Multiple anthropogenic stressors have inconsistent cumulative effects across a large spatial gradient

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Abstract (150-word limit)
Biodiversity is declining, typically because of multiple anthropogenic stressors. Cumulative effects of multiple stressors are classified as additive, when cumulative effects are as expected from the stressor's singular effects, synergistic when greater than additive or antagonistic when less than additive. Less attention has been given to the consistency of cumulative effects. We analysed stream insects, Ephemeroptera, Plecoptera and Trichoptera (EPT) data from two habitats spanning a 3,600 km latitudinal (S11°-S43°) gradient in eastern Australia. We found that the cumulative effect of salinity and suspended sediments on EPT family richness was inconsistent with additive, synergistic or antagonistic effects, and the reduction EPT family richness from increasing both stressors varied (48-70%) depending on habitat (riffle vs. edge), water temperature and terrain slope. Studies of cumulative effects of multiple stressors at one location risk not describing cumulative effects elsewhere and ecologists should consider the spatial consistency of multiple stressors.

Introduction

Biodiversity is increasingly subject to the cumulative effects of multiple stressors including land-use change, pollution, invasive species, and overharvesting (Vitousek 1994; Sánchez-Bayo & Wyckhuys 2019). There is widespread recognition that understanding interactions among multiple stressors is key to identifying and managing their cumulative impacts on biodiversity (Vörösmarty *et al.* 2010; Halpern *et al.* 2015), particularly as climate change is adding additional stress to ecosystems (Pounds *et al.* 2006; Mora *et al.* 2007; Brook *et al.* 2008; Mantyka-Pringle *et al.* 2012; Mantyka-Pringle *et al.* 2014; Mantyka-Pringle *et al.* 2015). Understanding stressor interactions is key because it could help identify how often and under what circumstances we expect 'ecological surprises': situations where the combined effect of stressors differs from the sum of their individual effects (an additive effect). For example, if stressors interact synergistically, their combined effects are amplified or magnified when they act together (Bliss 1939; Wedemeyer 1970; Hyslop 1976; Breitburg *et al.* 1999; Folt *et al.* 1999; Orr *et al.* 2020), potentially accelerating biodiversity loss. Alternatively, stressors can interact antagonistically, meaning their combined effect is less than the sum of 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).

Given that the nature of interactions can play a critical role in amplifying or dampening the cumulative effect of multiple stressors on biodiversity, much attention has focused on identifying and classifying stressor interactions (Birk *et al.* 2020), especially using the interaction typology above (e.g. additive/synergistic/antagonistic), evaluating the frequency of these different types of interactions in different ecosystems (Heugens *et al.* 2001; Crain *et al.* 2008; Piggott *et al.* 2015b; Jackson *et al.* 2016), and trying to identify mechanisms that might explain how and why stressors interact in these different ways (Vinebrooke *et al.* 2004;

Hodgson *et al.* 2017; De Laender 2018; Schäfer & Piggott 2018; Verberk *et al.* 2020; Franklin & Hoppeler 2021; Jackson *et al.* 2021). Much research into multiple stressor impacts has used this typology, but less widely explored is the consistency of the interaction between 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. In the case of two stressors, 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 organisation 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 measure the interaction between two stressors, and hence determine the nature of their 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 generalising the findings from individual studies, allowing results obtained at one location and time to be extrapolated to other locations and times.

Nevertheless, there are good reasons to anticipate inconsistencies in a stressor interaction 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 gradients in temperature or water availability. Such inconsistency would imply a higher-order interaction, whereby the form of the two-way stressor interaction changes in response to a change in one or more 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 under different circumstances. Such inconsistency could limit the scope for understanding and predicting multiple stressor impacts by focusing on the additive/synergistic/antagonistic typology, suggesting a need to expand focus to evaluate the consistency of outcomes across space and time, and the role of higher-order interactions, which may not necessary be linear (Duncan & Kefford 2021). However, few multiple stressor studies have examined these issues because most are of limited spatial and/or temporal extent, and most consider only two-way interactions among multiple stressors (but see (Piggott *et al.* 2015a; Beermann *et al.* 2018; Beermann *et al.* 2021) for rarer examples of considering three-way interactions).

Our aim is to use a near continent-wide dataset (4,658 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 EPT families (Barbour *et al.* 1996; Eriksen *et al.* 2021)), and to quantify how the interaction, and the resulting cumulative effects of the two stressors, varies across two major environmental gradients in the region: changes in mean temperature and terrain slope. We use methods that account for statistical issues associated with estimating interactions, including the possibility that any interactions are non-linear (Duncan & Kefford 2021) to uncover the form of the turbidity-salinity stressor interaction, and to evaluate the consistency of this interaction. We show that the stressor interaction is inconsistent, with the form of the interaction changing along environmental gradients, and hence whether the combined effect is described by an additive, synergistic or antagonistic type effect varies spatially. Consequently, a study at a single location is insufficient to understand the cumulative impact of these two stressors on freshwater biodiversity. Our findings suggest that research on multiple stressors would benefit from a shift in focus toward understanding the consistency of the cumulative impacts of stressors, which implies greater consideration of higher-order interactions.

Methods

Field data collection.

We analysed stream macroinvertebrate data and associated environmental variables collected at 4,658 sites located throughout the Australian jurisdictions of Tasmania, Victoria, New South Wales (NSW), Australian Capital Territory (ACT) and Queensland (Figure 1a). The sites covered a wide latitudinal gradient from tropical to temperate (latitude S11°-S43°, spanning approximately 3,600 km) and were at elevations ranging from sea level to 2020 m above sea level.

