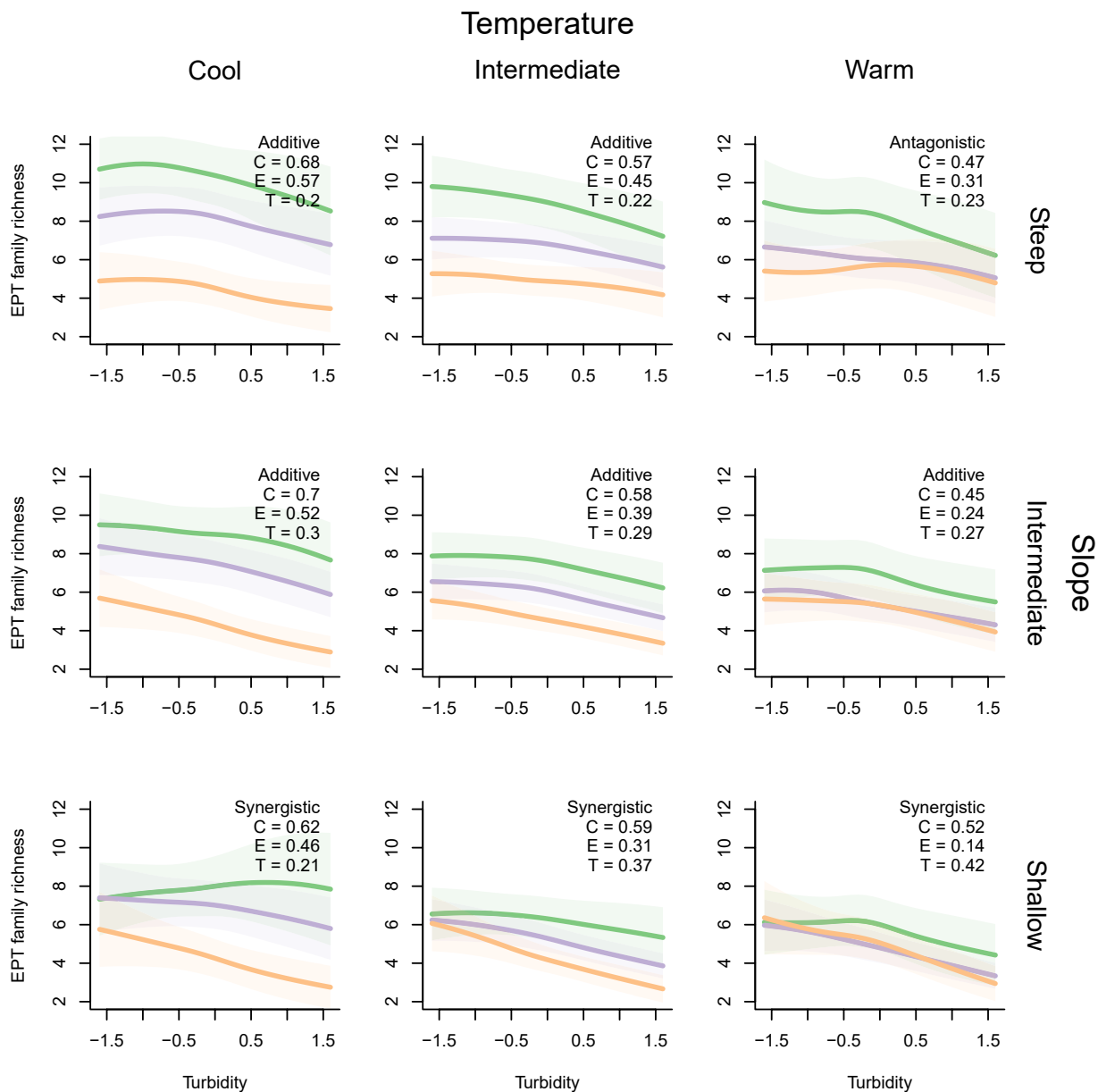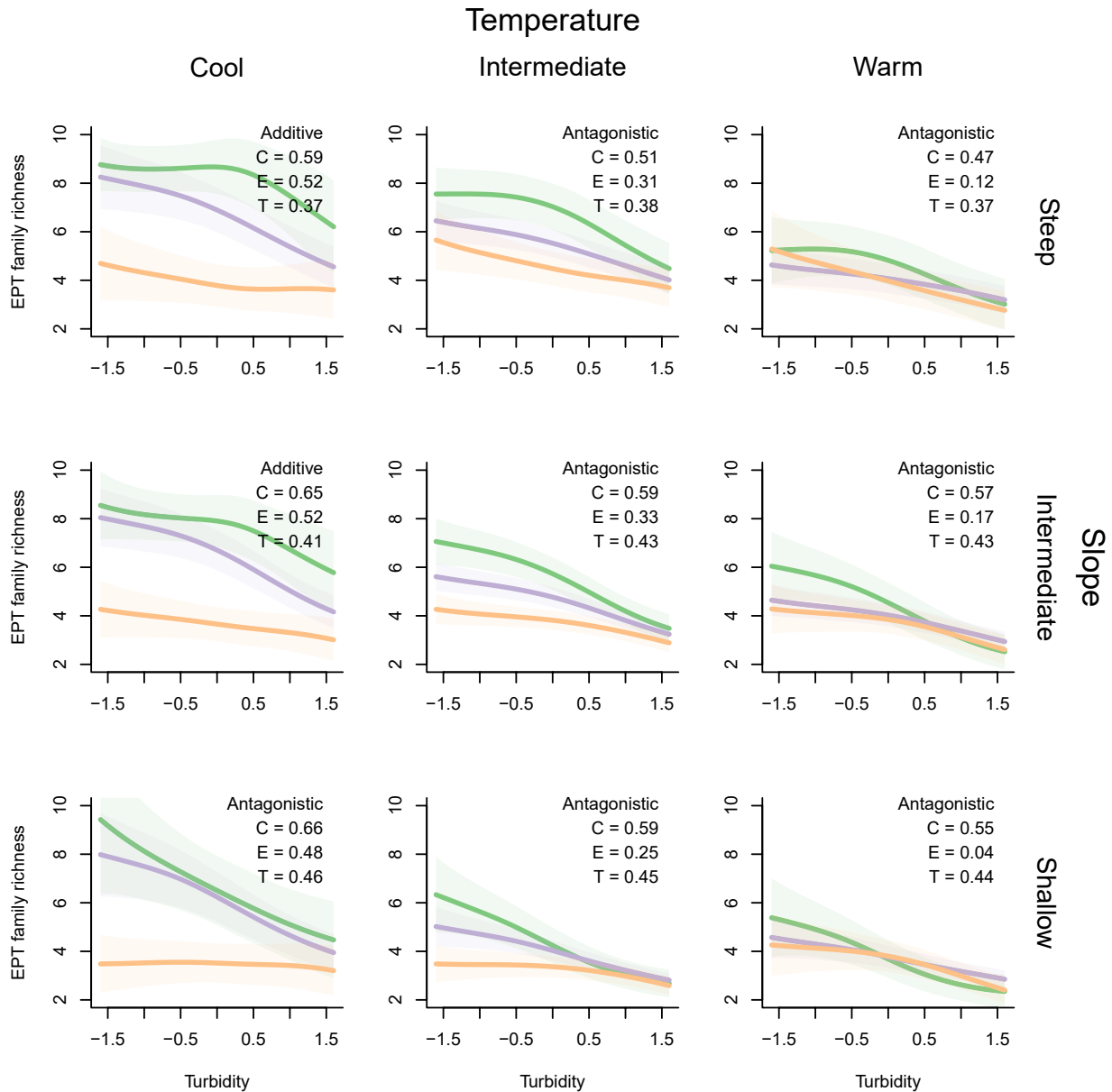
FIGURE 3 Predicted values of Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness from the generalized additive model (GAM) fitted to the riffle data (see Table 1). Each panel shows the relationship between EPT family richness (y-axis) and turbidity (x-axis) at three values of electrical conductivity (EC): low (EC = −1.6, coloured green), intermediate (EC = 0, coloured purple) and high (EC = 1.6, coloured orange). Shading shows the 95% confidence intervals around the predicted values. Each of the nine panels shows the predicted relationship between EPT family richness, turbidity and EC at a different combination of values of temperature and slope. In the top right of each panel is shown the form of the interaction between turbidity and EC (either additive, synergistic or antagonistic, see text for details) and the proportional reduction in EPT family richness in shifting from low to high values of: both turbidity and EC (C = the cumulative effect), EC alone (E) and turbidity alone (T; see text for details).

