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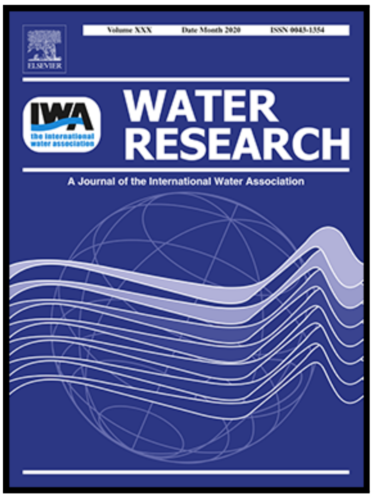

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Perceived multiple stressor effects depend on sample size and stressor gradient length

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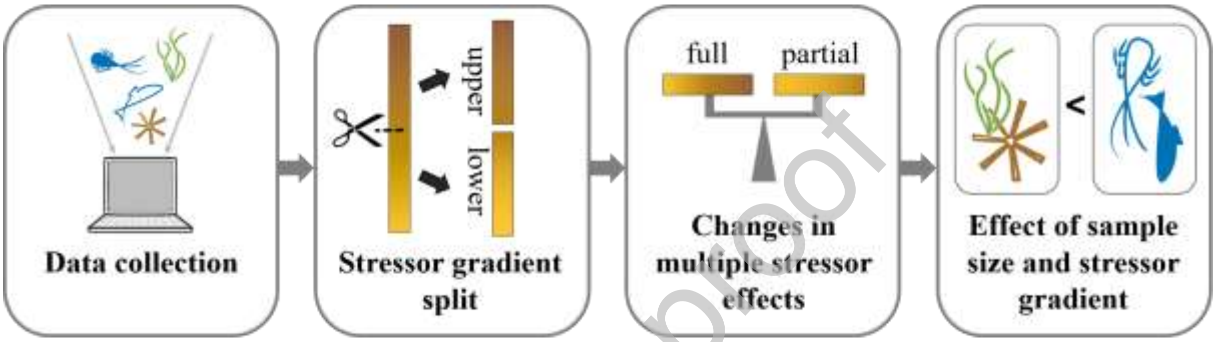
Highlights

- Multiple stressor effects depend on sample size and stressor gradient length
- Dependence on gradient length increases with increasing trophic level
- Monitoring programmes need to provide sufficient stressor gradient coverage
- The findings highlight the importance of adaptive environmental management

Graphical Abstract



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Abstract

Multiple stressors are continuously deteriorating surface waters worldwide, posing many challenges for their conservation and restoration. Combined effect types of multiple stressors range from single-stressor dominance to complex interactions. Identifying prevalent combined effect types is critical for environmental management, as it helps to prioritise key stressors for mitigation. However, it remains unclear whether observed single and combined stressor effects reflect true ecological processes unbiased by sample size and length of stressor gradients. Therefore, we examined the role of sample size and stressor gradient lengths in 158 paired-stressor response cases with over 120,000 samples from rivers, lakes, transitional and marine ecosystems around the world. For each case, we split the overall stressor gradient into two partial gradients (lower and upper) and investigated associated changes in single and combined stressor effects.

Sample size influenced the identified combined effect types, and stressor interactions were less likely for cases with fewer samples. After splitting gradients, 40 % of cases showed a change in combined effect type, 30 % no change, and 31 % showed a loss in stressor effects. These findings suggest that identified combined effect types may often be statistical artefacts rather than representing ecological processes. In 58 % of cases, we observed changes in stressor effect directions after the gradient split, suggesting unimodal stressor effects. In general, such non-linear responses were more pronounced for organisms at higher trophic levels.

We conclude that observed multiple stressor effects are not solely determined by ecological processes, but also strongly depend on sampling design. Observed effects are likely to change when sample size and/or gradient length are modified. Our study highlights the need for improved monitoring programmes with sufficient sample size and stressor gradient coverage. Our findings emphasize the importance of adaptive management, as stress reduction measures or further ecosystem degradation may change multiple stressor-effect relationships, which will then require associated changes in management strategies.

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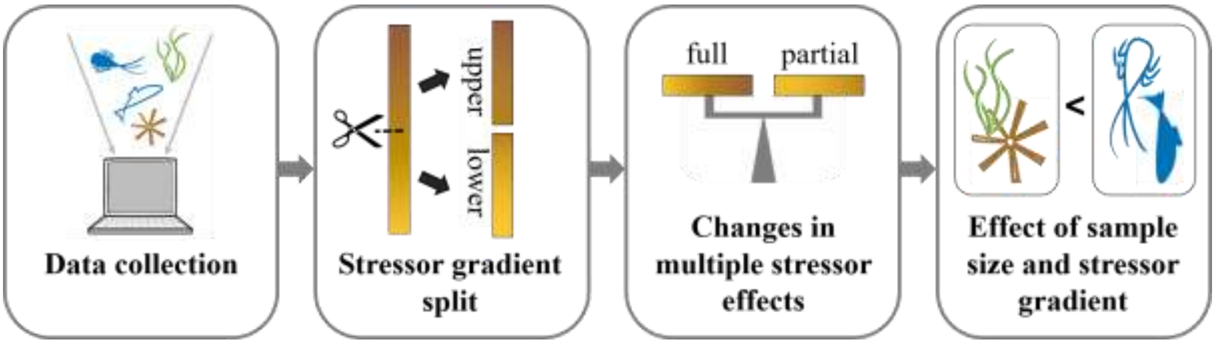
Keywords: multiple stressor effect sizes, multiple stressor effect types, stressor levels, dose dependence, adaptive management, sampling design

Graphical abstract



1 Introduction

Multiple stressors are damaging ecosystems worldwide. Hence, for successful conservation and restoration of surface waters, these need to be addressed in concert (Nõges et al., 2016). Human-induced stressors operate locally (e.g. modified land use) to globally (climate change), all leading to critical declines in biodiversity and functioning of aquatic ecosystems (Dirzo et al., 2014). Surface waters are particularly vulnerable ecosystems which suffer from various stressors, such as nutrient and contaminant loadings, hydro-morphological alterations, rising temperatures and acidification (EEA, 2018; IPCC, 2022). Most aquatic ecosystems are therefore affected by multiple, co-occurring stressors, which can interact and thereby, change their combined effects on biological communities (Breitburg and Riedel, 2005; Schinegger et al., 2016; Grizzetti et al., 2017; Reid et al., 2019). Conceptually, ecologists distinguish dominant, additive and interactive (synergistic or antagonistic) combined effect types (Folt et al., 1999). Interactions can occur when one stressor modifies the effect of another stressor or modifies the sensitivity of the affected organism to another stressor. Identifying stressor interactions is particularly important for the design of effective mitigation measures in environmental management, as different interaction types require different management approaches (Côté et al., 2016; Ormerod et al., 2010; Spears et al., 2021). Mitigating a stressor that interacts synergistically with other stressors can have a pronounced positive effect on ecosystem health. For antagonistic stressor interactions, by contrast, the management of a single stressor may lead to further ecological degradation (Spears et al., 2021). Despite

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several studies investigating the occurrence of stressor interactions (Jackson et al., 2016; Côté et al., 2016; Kroeker et al., 2017; Birk et al., 2020), they cannot yet be predicted for certain, which makes it difficult to suggest appropriate and effective mitigation measures.