The data were collected by staff from relevant government agencies as part of a standardised monitoring program to assess stream condition in each jurisdiction, with streams chosen for sampling from a wide range of land-use categories. Stream sampling was carried out by trained staff using standardised methods with quality assurance procedures (Nichols & Norris 2006). The detailed sampling protocols are available here: https://ausrivas.ewater.org.au/index.php/manuals-a-datasheets. Briefly, at each site macroinvertebrate samples were collected from stream edge habitat (defined as slow flowing or still waters adjacent to the stream bank, preferably with overhanging or emergent vegetation, undercut banks, root mats or other suitable habitat providing cover and refuge for macroinvertebrates) by sweeping a hand-held net (250µm mesh) through the water column including though submerged vegetation and other habitat present in the water (sweep sampling). At sites where riffle habitat was present (defined as broken water flowing over gravel, pebbles, cobbles, boulders, or bedrock with water depth generally between 10-30 cm), macroinvertebrates were sampled from this habitat by placing the frame of the hand-held net on the steam bed with the mesh net trailing downstream. The operator stood upstream of the net disturbing the substrate with their feet to dislodge material, including invertebrates, that were washed into the net (kick sampling). In both habitats, a sample was obtained from about a 10m section of stream. The macroinvertebrates collected in each sample were then sub-sampled to obtain approximately 200 individuals and the taxa in the sub-sample were identified to family with the aid of a stereomicroscope. Of the 4,658 sample sites, edge habitat was sampled at 4,339 sites and riffle habitat was sampled at 2,533 sites, with both habitats sampled at 2,214 sites. A greater number of edge habitats were sampled because sites sampled on streams and rivers traversing predominantly flat plains, especially in inland (western) NSW and Victoria, typically lacked riffle habitat (Figure 1a).

For each sample taken from each habitat, we calculated the number of families in the freshwater insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (EPT family richness). This biodiversity measure is widely used as an index of stream "health" because EPT family richness is known to be sensitive to a variety of stressors in freshwater ecosystems and is used globally as an indicator of water quality (Barbour *et al.* 1996; Eriksen *et al.* 2021). EPT family richness contain the same statistical information as EPT genus and EPT species richness (Kitchin 2005).

Concurrent with macroinvertebrate sampling, several environmental variables were measured in each habitat at each site using standardised methods and calibrated instruments, including water temperature (°C), electrical conductivity (EC, µS/cm @ 25°C) and turbidity (NTU). Electrical conductivity is a proxy for salinity, which is the total concentrations of multiple anions and cations (Williams & Sherwood 1994). 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 such things as dissolved organic matter and algae blooms, the major cause of high turbidity in Australian streams is suspended sediments (Harrison *et al.* 2011). In eastern Australia, as elsewhere, levels of both salinity and suspended sediments can vary naturally with geology, climate, and deposition of oceanic aerosols, but the dominant driver of variation in salinity and turbidity is human land-use modification, particularly vegetation clearance since European settlement (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) on EPT family richness because these two stressors have major impacts in Australian and other freshwater systems driven primarily by human activities.

We examined how the cumulative impacts of EC and turbidity on EPT family richness varied in relation to mean water temperature and terrain slope, two factors that varied widely across the study region and that have the potential to affect EPT family richness. Mean temperature varied widely because of the wide latitude and elevation range across the region. Temperature could affect the diversity of 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), although little is documented about how EPT family richness varies in response to temperature at large spatial scales. Terrain slope also varied widely across the region because sample sites ranged from flat coastal and inland plains to mountainous areas. Terrain slope is a likely surrogate for other factors that could directly affect EPT taxa, including, water velocity (m/s) and related flow characteristic like turbulence, sheer stress, which influence such things as levels of dissolved oxygen and stream bed substrate. Because many EPT taxa favour fast-flowing water, we used an index of terrain slope as a surrogate to capture variation in flow, dissolved oxygen and related factors.

Data analysis.

Sixty percent of sites had been sampled on more than one occasion (range from 1-38 sampling occasions) over the period of data collection (1990-2016). For each habitat at each site sampled more than once, we calculated the means for EPT family richness, EC, and turbidity, averaged over the sampling occasions, and used these values in our analysis.

We used two methods to derive a comparative measure of temperature at each site. First, water temperature (°C) was measured directly in each habitat at each site on each sampling occasion. To correct for seasonal variations in water temperature sampled at different times of the year, 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 effect 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 to extract the mean annual air temperature (°C) given each site's latitude and longitude. Within habitats, our measure of scaled temperature was strongly positively correlated with WorldClim mean annual temperature (riffle: $r = 0.91$, edge: $r = 0.84$, Supplementary Figure S1). We used scaled temperature in our analyses because this measure was derived from direct measurements of water temperature in each habitat at each site, accounts for elevation and captures temperature differences associated with variation in local site conditions, for example shading by overhanging vegetation (Rutherford *et al.* 2004).

We calculated an index of terrain slope at each site. To do this, we obtained elevation data from the SRTM 90m digital elevation database using the getData function in the R package raster and then used the terrain function in that package to calculate the slope at each site in radians. We then calculated a slope index as: log(1/tan(slope in radians)), which equates to 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 ($r = 0.44$), in part because the predominantly flat sites of the inland plains tend to have warmer temperatures. Nevertheless, this moderate correlation implies that the two measures will capture at least some independent aspects of environmental variation that could influence EPT family richness at each site. We log transformed values for EC and turbidity and then scaled each of the four explanatory variables to mean zero and standard deviation one prior to including them in the analyses below.

We first examined univariate relationships between EPT family richness and each of the four explanatory variables (EC, turbidity, temperature, and slope index) within each habitat. We did this to assess the degree of non-linearity in these relationships, which is an important factor to consider when fitting statistical models with interactions. 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 generalized additive models (GAMs) to the data, fitting two models to each dataset: 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 values 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.