anthropogenic salinization, relative to the cooler south, but see Dunlop et al. (2008).

Multiple stressor studies have frequently focused on the nature of stressor interactions in the context of the additive/antagonistic/synergistic typology, largely because such interactions are viewed as key to identifying the cumulative impacts of stressors on biodiversity and avoiding potential 'ecological surprises' (Brook et al., 2008; Côté et al., 2016). While the two anthropogenic stressors in this study interacted, the nature of their interaction varied across the

landscape and eluded a simple additive/antagonistic/synergistic classification. As, or more, significant was the degree to which the impact of individual stressors varied by habitat (turbidity) and in relation to temperature (EC), with the two-way interaction between EC and temperature particularly important (Table 1). These interactions arose from stressors having inconsistent impacts across the landscape, which appeared capable of generating larger ecological surprises than outcomes resulting from synergistic or antagonistic interactions between the two focal stressors. Other studies have

Temperature

FIGURE 4 Predicted values of Ephemeroptera, Plecoptera and Trichoptera (EPT) family richness from the generalized additive model (GAM) fitted to the edge data (see Table 1). Each panel shows the relationship between EPT family richness (*y*-axis) and turbidity (*x*-axis) at three values of electrical conductivity (EC): low (EC = −1.6, coloured green), intermediate (EC = 0, coloured purple) and high (EC = 1.6, coloured orange). Shading shows the 95% confidence intervals around the predicted values. Each of the nine panels shows the predicted relationship between EPT family richness, turbidity and EC at a different combination of values of temperature and slope. In the top right of each panel is shown the form of the interaction between turbidity and EC (either additive, synergistic or antagonistic, see text for details) and the proportional reduction in EPT family richness in shifting from low to high values of: both turbidity and EC (C = the cumulative effect), EC alone (E) and turbidity alone (T; see text for details).

shown that the singular effect of stressors can vary across climate gradients (Guo et al., 2018; Karp et al., 2018; Lorenzen et al., 2011; Peters et al., 2019; Pounds et al., 2006), experimentally imposed drought gradients (Stampfli et al., 2013), and in relation to vegetation cover (Orlinskiy et al., 2015; Schäfer et al., 2012). For example, Peters et al. (2019) observed that land-use change tended to have less impact on terrestrial biota at higher relative to lower elevation sites. Our novelty is in demonstrating that the cumulative effects of multiple stressors can also be inconsistent across the landscape.

The findings of our study are consistent with others regarding the overall negative impacts of turbidity and EC on EPT family richness, but they differ in other important respects (Beermann et al., 2018, 2021; Piggott et al., 2012, 2015a). For example, we found little evidence of a strong interaction between turbidity and temperature, in contrast to two mesocosm experiments where increased sedimentation and higher temperature had a strong antagonistic effect on EPT genus richness (Piggott et al., 2012, 2015a). Similarly, the greater effect of EC (salinity) on EPT family richness

we observed at cooler relative to warmer sites is in apparent contradiction to single species short-term laboratory studies that show a greater effect of salinity at higher temperatures (Cañedo Argüelles et al., 2013; Jackson & Funk, 2019; Moulding et al., 2022). Such inconsistencies between experiments and our findings reinforce the general point that context matters in determining the cumulative effects of multiple stressors. Laboratory and mesocosm environments differ from natural environments (Cairns, 1983; Cairns Jr, 1986; Petersen et al., 1999; Quinn & Keough, 1993; Rohr et al., 2006) likely altering the nature of interactions between stressors.

Inconsistencies in the effects of EC and turbidity that we observed across the landscape suggest that a focus on using the additive/synergistic/antagonistic typology to classify stressor interactions could overlook other important aspects of multiple stressor impacts. Our results show that the nature of two-way stressor interactions need not be fixed, and that the cumulative impact of two stressors can vary greatly depending on environmental context (Figures 3 and 4). This latter outcome may be more important in understanding and predicting stressor impacts than knowing the form of the interaction, at least when two or more stressors have a common driver. In Australia, sedimentation and salinization of streams often result from agriculture activities that include clearing of native vegetation, grazing and cultivation (Harrison et al., 2011; NLWRA, 2001; Sauer et al., 2016). Consequently, restoration practices that aim to mitigate the impacts of one stressor can also alleviate other stresses, thereby reducing their cumulative impacts. In such circumstances, understanding how the magnitude of the cumulative impact varies across the landscape should assist in identifying areas most likely to benefit from restoration. In contrast, when different stressors have different drivers, managers may have to decide which stressor(s) they prioritize in mitigation. Here, understanding both variation in the magnitude of impact and the nature of interactions should help in prioritization. Where cumulative effects are synergistic, for example, there will be advantages in reducing all stressors (Simmons et al., 2021).