Several factors that influence the effects of multiple stressors on species communities and ecosystems have already been identified. In particular, the specific stressors and the affected organisms determine multiple stressor effects (Ban et al., 2014; Côté et al., 2016). In addition to characteristic stress sensitivity of response organisms, factors such as the level of biological organisation (from the individual over population and community to ecosystem level; Thompson et al., 2018a; Turschwell et al., 2022), biotic interactions (Kroeker et al., 2017; Thompson et al., 2018b) and adaptive evolution of organisms (Cambronero et al., 2018; Zhang et al., 2018; Orr et al., 2021) can play a vital role. Independent of stressor pairs and organism groups, framing conditions such as the timing, sequence and duration of stressors (Debecker et al., 2017; Jackson et al., 2021; Lange et al., 2018, Brooks and Crowe, 2019), ecosystem type and spatial scale (Birk et al., 2020) can also be important.

The dependence of combined effect types on scales suggests that the observed combined effect types are not solely dependent on the environmental setting, but also on the sampling strategy. An increase in scale can be associated with an increase in the size of datasets or the stressor gradient length (e.g. an increase in the temperature gradient length from 15 – 22 °C to 15 – 31 °C). Feld et al. (2016) showed that sample size and stressor gradient in survey-based multiple stressor studies needed to be sufficient to accurately detect the combined stressor effect type (sample size ≥ 150 and gradient length ≥ 75 % of the prevalent gradient). However, systematic analyses of the role of the stressor gradient length on multiple stressor effects are lacking. Such knowledge is needed to support the conceptual and operational understanding of multiple stressor-effect relationships and the design of novel frameworks in multiple stressor research. Ultimately, this knowledge can improve the prediction of stressor

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mitigation effects in environmental monitoring, as stressor mitigation often leads to a shortening of the stressor gradient length.

Our aim was to elucidate how sample size and stressor gradient length influence observed multiple stressor effects, in order to advance multiple stressor understanding and support environmental management. We collected existing datasets representing 158 cases of stressor pairs affecting aquatic phototrophs (hereafter referred to as ‘plants’) or animals from rivers, lakes, transitional and marine ecosystems. For each original case (covering the entire stressor gradient), we divided the gradient of the first stressor (the one with the greater effect) into two equal parts, creating a lower and an upper gradient (representing lower and higher first stressor levels; Figure 1). To identify patterns of whether and how multiple stressor effects change with sample size and along the first stressor gradient, we examined the changes in multiple stressor effects from the full gradient compared to each of the partial gradients. In particular, we investigated changes in combined stressor effect types and changes in the individual stressor effect sizes and directions. Furthermore, we investigated if these changes in stressor effects depended on specific grouping categories, including ecosystem domain, water category, response organism group and kingdom, response category, stressor categories, and effect types. We did not formulate specific hypothesis, as we expected effects but the nature of these effects was obscure prior to our analysis and could not be retrieved from the relevant literature.

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2 Materials and Methods

A graphical summary of the methodological approach of this study is presented in Figure 1.

