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Multiple-stressor effects on freshwater fish: Importance of taxonomy and life stage

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Abstract

Interactions among multiple anthropogenic stressors threaten freshwater fish and pose challenges for fisheries management and conservation. Previous studies of multiple-stressor effects on freshwater fish suggest a prevalence of antagonistic interactions. However, taxonomy, life stage and/or environmental context likely modify the magnitude and direction of fish responses to multiple stressors. Stressor intensity, impact mechanism, exposure time and ecosystem size may further affect interaction outcomes. Large-scale studies quantifying how these variables moderate stressor interactions are lacking. To address this knowledge gap, we performed a meta-analysis of 29 factorial multiple-stressor experiments to examine the influence of seven potential moderator variables on the magnitude and direction of stressor interactions. Using weighted random-effects meta-analytic models, we demonstrate the importance of taxonomic identity and life stage for interaction outcomes. In particular, Cypriniformes showed stronger antagonisms than Salmoniformes, as did larval fish compared to juveniles. Interaction outcomes also varied among the measured fish responses with survival yielding stronger antagonisms than biomass. Increasing experimental duration and volume of the experimental units both drove interactions towards synergisms, supporting findings from previous studies that synergisms take time and space to develop. In an era when the number of stressors affecting freshwater systems is increasing rapidly, our study provides a vital step towards identifying generalities in multiple-stressor outcomes and thus improved predictions of multiple-stressor impacts. Furthermore, our meta-analysis complements studies in real streams, rivers and lakes by providing an experimentally derived context for the growing number of multiple-stressor assessments in research, management and conservation of freshwater fish.

KEYWORDS
antagonism, moderator variables, synergism, weighted random-effects meta-analysis

1 | INTRODUCTION

Freshwater fish populations are declining at unprecedented rates across the globe (Closs, 2016; Gordon et al., 2018). While single anthropogenic actions may have predictable impacts on freshwater fish, we now know that most freshwater ecosystems are subject to multiple stressors, which poses enormous challenges for conservation and freshwater management (Closs, 2016; Côté, Darling, & Brown, 2016). Currently, there is much uncertainty whether stressors act in an additive, antagonistic or synergistic manner because

interactions may depend on the biological or environmental context. Predicting multiple-stressor outcomes and singling out the factors driving their interaction is therefore crucial for biodiversity conservation.

Multiple stressors may interact simultaneously or sequentially and thus result in non-additive responses of organisms or ecological processes that cannot be predicted from the sum of individual stressor effects (Christensen et al., 2006; Crain, Kroeker, & Halpern, 2008; Folt, Chen, Moore, & Burnaford, 1999). If stressors interact synergistically, the response is greater than predicted, whereas antagonistic interactions result in smaller than predicted responses (Figure 1; Folt et al., 1999). The development of synergisms and antagonisms depends on the mechanisms of stressor effects (Segner, Schmitt-Jansen, & Sabater, 2014). Synergisms might ensue in situations where stressed individuals have a lower resistance to additional stressors acting through different mechanisms (Bruder, Salis, Jones, & Matthaei, 2017; Christensen et al., 2006; Darling & Côté, 2008). Conversely, if stressors act through similar mechanisms, certain physiological adaptations or behavioural responses could be beneficial against exposure to additional stressors and thus lead to co-tolerance, resulting in antagonistic responses (Vinebrooke et al., 2004).

Generalizations of multiple-stressor effects are difficult due to various factors defining the biological or environmental context of stressor interactions. In particular, effects of stressors and their interactions may depend on intrinsic and extrinsic moderator variables across temporal and spatial gradients (Segner et al., 2014). Fish taxa (i.e. an intrinsic moderator variable) vary in their susceptibility and response to multiple stressors due to species-specific adaptations to environmental conditions and flexibility therein (reviewed in Segner et al., 2014). For instance, salmonids appear to be more sensitive to habitat degradation than cyprinids (Alabaster & Lloyd, 2013; Pont et al., 2006), or are particularly sensitive to current trajectories of environmental change, such as loss of habitat connectivity and warming of river systems (Ruhí, Olden, & Sabo, 2016). Susceptibility may also depend on life-history characteristics (another intrinsic moderator variable), with embryo-larval stages usually being more sensitive than juveniles or adults (McKim, 1977; Power, 1997).

The prevalence of stressor interaction types may also depend on the fish responses assessed (i.e. fish survival, growth, reproduction, or physiology, Segner et al., 2014). For instance, physiological responses (e.g. cortisol levels, oxygen consumption, nitrogenous waste excretion) may respond rapidly to acute stress (Barton, 2002), whereas growth integrates the effects of chronic stress, and changes in survival may reflect the consequence of both acute and chronic stress (Bruder et al., 2017; Salazar-Lugo et al., 2009). The occurrence of stressor interactions may also depend on variation in intensity and temporal and/or spatial extent of stressors. For instance, the duration of exposure to stress can affect multiple-stressor outcomes if the energetic costs for organisms to tolerate stressors increase with time, thus intensifying negative

physiological responses and producing synergisms (Darling & Côté, 2008; Segner et al., 2014).