Recently, various authors have advocated developing a theoretical understanding of the mechanisms that underlie stressor effects and their interactions (De Laender, 2018; Dey & Koops, 2021; Franklin & Hoppeler, 2021; Griffen et al., 2016; Jackson et al., 2021; Schäfer & Piggott, 2018; Thompson et al., 2018a, 2018b), in particular to predict whether interactions are likely to be synergistic, additive or antagonistic. This approach, and typology, focuses on two-way interactions between stressors. However, the presence of higher-order and non-linear interactions among stressors and environmental factors (Table 1) identifies a need for multiple stressor studies to expand their focus beyond the current typology and two-way interactions. Analysis of large spatial (or temporal) scale datasets, as in the current study, may be useful for evaluating the consistency of stressor effects by evaluating higher-order interactions, although such studies suffer from an inability to establish cause–effect relationships. Experimental studies can isolate cause and effect but the increasing size and complexity of experiments required to study interactions among three (Beermann et al., 2018;

Elbrecht et al., 2016; Piggott et al., 2012, 2015a), four (Juvigny-Khenafou et al., 2021) or more factors is challenging. An alternative is distributed experiments, where the same experiment is repeated at different locations, allowing stressor interactions to be explored along climatic and other gradients. Despite their challenges (Borer et al., 2014; Fraser et al., 2013; Knapp et al., 2017; Yahdjian et al., 2021), distributed experiments offer many advantages for understanding the cumulative impacts of stressors across the landscape.

Also useful is the identification of metrics to evaluate the consistency of relationships among stressors. Such metrics could quantify different aspects by which the cumulative impacts of stressors vary across the landscape, as we have done here. These metrics could assist in identifying outcomes that result in ecological surprises. In our study, we suggest it is variation in the magnitude of cumulative impacts, rather than variation in the nature of the interactions, that is key to identifying sites most vulnerable to increasing levels of turbidity and EC. For different response variables, locations and stressors, other factors may be more important.

AUTHOR CONTRIBUTIONS

Provision of data: Ben J. Kefford and Susan J. Nichols; conception of the idea: Ben J. Kefford and Richard P. Duncan; data analysis: Richard P. Duncan; wrote first draft: Ben J. Kefford and Richard P. Duncan; discussing of issues covered, finding literature, editing and approval of final MS: all.

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CONFLICT OF INTEREST

We have no known conflict of interest.

DATA AVAILABILITY STATEMENT

R code used to reproduce all analyses and the data analysed in this paper are available at https://zenodo.org/record/7080635#.YyKvn bRByUl.

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REFERENCES

Akamagwuna, F. C., Mensah, P. K., Nnadozie, C. F., & Odume, O. N. (2019). Evaluating the responses of taxa in the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) to sediment stress in the Tsitsa

River and its tributaries, Eastern Cape, South Africa. *Environmental Monitoring and Assessment*, 191, 1–15.

Barbour, M., Gerritsen, J., Griffith, G., Frydenborg, R., McCarron, E., White, J., & Bastian, M. (1996). A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society*, 15, 185–211.

Beermann, A. J., Elbrecht, V., Karnatz, S., Ma, L., Matthaei, C. D., Piggott, J. J., & Leese, F. (2018). Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. *Science of the Total Environment*, 610, 961–971.

Beermann, A. J., Werner, M.-T., Elbrecht, V., Zizka, V. M., & Leese, F. (2021). DNA metabarcoding improves the detection of multiple stressor responses of stream invertebrates to increased salinity, fine sediment deposition and reduced flow velocity. *Science of the Total Environment*, 750, 141969.

Birk, S., Chapman, D., Carvalho, L., Spears, B. M., Andersen, H. E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., & Beklioğlu, M. (2020). Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nature Ecology & Evolution*, 4, 1060–1068.

Bliss, C. I. (1939). The toxicity of poisons applied jointly. *Annals of Applied Biology*, 26, 585–615.

Borer, E. T., Harpole, W. S., Adler, P. B., Lind, E. M., Orrock, J. L., Seabloom, E. W., & Smith, M. D. (2014). Finding generality in ecology: A model for globally distributed experiments. *Methods in Ecology and Evolution*, 5, 65–73.

Bray, J. P., Reich, J., Nichols, S. J., King, G. K. K., Nally, R. M., Thompson, R., O'Reilly-Nugent, A., & Kefford, B. J. (2019). Biological interactions mediate context and species-specific sensitivities to salinity. *Philosophical Transactions of the Royal Society of London B*, 374, 20180020.

Breitburg, D. L., Sanders, J. G., Gilmour, C. C., Hatfield, C. A., Osman, R. W., Riedel, G. F., Seitzinger, S. P., & Seitzinger, S. P. (1999). Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. *Limnology and Oceanography*, 44, 837–863.