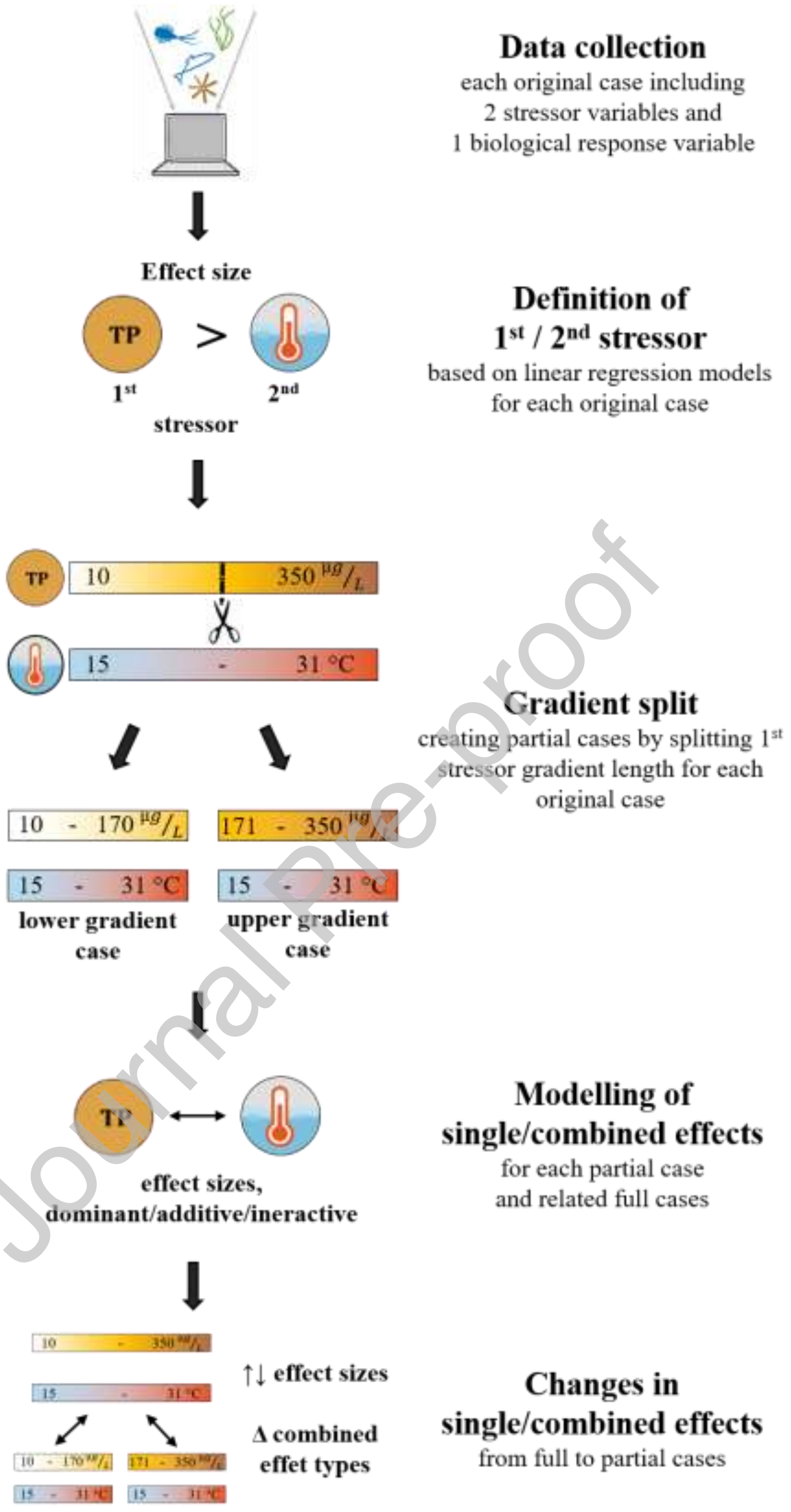

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Figure 1: Summary of the methodological approach of the study. After data collection, linear regression analysis was run on each original case to assign first and second stressor identity (in this example, total phosphorous and temperature) based on their standardised effect sizes. Then, the gradient length of the first stressor was split in half, creating a lower and an upper gradient case (=partial cases). Single and combined stressor effects were modelled for partial and full cases (covering entire stressor gradients but having the same sample size as partial cases). Finally, changes in single and combined stressor effects from full to partial cases were examined.

2.1 Data collection and characterisation

We searched for primary data on multiple stressors and their biological effects in surface waters to collect paired-stressor response combinations (hereafter referred to as 'cases') fulfilling the following criteria: a) data originating from field measurements, b) at least two stressors related to land use and/or climate change, c) more than four stressor levels for each stressor, d) plants or animals as response variables, and e) lakes, rivers, marine waters or transitional waters (surface water bodies at the transition zone from rivers to coastal areas, which are partly saline and substantially influenced by freshwater flows; European Communities, 2000) as water categories.

We define a stressor as an anthropogenic perturbation to a system which is either unfamiliar to that system or natural to that system but applied at levels exceeding the natural variability (Barrett et al., 1976). Stressors included in this study belonged to seven categories (Table 1): i) nutrient stressors, including concentrations of nitrogen and phosphorus components, ii) thermal stressors, including water and air temperatures, iii) morphological stressors, including morphological modifications of water bodies and their surroundings, iv) hydrological stressors, including modifications of the hydrological regime, v) physico-chemical stressors, including dissolved oxygen, pH, salinity and chloride, vi) toxic stressors, including xenobiotic compounds such as heavy metals and pesticides, and vii) light stressors, including alterations in irradiance.

Response organisms included metrics on five organism groups: i) benthic flora (20 cases), ii) phytoplankton (53 cases; including some specimens of the kingdom *Chromista*), iii) zooplankton (5 cases), iv) benthic invertebrates (61 cases), and v) fish (19 cases). The metrics belonged to the categories a) biodiversity metrics, including indices that reflect

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proportions of taxonomic groups in a community, b) biomass/abundance, including biomass or total abundance measures such as counts, concentrations, density or coverage, and c) functional traits, including absolute or relative abundances of functional groups of phytobenthos, benthic invertebrates and fish.

Data of individual cases are openly available in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis. An overview of the analysed cases and their references is given in Supplementary Material 1 (Table S1).

Table 1: Overview of the number of cases with specific stressor combinations. Freshwater cases included lakes and rivers, salt water cases included transitional and marine waters.

	Number of cases	
Paired stressors	**Freshwater**	**Saltwater**
Nutrient | Morphological	41	3
Nutrient | Thermal	23	7
Nutrient | Physico-Chemical	9	20
Nutrient | Hydrological	9	0
Nutrient | Toxic	6	2
Thermal | Physico-Chemical	8	17
Thermal | Hydrological	4	0
Physico-Chemical | Physico-Chemical	1	2
Physico-Chemical | Hydrological	1	0
Physico-Chemical | Light	0	3
Morphological | Hydrological	1	0
Morphological | Toxic	1	0

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2.2 Modelling multiple stressor effects

The single and combined effects of the stressors on biological responses were determined by linear regression modelling, which has been widely used in studies analysing multiple stressor impacts of aquatic biomonitoring data (e.g. Piggott et al., 2015; Jackson et al., 2016; Ellis et al., 2017; Verbeek et al., 2018; Birk et al., 2020; Spears et al. 2021). All analyses were conducted in R (version 4.0.3, R Core Team) based on the approach suggested by Feld et al. (2016) to assess the impacts of multiple stressors and the analytical procedure detailed in Birk et al. (2020). The following provides a short overview of the data processing, modelling, model evaluation and statistical synthesis. The codes to run the linear regression model and the gradient split are openly available in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis.

Data processing included transformation and standardisation of continuous stressor and response variables to a near-normal distribution (centred and scaled to have a mean of zero and variance of one) using Box-Cox transformation (Fox and Weisberg, 2019). We identified the two key stressor variables for each analytical case: in datasets with three to six stressors, we applied the dredge function for automated model selection, identifying the two stressors which provide the best account of the data (Barton, 2020). In datasets with more than six stressors, Random Forest analysis (Liaw and Wiener, 2002) was performed to identify the six most relevant stressors, followed by application of the dredge function. Further, stressor correlation was investigated using a correlation matrix chart (Peterson and Carl, 2020). Cases with a Spearman correlation of ≥ 0.7 were excluded to avoid collinearity problems (Feld et al., 2016).

Linear regression modelling was conducted to identify the effect of each stressor and the potential stressor interaction on the biological response. Following the criteria and statistical procedure in Birk et al. (2020), we used generalised linear models (GLM) or generalised linear mixed models (GLMM) for regression modelling. Model evaluation was conducted using the coefficient of determination explained by the stressor effects (marginal R^2). Models with an $R^2 < 0.2$ (weak relationships) were excluded from the analysis.

2.3 Identification of first stressor and classification of stressor effects

All single and combined stressor effects were modelled within this study to ensure that stressor effects for analysis were all based on the same defined approach. Multiple stressor effects were evaluated using standardised effect sizes (= regression coefficients) and their significance (t-test, $p < .05$; Table 2). The stressor with the greater standardised effect size

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was identified as the ‘first stressor’. Dominance was assigned to cases with only the first stressor showing a significant effect. An additive effect was assigned to cases with both stressors showing significant effects but a non-significant interaction. Interaction was assigned to cases with the stressor interaction showing significant effects, regardless of whether the first and second stressor main effects were significant or not.