Extrinsic moderator variables can also modify multiple-stressor interactions. For instance, larger habitats may allow for more complexity and a greater number of behavioural responses to stressors (Stewart et al., 2013; Woodward, Perkins, & Brown, 2010), thus increasing the occurrence of antagonisms. Brown trout (*Salmo trutta*, Salmonidae), for instance, may select deeper—and cooler—water layers in pools during summer, despite oxygen concentrations being lower than in surface waters, in an attempt to reduce overall stressor effects (Elliott, 2000). However, increasing ecological complexity coupled with larger spatial scales might also increase the number of indirect stressors and in turn increase the occurrence of synergisms under the assumption that diverse stressors act through different mechanisms (Bruder et al., 2017; Segner et al., 2014).

Knowledge of moderator variables affecting the prevalence of interaction types is required to predict multiple-stressor interactions and to efficiently inform management and conservation (Côté et al., 2016; Segner et al., 2014). The only previous meta-analysis of multiple-stressor effects on freshwater fish (Matthaei & Lange,

2016) determined directions of stressor interactions but included no moderator variables. By testing moderator variables (based on fish characteristics and experimental design), our meta-analysis of published multiple-stressor experiments with full-factorial designs aims at reducing this crucial knowledge gap. With the available studies, we were able to test seven moderator variables on the direction and magnitude of stressor interactions: fish taxonomy, life stage, stressor intensity, the measured biological endpoint (response type), ecosystem type (lentic vs. lotic), and experimental conditions (volume of experimental units, ranging from aquaria to ponds and duration of the experiment). By doing so, we were also able to tentatively extrapolate our findings to real freshwater ecosystems, for which factorial multiple-stressor experiments on fish are lacking. Our approach thus has three strengths: it makes use of data available from a range of highly controlled factorial experiments, allows extrapolation of our findings to real ecosystems and permits identifying research needs.

2 | METHODS

2.1 | Data collection

We restricted our analysis to manipulative experiments testing at least two stressors and their interactions; manipulative experiments (rather than surveys or modelling studies) are arguably the most powerful tool for studying interactions between multiple stressors (see Crain et al., 2008; Townsend, Uhlmann, & Matthaei, 2008). Data were obtained by searching the *ISI Web of Knowledge* (from 1960 to December 2014) using the following search terms: (fish*) AND (experiment*) AND (factorial) AND stressors (nutrient*, fine sediment*, water temperature, pH, flow reduction, radiation, density, toxi*, parasit*, hypoxia OR invasive). The initial search yielded 7,825 articles, of which 28 studies fulfilled the following selection criteria: (a) published in English, (b) freshwater or pre-smolting juvenile anadromous fish as focal species, (c) quantified effects on survival, biomass and/or physiology, (d) treatments with true replicates (sensu Hurlbert, 1984), (e) sample size (*n*), mean and variance (standard error or standard deviation) for each treatment level (e.g. control, stressor 1, stressor 2 and combined stressors) obtainable from text, tables or figures (using WebPlotDigitalizer; Rohatgi, 2014).

From each study, we extracted data for all reported biotic response types (e.g. survival, biomass—specific growth rate; physiology—plasma cortisol, blood pH, oxygen consumption; Supporting Information Table S1). If studies manipulated stressors at more than two levels, we extracted responses for the lowest and highest stressor intensity only. For each response, stressor intensities were then characterized as *low* (stressor 1 low × stressor 2 low), *medium* (stressor 1 low × stressor 2 high, or stressor 1 high × stressor 2 low) or *high* (stressor 1 high × stressor 2 high). We also obtained data for the seven moderator variables (see Supporting Information Table S1) from the original studies related to biological characteristics (taxon, life stage) and experimental design (response type, experimental duration and volume of experimental unit, simulated habitat type [i.e. lentic vs. lotic habitat] and stressor intensity).

## 2.2 | Calculation and classification of interactive effect sizes

Interaction strength between two stressors was computed according to the methods for factorial meta-analysis (Crain et al., 2008; Gurevitch, Morrison, & Hedges, 2000, for detailed methods see Appendix S1 in Supporting Information). We used the additive model for testing interactions because it is best suited for interpreting data derived from manipulative experiments (Côté et al., 2016; Folt et al., 1999). Stressor interactions were classified as *additive* if the 95% confidence interval of the interactive effect size included zero (Figure 1), i.e. was not significantly different from the sum of the individual stressor effects (Crain et al., 2008; Nakagawa & Cuthill, 2007). A *synergism* was defined as the interactive effect exceeding the sum of individual effects (i.e. the additive outcome). Here, exceedance refers to the direction of the deviation of the additive outcome from the control. An *antagonism* was defined as the interactive effect being smaller than the sum of the individual effects (Folt et al., 1999), i.e. a smaller deviation from the control compared to the additive outcome. To complement our meta-analysis, we also conducted a simple vote-counting procedure to assess the frequency of interaction types (see Jackson, Loewen, Vinebrooke, & Chimimba, 2016). Moreover, we identified all reversals among the antagonisms. A *reversal* was defined as the interactive effect being opposite to the sum of the individual effects (Jackson et al., 2016).