Brook, B. W., Sodhi, N. S., & Bradshaw, C. J. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23, 453–460.

Cairns, J. (1983). Are single species toxicity tests alone adequate for estimating environmental hazard? *Hydrobiologia*, 100, 47–57.

Cairns, J., Jr. (1986). The myth of the most sensitive species; multispecies testing can provide valuable evidence for protecting the environment. *Bioscience*, 36, 670–672.

Cañedo Argüelles, M., Kefford, B. J., Piscart, C., Prat, N., Schäfer, R. B., & Schulz, C.-J. (2013). Salinisation of rivers: An urgent ecological issue. *Environmental Pollution*, 173, 157–167.

Cañedo-Argüelles, M., Hawkins, C. P., Kefford, B. J., Schäfer, R. B., Dyack, B., Brucet, S., Buchwalter, D., Dunlop, J., Frör, O., Lazorchak, J., Coring, E., Fernandez, H. R., Goodfellow, W., González Achem, A. L., Hatfield-Dodds, S., Karimov, B. K., Mensah, P., Olson, J. R., Piscart, C., ... Timpano, A. J. (2016). Saving freshwater from salts. *Science*, 351, 914–916.

Clements, W. H., & Kotalik, C. (2016). Effects of major ions on natural benthic communities: An experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. *Freshwater Science*, 35, 126–138.

Colinet, H., Sinclair, B. J., Vernon, P., & Renault, D. (2015). Insects in fluctuating thermal environments. *Annual Review of Entomology*, 60, 123–140.

Conroy, E., Turner, J., Rymszewicz, A., Bruen, M., O'Sullivan, J., Lawler, D., Lally, H., & Kelly-Quinn, M. (2016). Evaluating the relationship between biotic and sediment metrics using mesocosms and field studies. *Science of the Total Environment*, 568, 1092–1101.

Côté, I. M., Darling, E. S., & Brown, C. J. (2016). Interactions among ecosystem stressors and their importance in conservation. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20152592.

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, 11, 1304–1315.

De Laender, F. (2018). Community-and ecosystem-level effects of multiple environmental change drivers: Beyond null model testing. *Global Change Biology*, 24, 5021–5030.

Dey, C. J., & Koops, M. A. (2021). The consequences of null model selection for predicting mortality from multiple stressors. *Proceedings of the Royal Society B*, 288, 20203126.

Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *The Journal of Experimental Biology*, 218, 1956–1967.

Duncan, R. P., & Kefford, B. J. (2021). Interactions in statistical models: Three things to know. *Methods in Ecology and Evolution*, 12, 2287–2229.

Dunlop, J. E., Horrigan, N., McGregor, G., Kefford, B. J., Choy, S., & Prasad, R. (2008). Effect of spatial variation on macroinvertebrate salinity tolerance in Eastern Australia: Implications for derivation of ecosystem protection trigger values. *Environmental Pollution*, 151, 621–630.

Elbrecht, V., Beermann, A. J., Goessler, G., Neumann, J., Tollrian, R., Wagner, R., Wlecklik, A., Piggott, J. J., Matthaei, C. D., & Leese, F. (2016). Multiple-stressor effects on stream invertebrates: A mesocosm experiment manipulating nutrients, fine sediment and flow velocity. *Freshwater Biology*, 61, 362–375.

Eriksen, T. E., Brittain, J. E., Søli, G., Jacobsen, D., Goethals, P., & Friberg, N. (2021). A global perspective on the application of riverine macroinvertebrates as biological indicators in Africa, South-Central America, Mexico and Southern Asia. *Ecological Indicators*, 126, 107609.

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302–4315.

Folt, C., Chen, C., Moore, M., & Burnaford, J. (1999). Synergism and antagonism among multiple stressors. *Limnology and Oceanography*, 44, 864–877.

Franklin, C. E., & Hoppeler, H. H. (2021). Elucidating mechanism is important in forecasting the impact of a changing world on species survival. *Journal of Experimental Biology*, 224(Suppl_1), jeb242284.

Fraser, L. H., Henry, H. A., Carlyle, C. N., White, S. R., Beierkuhnlein, C., Cahill, J. F., Jr., Casper, B. B., Cleland, E., Collins, S. L., & Dukes, J. S. (2013). Coordinated distributed experiments: An emerging tool for testing global hypotheses in ecology and environmental science. *Frontiers in Ecology and the Environment*, 11, 147–155.