The type of interaction for interactive cases was classified based on whether combined stressor effects (sum of effect sizes of both stressors and their interaction) was greater or smaller than the additive stressor effect (sum of first and second stressor effect sizes). Synergistic effects were assigned to cases where the combined effect was greater than the additive effect, and antagonistic effects were assigned to cases where the combined effect was smaller than the additive effect (Table 2).

Table 2: Classification of combined stressor effect types and interaction types. Classification depends on the standardised effect sizes of the first stressor (b_1), the second stressor (b_2) and the stressor interaction (b_3). For combined effect types, ‘**y**’ denotes a significant effect (t-test, $p < .05$), whereas ‘**-**‘ denotes a non-significant effect.

	b_1	b_2	b_3	**Classification of multiple stressor effect type**
Combined stressor effect type	y	-	-	stressor dominance
	y	y	-	additive stressor effects
			y	interaction between stressors
Type of interaction	$\|b_1 + b_2\| < \|b_1 + b_2 + b_3\|$			synergistic interaction
	$\|b_1 + b_2\| > \|b_1 + b_2 + b_3\|$			antagonistic interaction

2.4 Gradient split

The original gradient (including all samples from the primary data) of each case was split into two ‘partial gradients’ (Figure 1). We conducted the gradient split by cutting the transformed data set of the original gradient at the median of the first stressor levels. Thereby, we created a lower and an upper gradient case with similar sample sizes, with the median values included in the lower gradient case. To ensure that the split primarily affected the first stressor gradient, we excluded 36 partial cases where the length of the second stressor gradient was reduced by

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more than one third, owing to a correlation between the two stressors. For the remaining cases, the median gradient length of the second stressor was reduced by only 6 %. Therefore, we can expect the changes in multiple stressor effects to be primarily related to the splitting of the first stressor gradient.

Initial analyses indicated that effect types were related to sample size. To rule out the possibility that observed changes in multiple stressor effects were due to the reduced sample size of the partial gradients compared to the original gradient, we created full gradients with halved sample sizes (referred to as the 'full gradient' henceforth). This was done by deleting every second measurement along the first stressor gradient of the original cases, resulting in a similar sample size of full and partial gradients (Table 3).

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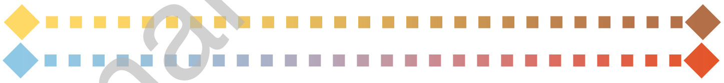
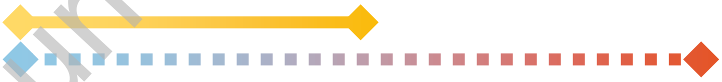
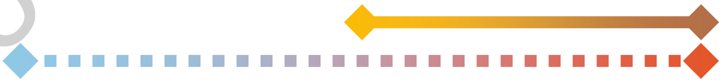
Table 3: Overview on the differences between original, full and partial cases, i.e. the stressor gradients covered and the sample size (N). The upper line (yellow/brown) depicts the first stressor and the lower line (blue/orange) the second stressor gradient length covered.

Case	Stressor gradient covered	Sample size
Original		N
Full		N/2
Lower		N/2
Upper		N/2

All partial gradients were analysed with the same modelling approach (GLM/GLMM) as for the respective full gradient, to estimate the changes in combined effect types as well as single effect sizes and directions (see sections 2.2 and 2.3 above). After the gradient split and regression analysis, 158 full cases and 275 partial (137 lower and 138 upper) cases remained for synthesis analysis.

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The purpose of the gradient split was to examine if observed stressor effects change with changing stressor gradient length. One split of the first stressor gradient was sufficient to investigate such a dependence and the splitting was not repeated to create further partial gradients of the first or second stressor. Such further splits might be used in future studies to examine how stressor effects change with changing gradient length.

2.5 Analysis of the impact of the gradient split on stressor effects

To study the dependence on sample size and stressor gradient length, we determined the changes in multiple stressor effects from full to partial gradients with similar sample sizes. The following analyses were performed:

1. Correlation between the sample size and combined effect type, by plotting a correlation chart (Peterson and Carl, 2020) and conducting pairwise Mann-Whitney U-tests.

2. Changes (e.g. from dominant to additive) in combined effect types or a loss of stressor effects after gradient splitting. A loss of stressor effects was defined as models with an explanatory power below 5 % or without any significant effects after splitting.

3. Switches in stressor effect directions, from stimulation to inhibition of the response organism or vice versa.

4. Changes in single stressor effect sizes. We conducted a meta-analysis on the changes in the standardised effect sizes of both stressors and their interaction upon gradient splitting using OpenMEE software (Wallace et al., 2017). Variance of each standardised effect size was calculated as the product of associated standard errors from GLM(M) and the square root of the sample size, raised to the power of two. Effect sizes of these comparisons and their variances were then computed for each of the stressor/interaction variables from each individual study as the differences between the full and the lower, as well as between the full and the upper gradient. The significance of these comparisons (*Z*-test, $p < .05$) was then tested across all studies and for different grouping categories (see below). Using the same approach,

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we also compared full gradients to the original gradients (with twice the number of measurements) to investigate if sample size alone affected the effect size. For the meta-analysis on effect size changes, we excluded cases with an explanatory power below 5 %.

5. To support the above analyses with information on increases or decreases in model performance, median changes in the explanatory power (marginal R^2) of models were compared using pairwise Mann-Whitney U-tests.

Finally, we investigated if the above changes in the single and combined stressor effects depended on the following grouping categories: a) the first stressor gradient part (lower versus upper partial gradient), b) ecosystem domain (fresh- or saltwater), c) water category (river, lake, transitional, marine), d) response organism kingdom (plants, including benthic flora and phytoplankton, or animals, including benthic invertebrates, zooplankton and fish), e) response organism group (benthic flora, phytoplankton, benthic invertebrates and fish; excluding zooplankton cases due to their low number), f) response category (biodiversity, biomass/abundance or functional traits), g) first stressor categories (nutrient stressors, thermal stressors, morphological stressors, hydrological stressors, physico-chemical stressors, toxic stressors; excluding light stressors due to their low number), and h) combined effect types (dominant, additive, synergistic, antagonistic) of the full cases. We tested for significant differences between the grouping categories using chi^2-tests.

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3 Results

After gradient splitting, we found pronounced changes in combined effect types and effect sizes. In a consistent pattern throughout all analyses, changes were significantly weaker for plants compared to animals, following the pattern phytoplankton/benthic flora < benthic invertebrates < fish. We therefore focused on differences between these response organism categories. Results regarding other grouping categories (i.e. first stressor gradient part, ecosystem domain, water category, response category, and first stressor category) are only reported in the following if considered noteworthy.