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, along with latitude and longitude to allow for spatial dependence. We used a variable selection procedure via the option select = TRUE in the gam function in mgcv, which serves to shrink a variable's smoothing function towards zero when that variable is weakly related to the outcome. This meant that we retained all main effect and interaction terms in the model, but terms associated with variables of little importance were shrunk towards zero and hence had little influence on the resulting 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 (Supplementary Figure S2), excluding combinations of explanatory variable values for which we had little or no data, and for which model predictions would be less reliable. 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 could assess 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 either 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. For relationships identified as non-additive, we then classified these as synergistic or antagonistic based on the form of the interaction.

The above approach allows us to classify and evaluate the consistency of the interaction between turbidity and EC. 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 a situation where both stressors had a low value to one 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. This measure of consistency may be as or more relevant than the form of the interaction between stressors. For example, 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: the same two stressors could have relatively little cumulative impact on biodiversity at some locations, but substantial impact at others.

Results

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 (see Figure 2). Each explanatory variable alone explained between 0.39-0.46 of the total deviance in each habitat.

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 (Supplementary Figure S3). Parameter estimates for the fitted models indicated there were important three-way interactions in both habitats that involved all four explanatory variables (Table 1). These higher-order interactions imply that the form of the relationship between turbidity and EC was inconsistent and varied depending on values of temperature and slope. 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 and ≤ 2 indicate a weakly non-linear relationship and values >2 a highly non-linear relationship (Hunsicker *et al.* 2016). Some parameters had EDF values <1 caused by the variable selection procedure we used: parameters with EDF <1 had been shrunk toward zero). Hence, the results show that, not only were three-way interactions important, but several three-way interactions were highly non-linear, as were several two-way interactions and main effects (Table 1 and see Figure 2).

For both riffle and edge habitats, these higher-order interactions resulted in the cumulative effects of turbidity and EC on EPT family richness varying under different temperature and slope conditions (Figures 3 and 4). For riffle habitat, the cumulative effects of turbidity and EC on EPT family richness were mostly additive for steep and intermediate slopes but shifted to synergistic for shallow slopes (Figure 3). In contrast, for edge habitats the cumulative effects were mostly antagonistic, shifting toward additive for cool sites on steeper slopes (Figure 4).

In addition to inconsistencies in the nature of the interaction, the magnitude of the cumulative impact of the two stressors varied markedly among sites. For both riffle and edge habitats, the major feature was that the cumulative impact of turbidity and EC increased along a gradient from warm to cool temperatures. That is, an increase in cumulative stress caused by increases in turbidity and EC had a larger impact on EPT family richness in cool relative to warm environments. In riffle habitat, for example, cool sites were predicted to lose about 70% of EPT families when turbidity and EC increased from low to high values, while warm sites were predicted to lose about 50% (Figure 3).

Discussion

Both EC and turbidity, had large negative effect on EPT family richness (Figure 2). These observations were broadly in agreement with other research on freshwater insect from the orders Ephemeroptera, Plecoptera and Trichoptera in our study region (Pettigrove 1990; Kefford *et al.* 2011; Bray *et al.* 2018) and elsewhere (Conroy *et al.* 2016; Akamagwuna *et al.* 2019). Depending on the habitat (edge or riffle), water temperature and terrain slope, the cumulative effect of EC and turbidity on EPT family richness was classified as additive, synergistic or antagonistic so the nature of the cumulative impact on biodiversity from EC and turbidity differed spatially and between habitats (Figure 3 and 4). Perhaps more importantly there was between 48% and 70% loss in EPT family richness occurred by moving from low to high levels of both EC and turbidity, depending on habitat, temperature and slope. Failure to consider spatial, and potentially temporal, inconsistency of cumulative effects between multiple stressors will mean that identified effects are applicable only under a narrow range of situations.

Singular effect of various stressors are inconsistent across natural gradients associated with climate (Pounds *et al.* 2006; Lorenzen *et al.* 2011; Guo *et al.* 2018; Karp *et al.* 2018; Peters *et al.* 2019), experimentally imposed drought (Stampfli *et al.* 2013) and occurrence of forests (Schäfer *et al.* 2012; Orlinskiy *et al.* 2015). For example, Peters et al. (2019) observed landuse changes tended to have less effect at higher elevational (i.e. colder) sites than lower (i.e. warmer) sites. The novelty of the current study is in showing that not only can singular effects of stressors be inconsistent across natural gradients, but that the cumulative effects of natural stressors also can be inconstant.

Some similar inconsistencies were observed in a three-factor German mesocosm experiment manipulating salinity, sedimentation and water velocity (Beermann *et al.* 2018; Beermann *et al.* 2021), which correspond to EC, turbidity and terrain slope, respectively, in the current study. Beermann's et al. experiment found that in streambed habitats there was a statistically significant three-way interaction between salinity, sedimentation and water velocity for EPT genus/family richness (Beermann *et al.* 2018) (in that study, Ephemeroptera were identified to family level, while Plecoptera and Trichoptera, generally to genus level). Beernmann et al. (2018) also found that in leaf litter habitat none of these three stressors and none of their interactions had a statistically detectable effect on EPT genus/family richness. So, in both our study and in Beermann et al. (2018), the effect of salinity and sediment was inconsistent across habitats. However, in the streambed habitat the relationship between the stressors and EPT richness was inconsistent with our results in both the riffles and edge habitats. As, Beermann (2018) found that in the presence of sedimentation and reduced water velocity, increasing salinity increased EPT genus/family richness, yet we did not observe EPT family richness to increase with increasing salinity (EC) nor increasing sedimentation (turbidity). In the presence of sedimentation, without decreasing water velocity, Beermann et al. (2018), observed that EPT genus/family richness declined with increased salinity, broadly consistent with our results. Thus, the response of EPT richness to salinity and sedimentation and the influence of water velocity/terrain slope on this response varied between the two studies possibly because in natural over extended periods terrain slope will influence habitat characteristics of streams such as size distribution of substrate, which will be unchanged in mesocosm experiments.