Gerth, W. J., & Herlihy, A. T. (2006). Effect of sampling different habitat types in regional macroinvertebrate bioassessment surveys. *Journal of the North American Benthological Society*, 25, 501–512.

Gippel, C. J. (1995). Potential of turbidity monitoring for measuring the transport of suspended solids in streams. *Hydrological Processes*, 9, 83–97.

Gordon, N. D., McMahon, T. A., Finlayson, B. L., Gippel, C. J., & Nathan, R. J. (2004). *Stream hydrology: An introduction for ecologists*. John Wiley and Sons.

Griffen, B. D., Belgrad, B. A., Cannizzo, Z. J., Knotts, E. R., & Hancock, E. R. (2016). Rethinking our approach to multiple stressor studies in marine environments. *Marine Ecology Progress Series*, 543, 273–281.

Guo, F., Lenoir, J., & Bonebrake, T. C. (2018). Land-use change interacts with climate to determine elevational species redistribution. *Nature Communications*, 9, 1315.

Halpern, B. S., Frazier, M., Potapenko, J., Casey, K. S., Koenig, K., Longo, C., Lowndes, J. S., Rockwood, R. C., Selig, E. R., & Selkoe, K. A. (2015). Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature Communications*, 6, 1–7.

Harrison, E. T., Nichols, S., Gruber, B., Dyer, F., Tschierschke, A., & Norris, R. (2011). *AUSRIVAS: Australia's in-stream biological health 2003–2010. 2011 State of the Environment reporting.* Report prepared for The Australian Government. Department of Sustainability, Environment, Water, Population and Communities, Canberra.

Heugens, E. H. W., Hendriks, A. J., Dekker, T., van Straalen, N. M., & Admiraal, W. (2001). A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. *Critical Reviews in Toxicology, 31,* 247–284.

Hewlett, R. (2000). Implications of taxonomic resolution and sample habitat for stream classification at a broad geographic scale. *Journal of the North American Benthological Society, 19,* 352–361.

Hijmans, R. J., Etten, J. V., Sumner, M., Cheng, J., Baston, D., Bevan, A., Bivand, R., Busetto, L., Canty, M., Fasoli, B., Forrest, D., Ghosh, A., Golicher, D., Gray, J., Greenberg, J. A., Hiemstra, P., Hingee, K., Ilich, A., Geosciences, I. F. M. A., ... Wueest, R. (2021). *raster: Geographic data analysis and modeling.* R package version 3.5-11.

Hodgson, E. E., Essington, T. E., & Halpern, B. S. (2017). Density dependence governs when population responses to multiple stressors are magnified or mitigated. *Ecology, 98,* 2673–2683.

Hunsicker, M. E., Kappel, C. V., Selkoe, K. A., Halpern, B. S., Scarborough, C., Mease, L., & Amrhein, A. (2016). Characterizing driver–response relationships in marine pelagic ecosystems for improved ocean management. *Ecological Applications, 26,* 651–663.

Hyslop, N. S. G. (1976). Some direct and indirect interactions of weather and topography with resistance to disease in animals. *International Journal of Biometeorology, 20,* 51–60.

Jackson, J. K., & Funk, D. H. (2019). Temperature affects acute mayfly responses to elevated salinity: Implications for toxicity of road de-icing salts. *Philosophical Transactions of the Royal Society B, 374,* 20180081.

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global Change Biology, 22,* 180–189.

Jackson, M. C., Pawar, S., & Woodward, G. (2021). The temporal dynamics of multiple stressor effects: From individuals to ecosystems. *Trends in Ecology & Evolution, 36,* 402–410.

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist, 101,* 233–249.

Johnson, B. R., Weaver, P. C., Nietch, C. T., Lazorchak, J. M., Struewing, K. A., & Funk, D. H. (2015). Elevated major ion concentrations inhibit larval mayfly growth and development. *Environmental Toxicology and Chemistry, 34,* 167–172.

Juvigny-Khenafou, N. P., Piggott, J. J., Atkinson, D., Zhang, Y., Macaulay, S. J., Wu, N., & Matthaei, C. D. (2021). Impacts of multiple anthropogenic stressors on stream macroinvertebrate community composition and functional diversity. *Ecology and Evolution, 11,* 133–152.

Karp, D. S., Frishkoff, L. O., Echeverri, A., Zook, J., Juárez, P., & Chan, K. M. (2018). Agriculture erases climate-driven β-diversity in Neotropical bird communities. *Global Change Biology, 24,* 338–349.

Kefford, B. J., Marchant, R., Schäfer, R. B., Metzeling, L., Dunlop, J. E., Choy, S. C., & Goonan, P. (2011). The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. *Environmental Pollution, 159,* 302–310.