The data presented in the results section can be found in Supplementary Material 2.

3.1 Influence of sample size on combined effect types

We found a significant influence of the sample size of the original and the partial gradients on the combined effect type (Figure 2). Cases with smaller sample sizes generally resulted in stressor dominance, while cases with larger sample sizes resulted more frequently in additive and interactive combined effect types (Kruskal-Wallis test, $p < .05$).

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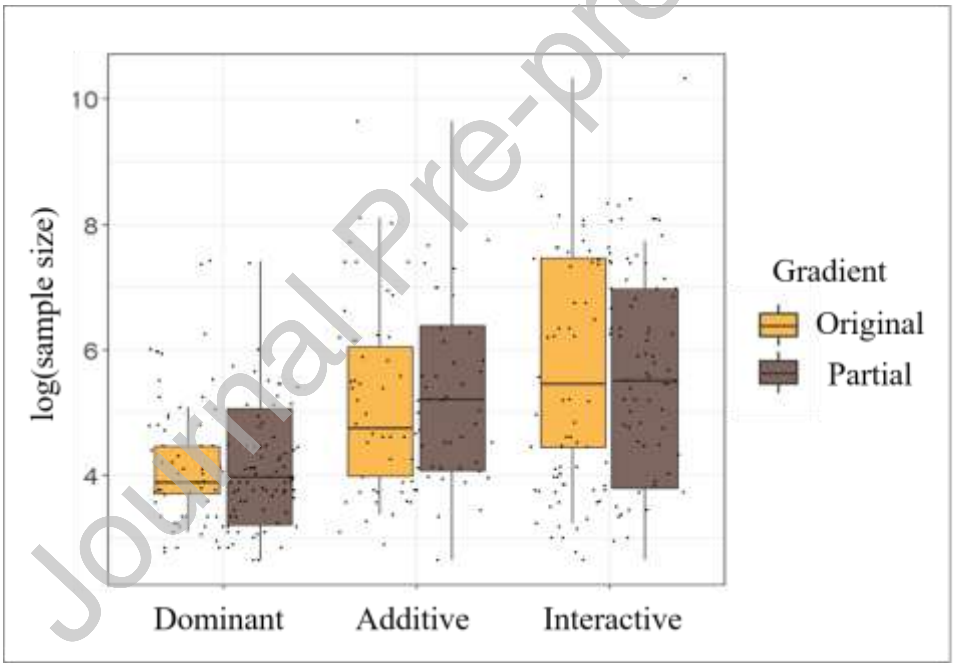

Figure 2: Sample sizes of cases with dominant, additive and interactive combined effect types, for the original cases before the gradient split and partial gradients (lower and upper partial gradients combined). The sample size significantly influenced the identified combined effect types (Kruskal-Wallis test, $p < .05$).

3.2 Gradient-dependent changes in combined effect types

From full compared to partial gradients, 40 % of cases showed a change in combined effect type, 30 % no change and 31 % showed a loss in stressor effects. We did not observe different patterns in combined effect type changes for the lower versus upper partial gradients

(Supplementary Material 1, Table S2). The frequency of changes depended on the combined effect type before the split (chi^2-test, $p < .05$, Figure 3): dominant effects mainly remained dominant or lost the stressor effect, with 38 % of cases still being dominant after the split, 23 % changing in combined effect type and 38 % showing a loss in effect. Additive cases changed in combined effect type most frequently and lost the stressor effects least frequently, with 31 % of cases not changing, 53 % changing and 16 % showing a loss in stressor effects. Synergistic and antagonistic effects mostly changed in combined effect type: 24 % and 19 % remained the same, 46 % and 43 % showed a change, and 30 % and 38 % lost the stressor effects, respectively.

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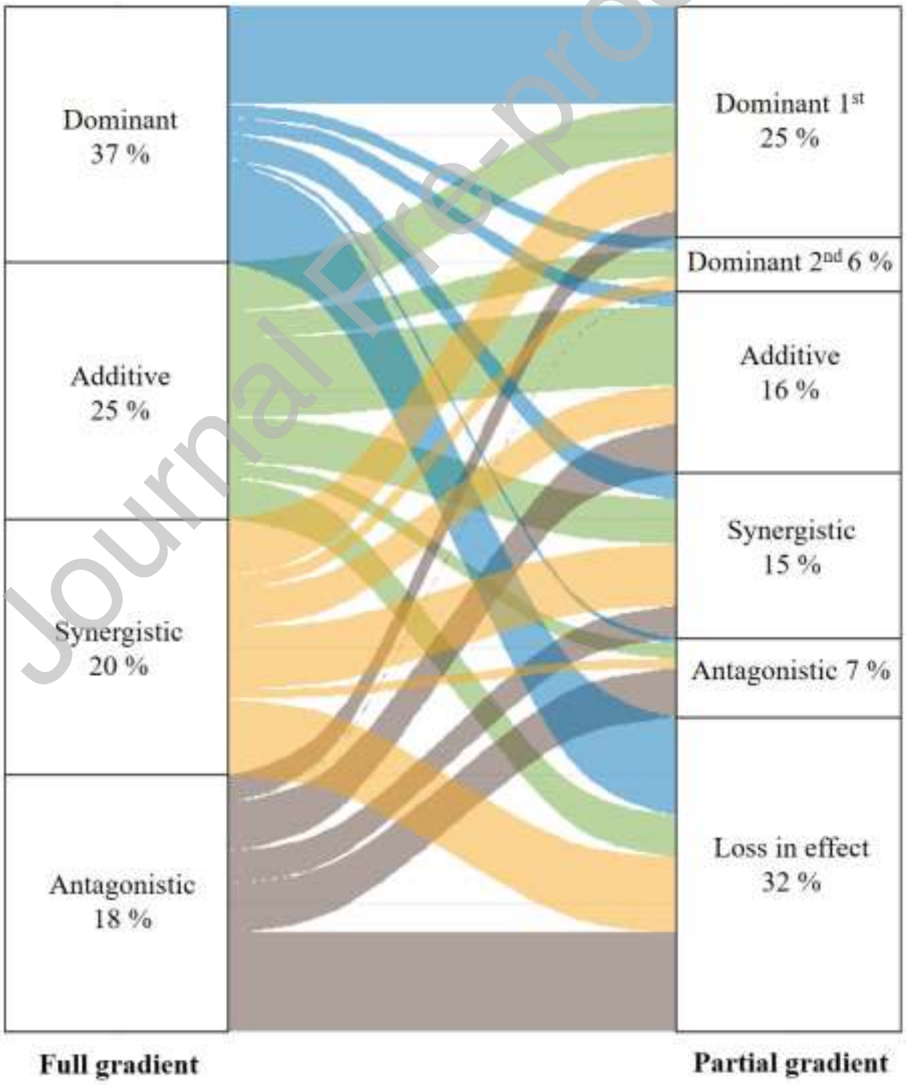

Figure 3: Changes in combined effect types from full to partial gradients (with both gradients having similar sample sizes). Dominant cases mainly remained dominant or lost the stressor effect in partial gradients. Additive cases mainly changed in combined effect type and lost stressor effects with the lowest frequency. Synergistic and antagonistic cases changed in combined effect type most often, followed by a loss in effect and non-changing cases.