There was another type of inconsistency in the cumulative effects of salinity, sedimentation and water velocity in Beermann's et al. experimental study, which we call inconsistency across taxonomic resolutions. The literature on different taxonomic resolutions for stream macroinvertebrates (Marchant *et al.* 1995; Wright 1995; Bailey *et al.* 2001; Buss & Vitorino 2010), including EPT richness (Kitchin 2005) finds very similar conclusions across species, genus and family levels of identification, which is not reflected in Beermann's et al. experiment. EPT richness was quantified by two methods. First, using traditional morphological identification of the EPT genus/family richness (Beermann *et al.* 2018) as discussed and second, using DNA metabarcoding to operational taxonomic units or OTU (Beermann *et al.* 2021) with different effects of the three stressors on EPT richness across these levels of taxonomic resolution. For streambed, EPT genus/family richness there was a significant three-way interaction (Beermann *et al.* 2018) indicating a complex effect of all three variables, which is difficult to quantify in the additive/synergistic/antagonistic typology. In contrast, the streambed EPT OTU richness was negatively affected by sedimentation and reduced water velocity and unaffected by salinity and nor were there any significant interactions, indicating an additive cumulative effect of sedimentation and reduced water velocity and no effect of salinity (Beermann *et al.* 2021). The inconsistencies between the effects of the three stressors between the two levels of taxonomic resolution can be explained by although certain combinations of the three stressors changed the total number of EPT families/genera, that these changes were compensated for by changes in the number of OTU within families/genera.

The inconsistency in cumulative effects suggests focusing on classifying stressor interactions using the additive/synergistic/antagonistic typology is likely to overlook important features of stressor interactions. Notably the form of the interaction is not fixed, nor is the magnitude of the cumulative impact of two stressors. The latter may be more important in understanding and predicting stressor impacts than the form of the interaction, at least, when two or more stressors have a common driver. In Australia, while the precise local causes are complex, both sedimentation and salinization of streams are largely a product agriculture activities, in particular clearing of native vegetation, over grazing and cultivation (NLWRA 2001; Harrison *et al.* 2011; Sauer *et al.* 2016). Consequently, in many cases management practices, e.g. planting of native vegetation, that address one of these stressors will address the other, at least at the spatial scale of our analysis. However, understanding the nature of the interactions becomes more important where stressors are the result of independent drivers and management practices that address one stressor do not appreciably affect other stressors. As in such circumstance, managers can decide which stressor or stressors they prioritize in reducing, and where effects are synergistic, there will be advantages of addressing all stressors (Simmons *et al.* 2021).

Recently, various authors have advocated developing a theoretical understanding of the mechanisms that cause particular effects and interactions (Griffen *et al.* 2016; De Laender 2018; Schäfer & Piggott 2018; Thompson *et al.* 2018a, b; Dey & Koops 2021; Franklin & Hoppeler 2021; Jackson *et al.* 2021). We agree that such theory would be a significant scientific advancement. Such theory would also be practically useful if it could predict, from relatively simple measurements, circumstances where cumulative effects are likely to be synergistic, additive or antagonistic. Knowing, for example, that certain stressors are likely to cause synergistic cumulative effects could led to prioritising their remediation with consequent biodiversity benefits. Our results, and those of Beermann (2018), however, suggest that a mechanistic understanding needs to consider more than the nature of stressors but also factors that cause the interactions between stressors to vary spatially.

Although not observed in the current study, non-linear interactions (Duncan & Kefford 2021) between multiple stressor or environmental factors have the potential to render the additive/synergistic/antagonistic typology not descriptive of cumulative effects of even two stressors. We were able to classify the non-linear interactions in the current study into additive, synergistic or antagonistic type effects (Figures 3 & 4), despite 50% of two-way and 60% of the three-way interactions (Table 1) being highly nonlinear (EDF >2 following Hunsicker et al. (2016)). However, it is plausible that two stressors might have an interaction of a form like Figure 5d, where there is an additive effect of the two stressors at low to moderate levels of stressor 1, but a synergistic effect at higher levels of stressor 1. Such interactions require a revision of the typology, to allow the type of effect to change across the levels of the stressors.

More complex still non-linear interactions between stressors can be imagined, which are not easily classified into the additive/synergistic/antagonistic typology (e.g. Figure 5e). Although how common such non-linear interactions, remains to be seen.

Our findings, and the potential for complex non-linear interactions (Figure 5d,e), emphasise the need for multiple stressor studies to expand their focus beyond the current typology. We appreciate this is difficult because it involves considering higher-order interactions among multiple stressors and environmental factors. Analysis of large spatial (or temporal) scale datasets, as we did in the current study, are useful for considering the consistency of cumulative effects between multiple stressors. Further studies of multi-stressor experiments manipulating three stressors along the lines of Beermann et al. (2018) would be useful but logistical constraints make investigating consistency of multiple stressors across a wide range of circumstances in a single experiment a considerable challenge. Rather, it may be more practical to repeat experiments at multiple locations along climatic and other gradients. Conducting single experiments at one location, as we have done e.g. (Bray *et al.* 2018; Bray *et al.* 2019; Brooks *et al.* 2021), while useful, is only the first step to understanding cumulative effects of stressors across a range of circumstances. Editors of journals should recognise the novelty (and value) of multi-stressor studies that repeat experiments or observational studies done elsewhere with the aim of determining the consistency of the cumulative effects, rather than see such repeat studies as merely conformational. Ultimately, meta-analysis should bring together multiple observational and/or experimental studies to uncover cumulative effects across a range of circumstance will likely be needed.