Kefford, B. J., Zalizniak, L., Dunlop, J. E., Nugegoda, D., & Choy, S. C. (2010). How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? *Environmental Pollution, 158,* 543–550.

Knapp, A. K., Avolio, M. L., Beier, C., Carroll, C. J., Collins, S. L., Dukes, J. S., Fraser, L. H., Griffin-Nolan, R. J., Hoover, D. L., & Jentsch, A. (2017). Pushing precipitation to the extremes in distributed experiments: Recommendations for simulating wet and dry years. *Global Change Biology, 23,* 1774–1782.

Knighton, D. (2014). *Fluvial forms and processes: A new perspective.* Routledge.

Lorenzen, E. D., Nogués-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K. A., Ugan, A., Borreegaard, M. K., Gilbert, M. T. P., & Nielsen, R. (2011). Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature, 479,* 359–364.

Mantyka-Pringle, C. S., Martin, T. G., Moffatt, D. B., Linke, S., & Rhodes, J. R. (2014). Understanding and predicting the combined effects of climate change and land-use change on freshwater macroinvertebrates and fish. *Journal of Applied Ecology, 51,* 572–581.

Mantyka-Pringle, C. S., Martin, T. G., & Rhodes, J. R. (2012). Interactions between climate and habitat loss effects on biodiversity: A systematic review and meta-analysis. *Global Change Biology, 18,* 1239–1252.

Mantyka-Pringle, C. S., Visconti, P., Di Marco, M., Martin, T. G., Rondinini, C., & Rhodes, J. R. (2015). Climate change modifies risk of global biodiversity loss due to land-cover change. *Biological Conservation, 187,* 103–111.

Marra, G., & Wood, S. N. (2011). Practical variable selection for generalized additive models. *Computational Statistics & Data Analysis, 55,* 2372–2387.

Metzeling, L., Tiller, D., Newall, P., Wells, F., & Reed, J. (2006). Biological objectives for the protection of rivers and streams in Victoria, Australia. *Hydrobiologia, 572,* 287–299.

Mora, C., Metzger, R., Rollo, A., & Myers, R. A. (2007). Experimental simulations about the effects of overexploitation and habitat fragmentation on populations facing environmental warming. *Proceedings of the Royal Society B: Biological Sciences, 274,* 1023–1028.

Moulding, B. J., King, G. K. K., Shenton, M., Bray, J. P., Nichols, S. J., & Kefford, B. J. (2022). Assessing the relative toxicity of different road salts and effect of temperature on salinity toxicity: LCx values versus No-Effect Concentration (NEC) Values. *Archives of Environmental Contamination and Toxicology, 82,* 281–293.

NLWRA. (2001). *Australian dryland salinity assessment 2000: Extent, impacts, processes, monitoring and management options* (p. 129). National Land and Water Resources Audit.

O'Connor, D. J., & Dobbins, W. E. (1958). Mechanism of reaeration in natural streams. *Transactions of the American Society of Civil Engineers, 123,* 641–666.

Orlinskiy, P., Münze, R., Beketov, M., Gunold, R., Paschke, A., Knillmann, S., & Liess, M. (2015). Forested headwaters mitigate pesticide effects on macroinvertebrate communities in streams: Mechanisms and quantification. *Science of the Total Environment, 524,* 115–123.

Orr, J. A., Vinebrooke, R. D., Jackson, M. C., Kroeker, K. J., Kordas, R. L., Mantyka-Pringle, C., Van den Brink, P. J., De Laender, F., Stoks, R., & Holmstrup, M. (2020). Towards a unified study of multiple stressors: Divisions and common goals across research disciplines. *Proceedings of the Royal Society B, 287,* 20200421.

Orr, S. E., & Buchwalter, D. B. (2020). It's all about the fluxes: Temperature influences ion transport and toxicity in aquatic insects. *Aquatic Toxicology, 221,* 105405.

Peters, M. K., Hemp, A., Appelhans, T., Becker, J. N., Behler, C., Classen, A., Detsch, F., Ensslin, A., Ferger, S. W., & Frederiksen, S. B. (2019). Climate–land-use interactions shape tropical mountain biodiversity and ecosystem functions. *Nature, 568,* 88–92.

Petersen, J. E., Cornwell, J. C., & Kemp, W. M. (1999). Implicit scaling in the design of experimental aquatic ecosystems. *Oikos, 85,* 3–18.