3.3 Gradient-dependent switches in effect directions

After gradient splitting, 58 % of cases showed a switch in the direction of at least one stressor/interaction effect from the full compared to the partial gradient. There were significant differences between organism kingdoms, with a switch in stressor direction in 73 % of animal cases and in 41 % of plant cases (Figure 4; chi^2-test, $p < .05$).

The first stressor effects only switched direction when reflecting nutrient or thermal stressors. Cases with physico-chemical, morphological, hydrological and toxic first stressors showed no switches. Moreover, the frequency of switches increased with phytoplankton/benthic flora < benthic invertebrates < fish (chi^2-test, $p < .05$).

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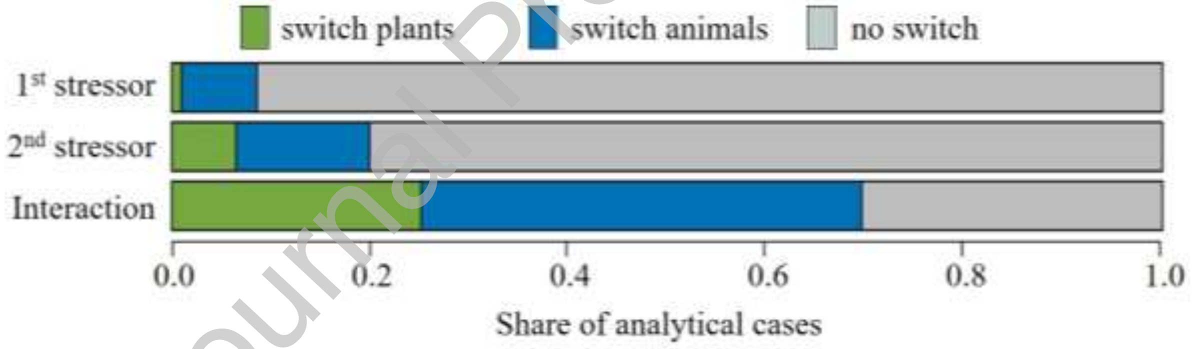

Figure 4: Switches in stressor effect directions upon gradient split. The bars show the proportion of cases with a switch/no switch in the effect direction of the stressors/interaction from full compared to partial gradients. Cases affecting animals account for a higher proportion of switches than those on plants (chi^2-test, $p < .05$).

3.4 Quantitative changes in effect sizes

When comparing the effect sizes of the original gradients (all samples included) to those of the full gradients (halved sample size), we found no significant differences. Thus, sample size alone did not influence the effect sizes of the individual studies.

Across all cases combined, the effect size of the first stressor did not significantly change with reduced gradient lengths, though there was a tendency of an increase in effect sizes from the full towards the upper gradient (Figure 5). Effect sizes of the second stressor significantly increased with reduced first stressor gradient, whereas the effect size of the stressor interaction only increased from full to the upper gradients (*Z*-test, $p < .05$).

In general, the changes in all stressor/interaction effect sizes (except for second stressor changes towards the lower gradient) showed the pattern phytoplankton/benthic flora < benthic invertebrates < fish cases for both partial gradients. From full to lower gradients, the first stressor effect size did not change for cases including phytoplankton or benthic flora, while it showed a pronounced increase in fish cases. From full to upper gradients, benthic flora cases showed a pronounced decrease, while benthic invertebrate and fish cases showed a pronounced increase.

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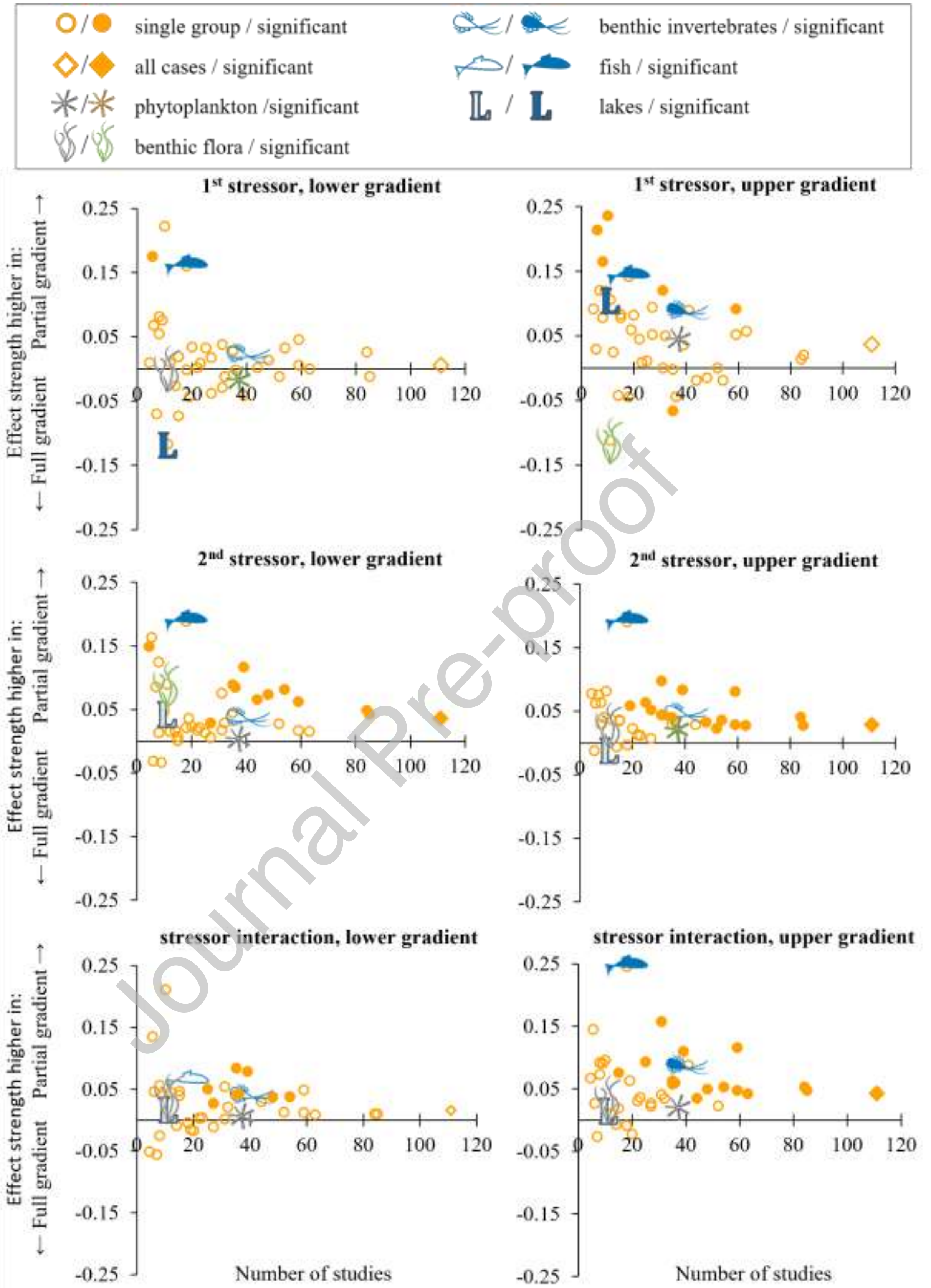