In addition to identifying higher-order interactions, a useful approach could be to identify metrics that evaluate the consistency of relationships between stressors. These could focus on different aspects regarding how the cumulative impacts of stressors vary across the landscape in the way we have done here (e.g. in terms of the form of interactions, or the magnitude of cumulative impact). This could enable identification of what is important in terms of generating ‘ecological surprises’ (Orr *et al.* 2020). In our study, we suggest it is variation in the magnitude of cumulative impacts rather than variation in the form of interactions that is key to identifying sites most vulnerable to increasing levels of turbidity and EC. For other response variable, ecosystems, locations and stressors, this may not necessary be the case.

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Author Contributions

Provision of data: BJK and SJN; conception of the idea: BJK and RPD, data analysis: RPD; wrote first draft: BJK and RPD; finding literature, editing and approval of final MS: all.

Data Availability

R code used to reproduce all analyses in this paper will be made available on publication. The data analysed will be made publicly available after one-year embargo, following publication.

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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 EPT family richness at each site. 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. 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. Statics for terms which are statistically significant (P<0.05) are in bold text.

	Riffle			Edge		
Term	EDF	Chi sq	P	EDF	Chi sq	P
s(temperature)	**3.5**	**31.4**	**<0.001**	**2.5**	**52.1**	**<0.001**
s(slope)	**1**	**34.5**	**<0.001**	**1**	**38**	**<0.001**
s(turbidity)	**2.7**	**40.6**	**<0.001**	**3.4**	**197.7**	**<0.001**
s(EC)	**3.6**	**153.3**	**<0.001**	**5.4**	**146.5**	**<0.001**
ti(temperature, slope)	0.7	1.2	0.14	**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**

Figure captions

Figure 1. A. Map of the sites sampled in eastern Australia for macroinvertebrates by habitat (riffle and edge) with each site coloured by observed EPT family richness. B. Distribution of EPT family richness at sample sites by habitat.

Figure 2. Univariate relationships between the four variables (scale temperature (derived from water temperature °C), slope, turbidity (NTU) and electrical conductivity (EC, µS/cm @ 25°C)) and EPT family richness for each habitat type (riffle and edge) with fitted GAMs. Grey circles are the raw data, red circles are the mean value for EPT family richness for equal-sized bins of the variable 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.

Figure 3. Predicted values of 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 expected when shifting from low to high values of both turbidity and EC (see text for details).

Figure 4. Predicted values of 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 expected when shifting from low to high values of both turbidity and EC (see text for details).

Figure 5. Stylised hypothetical examples of potential cumulative effects of two stressors. (a) No effect of ether stressor, (b) negative singular effect of both stressors and not interactive effect of the two stressors, i.e. with an additive cumulative effect, (c) negative singular effect of both stressors and with linear interactive effect of the two stressors, in this example a synergistic cumulative effect, (d) negative singular effect of both stressors and with non-linear interactive effect of the two stressors, in this example a at low to moderate levels of stressor 1 the cumulative effect is additive but at higher levels of stressor 1 the cumulative effect becomes synergistic, and (e) complex effects of two stressors with a non-linear interaction, which cannot be fitted into the additive/antagonistic/synergistic typology.