Pettigrove, V. (1990). The importance of site selection in monitoring macroinvertebrate communities of the Yarra River, Victoria. *Environmental Monitoring and Assessment, 14,* 297–313.

Piggott, J. J., Lange, K., Townsend, C. R., & Matthaei, C. D. (2012). Multiple stressors in agricultural streams: A mesocosm study of interactions among raised water temperature, sediment addition and nutrient enrichment. *PLoS ONE, 7,* e49873.

Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015a). Climate warming and agricultural stressors interact to determine stream

macroinvertebrate community dynamics. *Global Change Biology, 21,* 1887–1906.

Piggott, J. J., Townsend, C. R., & Matthaei, C. D. (2015b). Reconceptualizing synergism and antagonism among multiple stressors. *Ecology and Evolution, 5,* 1538–1547.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., & Puschendorf, R. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature, 439,* 161–167.

Quinn, G., & Keough, M. J. (1993). Potential effect of enclosure size on field experiments with herbivorous intertidal gastropods. *Marine Ecology Progress Series. Oldendorf, 98,* 199–201.

Rohr, J. R., Kerby, J. L., & Sih, A. (2006). Community ecology as a framework for predicting contaminant effects. *Trends in Ecology & Evolution, 21,* 606–613.

Rutherford, J. C., Marsh, N. A., Davies, P. M., & Bunn, S. E. (2004). Effects of patchy shade on stream water temperature: How quickly do streams heat and cool? *Marine and Freshwater Research, 55,* 737–748.

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation, 232,* 8–27.

Sauer, F. G., Bundschuh, M., Zubrod, J. P., Schäfer, R. B., Thompson, K., & Kefford, B. J. (2016). Effects of salinity on leaf breakdown: Dryland salinity versus salinity from a coalmine. *Aquatic Toxicology, 177,* 425–432.

Schäfer, R. B., & Piggott, J. J. (2018). Advancing understanding and prediction in multiple stressor research through a mechanistic basis for null models. *Global Change Biology, 24,* 1817–1826.

Schäfer, R. B., von der Ohe, P. C., Rasmussen, J., Kefford, B. J., Beketov, M. A., Schulz, R., & Liess, M. (2012). Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environmental Science and Technology, 46,* 5134–5142.

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology, 218,* 1856–1866.

Simmons, B. I., Blyth, P. S., Blanchard, J. L., Clegg, T., Delmas, E., Garnier, A., Griffiths, C. A., Jacob, U., Pennekamp, F., & Petchey, O. L. (2021). Refocusing multiple stressor research around the targets and scales of ecological impacts. *Nature Ecology & Evolution, 5,* 1478–1489.

Stampfli, N. C., Knillmann, S., Liess, M., Noskov, Y. A., Schäfer, R. B., & Beketov, M. A. (2013). Two stressors and a community – Effects of hydrological disturbance and a toxicant on freshwater zooplankton. *Aquatic Toxicology, 127,* 9–20.

Thompson, P. L., MacLennan, M. M., & Vinebrooke, R. D. (2018a). An improved null model for assessing the net effects of multiple stressors on communities. *Global Change Biology, 24,* 517–525.

Thompson, P. L., MacLennan, M. M., & Vinebrooke, R. D. (2018b). Species interactions cause non-additive effects of multiple environmental stressors on communities. *Ecosphere, 9,* e02518.

Verberk, W. C., Buchwalter, D. B., & Kefford, B. J. (2020). Energetics as a lens to understanding aquatic insect's responses to changing temperature, dissolved oxygen and salinity regimes. *Current Opinion in Insect Science, 41,* 46–53.

Vinebrooke, R. D., Cottingham, K. L., Norberg, J., Scheffer, M., Dodson, S. I., Maberly, S. C., & Sommer, U. (2004). Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. *Oikos, 104,* 451–457.

Vitousek, P. M. (1994). Beyond global warming: Ecology and global change. *Ecology, 75,* 1861–1876.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., & Liermann, C. R. (2010). Global threats to human water security and river biodiversity. *Nature, 467,* 555–561.

Waters, T. F. (1995). *Sediments in streams, sources, biological effects and control.* American Fisheries Society.

Wedemeyer, G. (1970). The role of stress in the disease resistance of fishes. *American Fisheries Society, 5,* 30–35.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology), 73,* 3–36.

Yahdjian, L., Sala, O. E., PiÑEiro-Guerra, J. M., Knapp, A. K., Collins, S. L., Phillips, R. P., & Smith, M. D. (2021). Why coordinated distributed experiments should go global. *Bioscience, 71,* 918–927.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.