Figure 5: Changes in effect sizes for the first stressor (top), second stressor (middle) and stressor interaction (bottom) upon gradient split. Symbols show the effect sizes of partial gradients minus the full gradients of specific groups (as in the different grouping categories), and thereby indicate if (and by how much) the effect size was stronger in the full (negative values) or the partial gradient (positive values). For example, for the 11 lake cases, the first stressor effect size decreased in lower gradients and increased in upper gradients upon gradient split.

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3.5 Changes in the explanatory power of models

After the gradient split, the median explanatory power of models decreased from 0.35 to 0.23 of explained variance. The magnitude of this decrease in explanatory power showed no significant differences between any grouping categories.

There were some cases (17 %) where explanatory power increased, but decreases (83 %) were much more frequent (Table 4). Organism groups and kingdoms revealed different patterns: in the lower gradients, the frequency of cases with increasing explanatory power was significantly higher for animal than for plant groups (chi^2-test, $p < .05$).

Table 4. Shares of cases with an increase or decrease in the explanatory power of models (R^2) for all cases as well as organism kingdoms and organism groups separately. Significant differences between kingdoms and organism groups are highlighted in **bold** (chi^2-test, $p < .05$).

Cases	Lower gradient		Upper gradient	
	increase	decrease	increase	decrease
All	0.19	0.81	0.15	0.85
Plants	**0.08**	**0.92**	0.14	0.86
Animals	**0.28**	**0.72**	0.17	0.83

4 Discussion

In general, our findings demonstrate that observed multiple stressor effects in survey-based studies are not only determined by ecological processes but also by sample size and stressor gradient length. The results of this study and implications for research are discussed in the following paragraphs, while important implications for resource managers are addressed in the concluding section of our paper.

4.1 Combined effect types often result from insufficient data or the statistical approach

The obvious relationship between sample size and combined effect type detected in our study highlights the need for careful interpretation of modelled combined effect types. Definition of combined effect types based on thresholds of *p*-values can be misleading because *p*-values are

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correlated with sample size (Greenland et al., 2016; Wasserstein and Lazar, 2016). This relationship was clearly observed in the cases included in our study; consequently, combined stressor effect types detected in many survey-based multiple stressor studies could be a result of the size of datasets rather than of ecological processes. We therefore agree with the widely cited recommendation that scientists should not rely solely on significance levels and categorical interpretations of combined effect types, but should put more emphasis on stressor effect sizes (Nakagawa and Cuthill, 2007; Spears et al., 2021).

We controlled for the influence of sample size in all gradient split comparisons by adjusting sample sizes of full and partial gradients. Examining the changes in combined effect types after gradient split indicated that, for dominant cases, the second stressor did not affect the response variable at all. The majority of dominant cases remained dominant or lost stressor effects when the stressor gradient was split, indicating that strong second stressor gradients were underrepresented in the data. Additive cases showed a large contrast to the dominant ones: a high share of cases changed in combined effect type, which can be explained by the small difference between first and second stressor effect sizes (Supplementary Material 1, Figure S1), as even small changes in effect sizes are likely to lead to switches in the stressor importance, potentially resulting in a changed combined effect type. Furthermore, there was only a low share of cases with a loss in stressor effects, which can be explained by the definition of additive cases (both stressors have to show a significant effect on the response for the individual stressors but lack one for their interactions): in case the effect of one stressor is lost, the other stressor still shows a significant effect. Interactive cases mainly changed towards dominant or additive combined effect types, likely due to the loss of the more extreme values at one end of the stressor gradient that results in less pronounced, non-interactive stressor effects (as assumed by Birk et al., 2020 in the case of stressor data spanning smaller spatial gradients). We conclude that, in addition to any ecological processes,

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perceived combined stressor effect types can be influenced by insufficient data or the statistics underlying the analysis.

This finding is consistent with a recent study by Segurado et al. (2022), where the effect of different sampling constraints on the identification of single and combined stressor effects was tested using simulated datasets. In general, the authors found a strong influence of the stressor gradient length on single and combined stressor effects, which is in line with our findings. One of the simulated scenarios was equal to our approach of halving the stressor gradient length of the first stressor. Specific changes in combined effect types, however, are hardly comparable due to differences in the methodological approach of Segurado et al. (2020) to our work.

4.2 Switching effect directions point to unimodal stressor effects

More than half the cases showed switching stressor effect directions, indicating non-linear multiple stressor-effect relationships. All these cases concerned either nutrients or temperature as the first stressor. This observation indicates that stressor impact is not always monotonously increasing with stressor intensity, as organisms show bell-shaped tolerance curves for certain environmental variables (e.g. Erofeeva, 2021; Harley et al., 2017). Favourable nutrient concentrations or temperatures stimulate productivity of animals and plants. However, excess nutrients or extreme temperatures can have inhibiting effects, which might result in adverse alterations in food web dynamics and structure due to the loss of sensitive animal and plant species (Odum et al., 1979). In line with Ellis et al. (2017), the empirical data presented in our study demonstrate the subsidy-stress effect of variables such as nutrients and temperature along their gradients. However, not all the switching cases showed the expected switch in direction for subsidy-stress responses, as the lower and upper gradient cases sometimes showed the same effect direction after the split (e.g. stimulating effect in the full case and inhibiting effect in lower and upper gradients, respectively). This

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might result from non-linear stressor effects, where more than one switch in stressor direction is present in the full gradient.