Figure 1

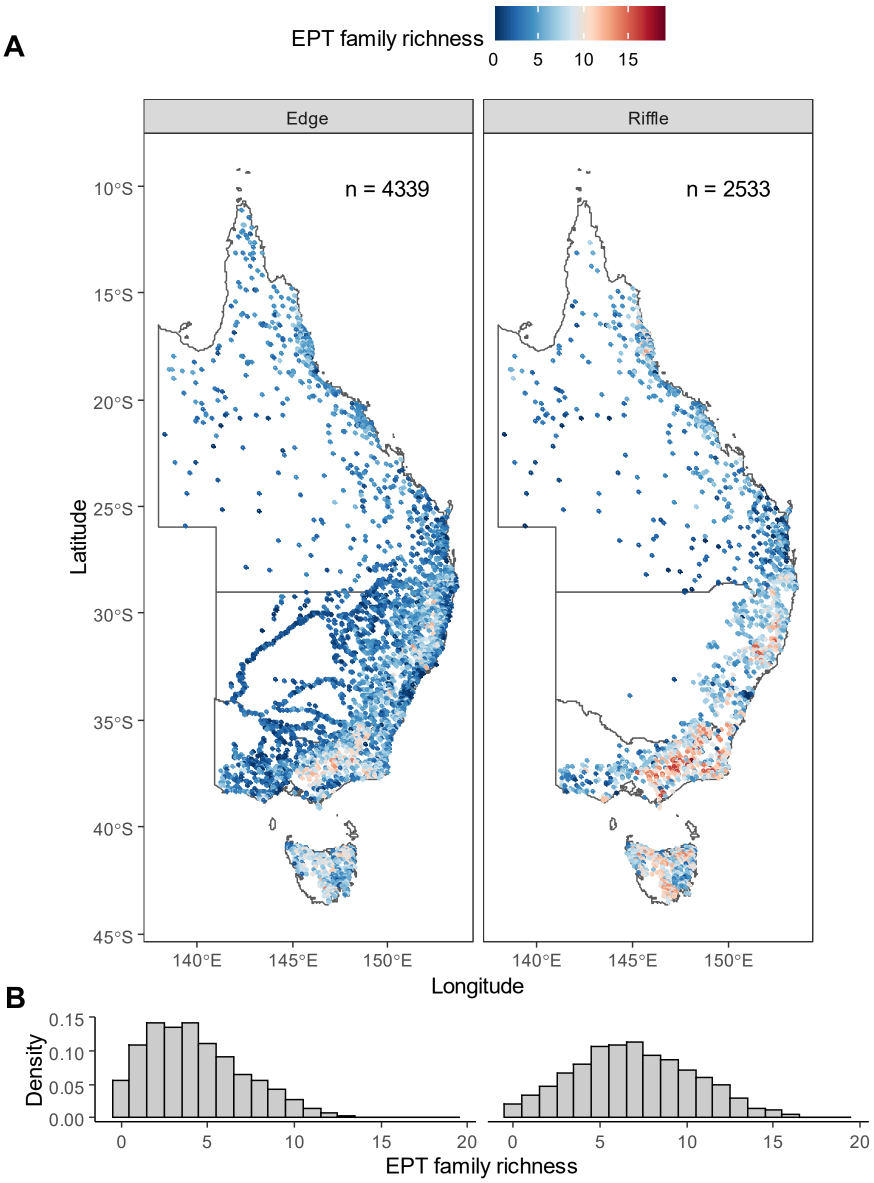

A
EPT family richness
0
5
10
15
Edge
Riffle
n = 4339
n = 2533
10°S
15°S
20°S
25°S
30°S
35°S
40°S
45°S
Latitude
140°E
145°E
150°E
140°E
145°E
150°E
Longitude
B
Density
0.15
0.10
0.05
0.00
0
5
10
15
20
0
5
10
15
20
EPT family richness

Figure 2.

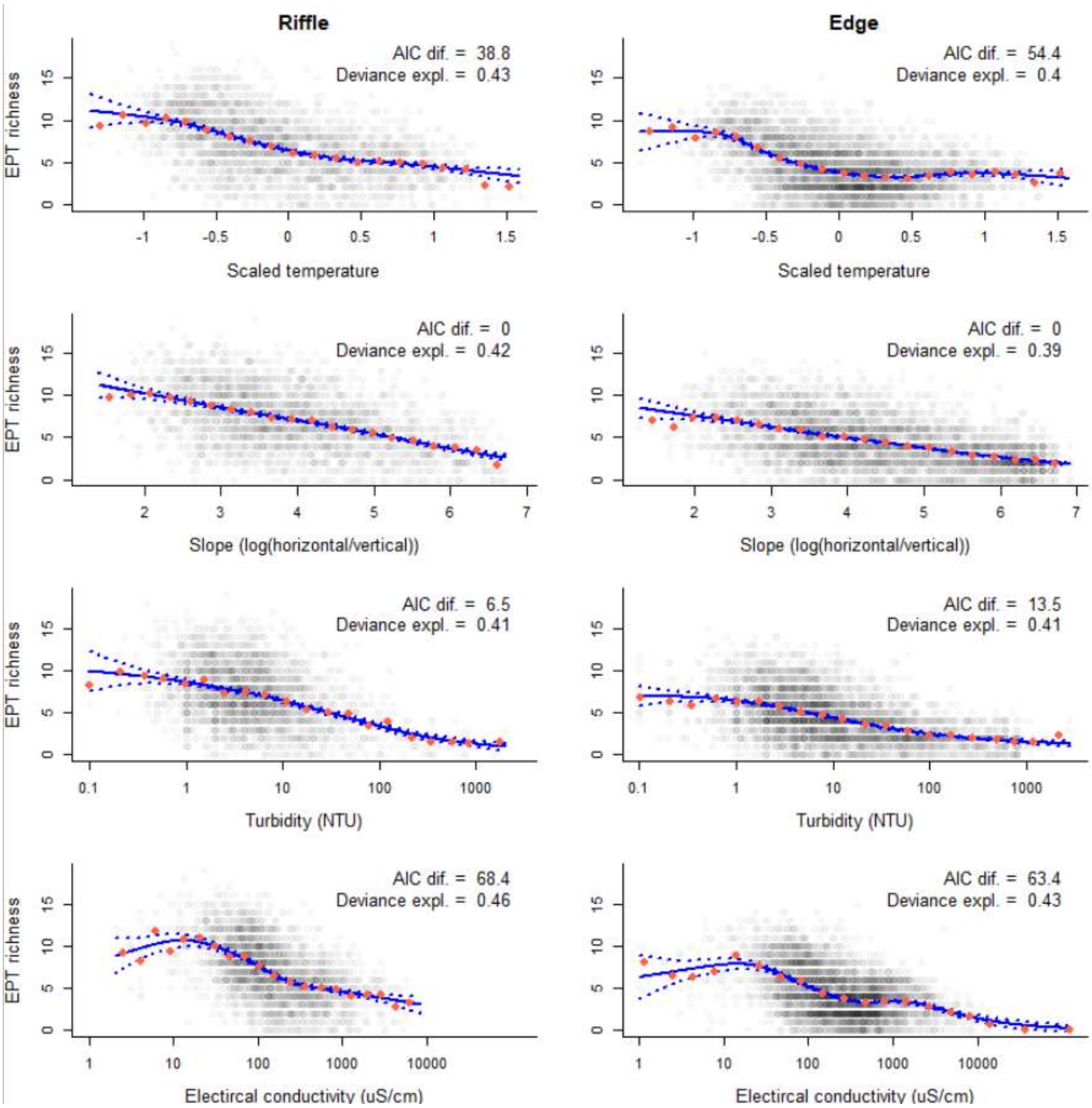

Riffle
Edge
AIC dif. = 38.8
Deviance expl. = 0.43
AIC dif. = 54.4
Deviance expl. = 0.4
EPT richness
0
5
10
15
-1
-0.5
0
0.5
1
1.5
Scaled temperature
AIC dif. = 0
Deviance expl. = 0.42
AIC dif. = 0
Deviance expl. = 0.39
2
3
4
5
6
7
Slope (log(horizontal/vertical))
AIC dif. = 6.5
Deviance expl. = 0.41
AIC dif. = 13.5
Deviance expl. = 0.41
0.1
1
10
100
1000
Turbidity (NTU)
AIC dif. = 68.4
Deviance expl. = 0.46
AIC dif. = 63.4
Deviance expl. = 0.43
1
10
100
1000
10000
Electircal conductivity (uS/cm)

Figure 3

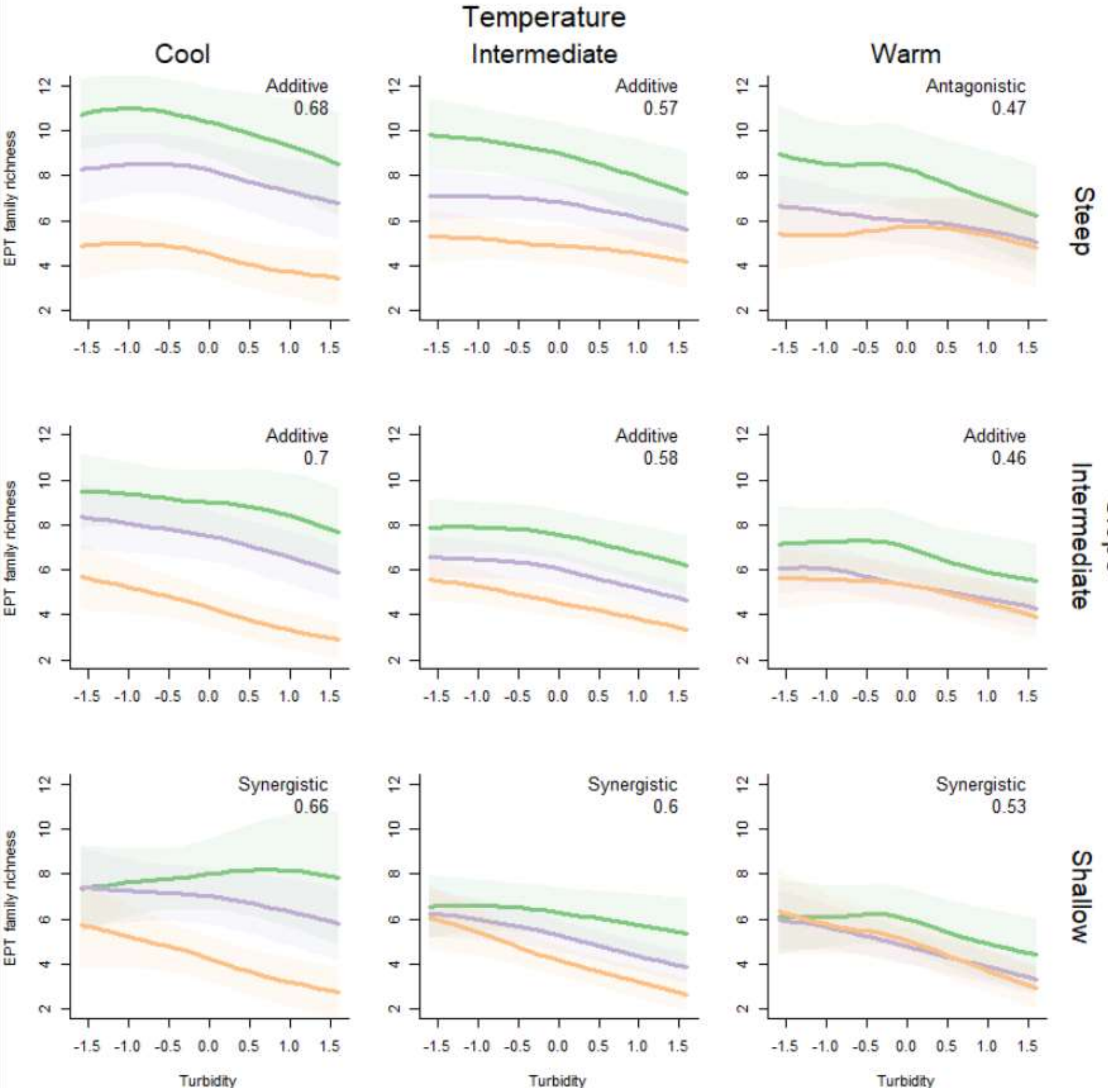

Temperature
Cool
Intermediate
Warm
Slope
Steep
Intermediate
Shallow
EPT family richness
Turbidity
2
4
6
8
10
12
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Additive
0.68
Additive
0.57
Antagonistic
0.47
Additive
0.7
Additive
0.58
Additive
0.46
Synergistic
0.66
Synergistic
0.6
Synergistic
0.53