4.3 Changes in effect sizes depend on response organism groups

Since we did not find any influence of the sample size on the effect sizes of the individual studies, we can attribute the observed changes in effect sizes to the reduced gradient length. Changes in effect sizes became especially interesting when investigating patterns of single grouping categories. Our findings indicate that along the first stressor gradient (i.e. with increasing first stressor levels), stressor effects on plants decrease, while they increase on benthic invertebrates and even more so on fish. Stressors can disrupt ecological processes governing dynamics of communities (Galic et al., 2018) and following this premise, we interpret the changes in effect sizes to be related to stressor effects cascading between different trophic levels in a community (Kagata and Ohgushi, 2005; Bruder et al., 2019; Beauchesne et al., 2021).

In our analysed cases, the decrease in stressor effects on plants can be an effect of switching stressor importance. For many plant-based metrics, already small changes in nutrient levels can cause a shift to a new state (Schernewski et al., 2008). With further increasing stress intensity, productivity might still be enhanced, whereas many metrics (e.g. species number, plankton over macrophyte dominance, share of cyanobacteria and chlorophytes in biomass structure) will only change to a minor degree (Scheffer et al., 1993). Animals, in contrast, respond to nutrient enrichment indirectly, e.g. through decreased oxygen concentration at night times or through enhanced food availability that favours few competitive animal species (Diaz and Rosenberg, 2008; Burkholder et al., 2013). Therefore, responses will only be manifested at higher stressor levels, once the plant assemblage has changed to a new state, and will continue with increasing stress levels.

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4.4 Higher non-linearity in multiple stressor effects for higher trophic levels

The changes in multiple stressor effects indicate that with increasing trophic level, organisms responded to stressors with increasing non-linearity. The changes in combined effect types, switches in the direction of single stressor effects, as well as changes in single stressor effect sizes showed the pattern of phytoplankton/benthic flora < benthic invertebrates < fish. Borja et al. (2016) observed a similar pattern when studying the responses of different organism groups to human stressors and management actions: the response of phytoplankton to the changing stressor levels was weak, while benthic invertebrates showed moderate to strong and fish showed strong responses.

Our interpretation is supported by the changes in the explanatory power of models: an increase in the explanatory power can indicate non-linear stressor effects, as the partial stressor gradients better reflect the stressor-effect relationships than the full gradients. Animals, which showed stronger changes in multiple stressor effects, also showed a significantly higher frequency of cases with increasing explanatory power compared to plants. Further, all cases with an increase in explanatory power also showed a change in combined effect type and/or a switch in stressor direction. The high share of non-linear responses of animal species is in line with observations of Hewitt et al. (2016) and Clark et al. (2021), who also found non-linear responses when analysing land use and climate change impacts on benthic invertebrates.

Non-linear stressor effects can also explain the simultaneous increase in a stressor effect from full to lower and upper partial gradients. In general, we expected the effect size to increase in one gradient part and to decrease in the other, when the stressor effect intensifies or weakens along the first stressor gradient. Increases in both partial gradients might result from non-linear stressor effects, where both partial gradients better reflect the stressor-effect relationships than the full gradient.

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4.5 The use of linear models

The use of linear regression models to study the effects of multiple stressors is common (e.g. Piggott et al., 2015b; Jackson et al., 2016; Ellis et al., 2017; Verbeek et al., 2018; Birk et al., 2020, Segurado et al., 2022), although non-linear effects are well known. Linear regression is based on the assumption that multiple stressor effects are persistent along the stressors' gradients. But it has been known for decades that single stressors can have non-linear effects, such as the unimodal effects of nutrients and temperature, which stimulate plant growth at low levels and inhibit it at high levels (Odum et al., 1979). Approaches capturing such non-linear effects of multiple stressors, such as Polynomial Regression (Ellis et al., 2017; Thrush et al., 2008), Boosted Regression Trees (Lemm et al., 2021) or Generalized Additive Models (Pedersen et al., 2019), are essential to provide more detailed information about the direction and strength of stressor effects along gradients. However, the interpretation of multiple stressor interactions is difficult when using non-linear approaches, as general frameworks are still lacking. The purpose of this study was to examine *if*, and not *how*, single and combined stressor effects depend on sample size and the stressor gradient length, and thereby, the use of linear regression represents a valid and sound approach.

5 Conclusions and Outlook

Having shown that identified multiple stressor effects are not exclusively inherent to any ecological processes but also depend on how we observe, our study highlights the importance of comprehensive monitoring programmes and adaptive management. Identifying the most prevalent multiple stressor effects is essential for the design of effective mitigation measures, as misguided stressor management can lead to unexpected outcomes and even a worsening of the water bodies' condition (Spears et al., 2021). We have shown that the identified multiple stressor effects can change due to shifts of stressor levels towards the lower or upper stress gradient. As these shifts can be based on the environmental setting and the sampling design,

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we can draw two important conclusions for management:

i) When based on insufficient data, identified multiple stressor effects in survey-based studies may be incorrect; therefore, monitoring programmes need to be designed to capture the full stressor gradients prevalent in the managed water body. A study conducted by Kreyling et al. (2018) indicated that monitoring programmes that include sampling a maximal number of locations without replication are better in capturing full stressor gradients and identifying non-linear multiple stressor effects than classical designs with replicated sampling at few locations.

ii) Changed environmental settings (actual shifts in stressor levels due to stressor mitigation or ecosystem degradation) can affect a change in multiple stressor-effect relationships, thus management actions need to be flexible enough to adapt to them by revising management approaches and measures. This especially holds true when management actions address organisms of higher trophic levels, as their responses to changed stressor gradients are more non-linear compared to lower trophic levels.

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Author contributions

LM, SB and DH developed the underlying idea. CFH, MP, JP, TC and DH contributed to the conceptualisation of the study and the search for data providers. LM coordinated the data acquisition. LM, CFH and MP contributed to the data acquisition and preparation. JA, JB, AB, DC, YFT, JK, CM, HP, AS, FOB, and AV conducted experimental investigations and/or provided data. LM conducted the formal analysis with the help of WK and MP. LM and SB conducted the synthesis of results and wrote the first manuscript draft. CFH, MP, JP, TC, WK and DH revised the first manuscript draft. All authors contributed to additional drafts of the article and approved the submitted version.

Data availability statement

The data on the individual cases (paired-stressor response combinations) and regression model outputs, as well as the codes to run the linear regression model and the gradient split are openly available in GitHub at https://github.com/leonimack/Multiple_stressor_gradient_analysis.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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