Figure 4

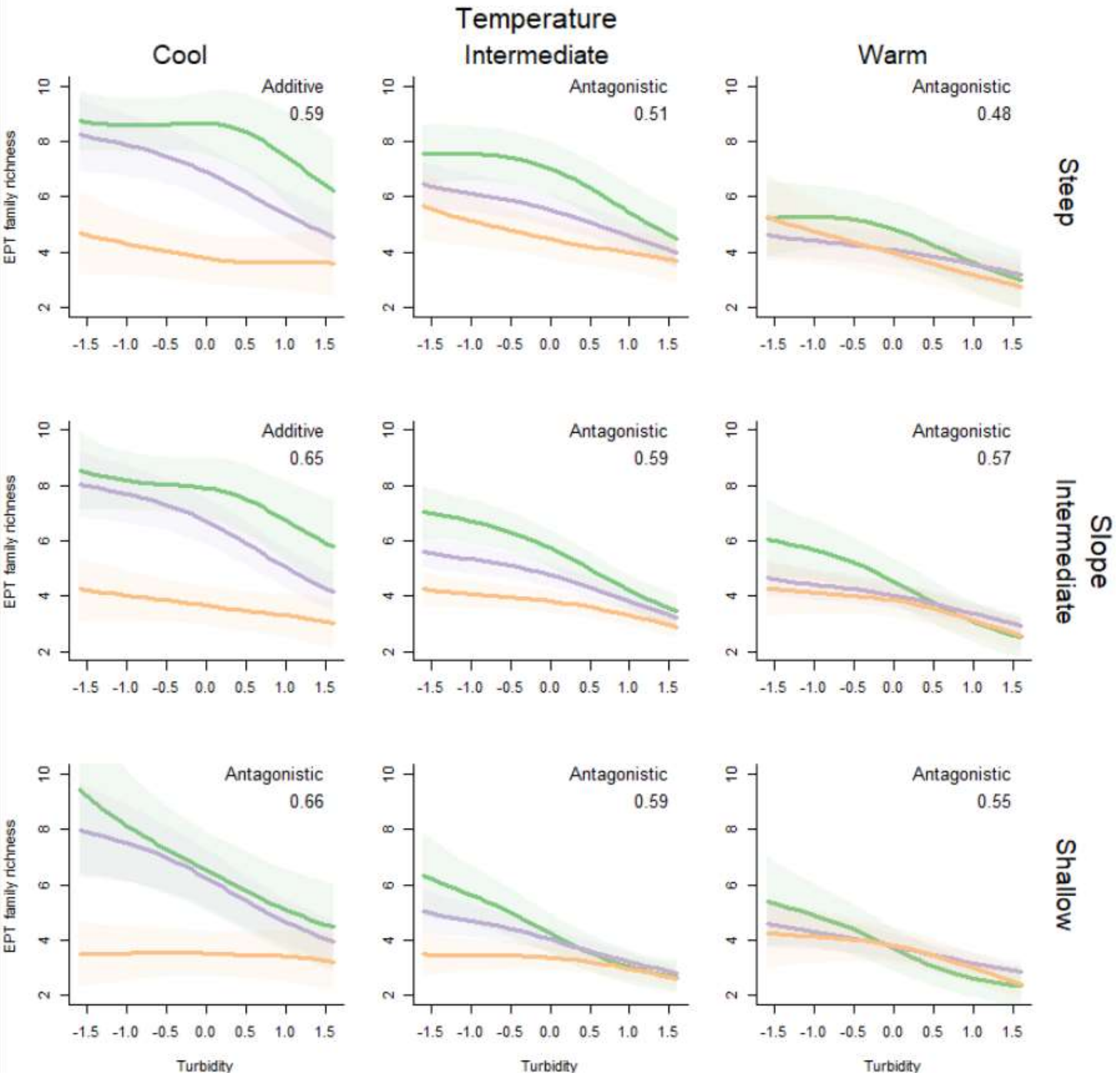
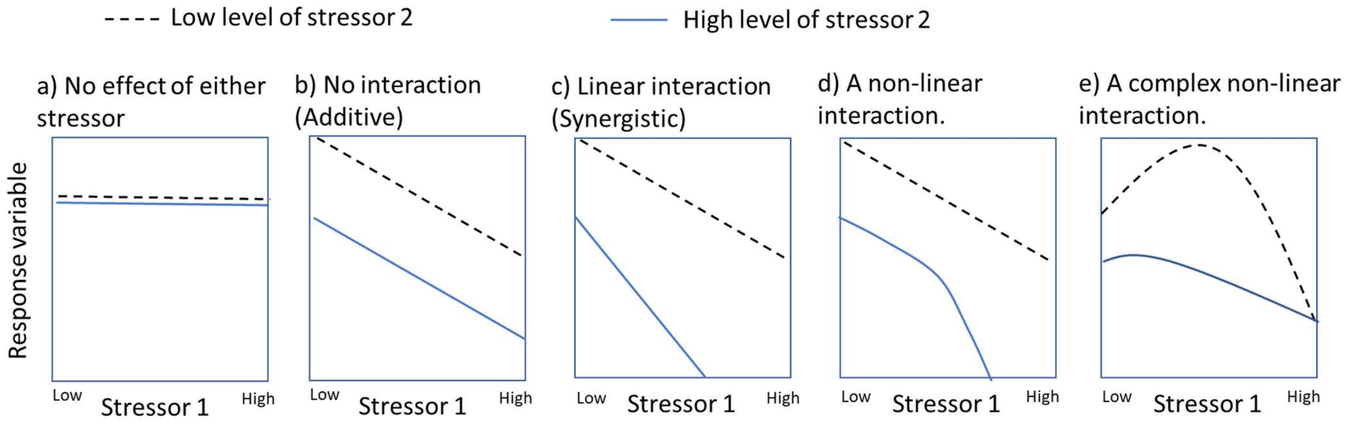

Temperature
Cool
Intermediate
Warm
Slope
Steep
Intermediate
Shallow
EPT family richness
Turbidity
Additive 0.59
Antagonistic 0.51
Antagonistic 0.48
Additive 0.65
Antagonistic 0.59
Antagonistic 0.57
Antagonistic 0.66
Antagonistic 0.59
Antagonistic 0.55
2
4
6
8
10
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5

Figure 5


- - - - Low level of stressor 2
High level of stressor 2
a) No effect of either stressor
b) No interaction (Additive)
c) Linear interaction (Synergistic)
d) A non-linear interaction.
e) A complex non-linear interaction.
Response variable
Low
Stressor 1
High