If individual effect sizes were opposing (Figure 1), the direction of the interactive effect was compared to that of the individual effect with the higher absolute value. Interactive effect sizes were inverted when both individual effects were negative or in cases with opposing effects where the negative effect had the higher absolute value. This approach allowed direct comparisons with stressor pairs where both stressors had positive or opposing individual effects and the positive effect had the higher absolute value (in contrast to Crain et al., 2008; where interactive effect sizes for cases with opposing individual effects were never inverted). Consequently, in cases where both individual effect sizes were positive, a positive interaction effect size indicated a synergism whereas a negative interaction effect size indicated an antagonism, and vice versa when both individual effects were negative (see Crain et al., 2008).

2.3 | Statistical analysis

All analyses were computed in R (version 3.0.2, R Development Core Team, 2013). Prior to analysis, funnel plots were used to detect outliers in the response variables. First, we calculated the overall effect size direction of our entire data set using a weighted linear mixed-effects model (intercept only, nlme:lme, version 3.1-118, Pinheiro, Bates, DebRoy, & Sarkar, 2014), fitted using restricted likelihood estimation. Study ID (i.e. source article) was included as a random factor to account for multiple effect sizes from the same article.

Second, to determine the effect of each moderator variable on stressor interactions, we incorporated each moderator variable into a separate weighted mixed-effects model. Continuous variables
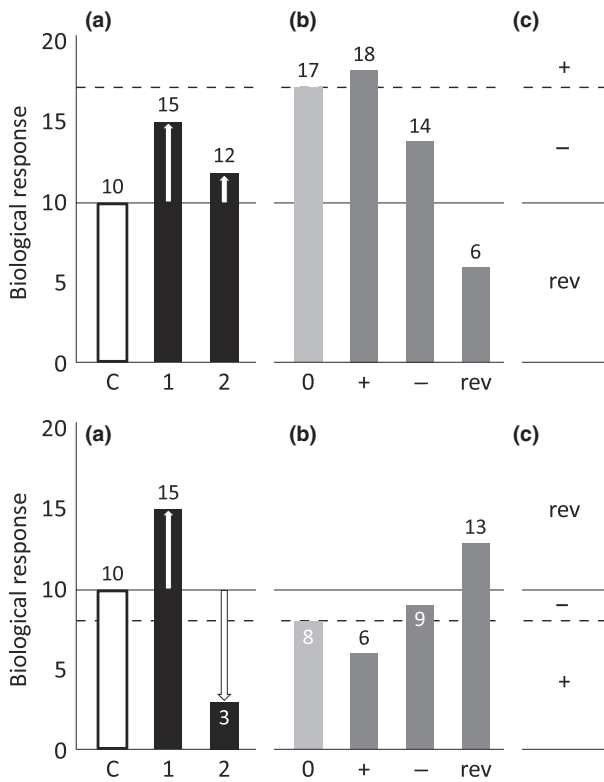
FIGURE 1 Conceptual model of interactions between two stressors (1 and 2, panel a) with unidirectional effects (white arrows; upper panel) compared to the control (C) and in a situation with opposing stressors (lower panel). Interactions (panel b) are defined as synergisms (+) if deviation from the control is larger compared to additivity (light grey bar and dashed line) and as antagonism (−) if deviation from the control is smaller. Interaction outcomes that are opposite compared to the sum of the individual effects are labelled as reversals (rev). Response spaces of interaction types are shown in panel c.

(experimental duration, volume) were centred on the mean and scaled by two standard deviations (Gelman, 2008). Outliers identified by plotting effect size as a function of each continuous variable were excluded from further analyses (e.g. Pilati, Vanni, Gonzalez, & Gaulke, 2009; large experimental ponds). Categorical variables with fewer than eight values per level (e.g. taxon: Clupeiformes, Perciformes, Osmeriformes; Supporting Information Table S2) were also excluded (Nakagawa & Cuthill, 2007). We report effect-size estimates with intercepts for categorical variables where we used contrast analysis to assess how the interactive effects changed for each moderator variable level (Paterson et al., 2013), whereas continuous variable effect sizes represent slopes. To calculate the degree of variation among studies (i.e. heterogeneity), we calculated the I^2 statistic (Higgins, Whitehead, Turner, Omar, & Thompson, 2001; Nakagawa & Santos, 2012), with I^2 values <25% suggesting high consistency among studies.

Third, to assess the relative importance of the seven moderator variables, the initial weighted linear mixed-effects model was extended to create a global model including all variables. From the set of all possible submodels created using MuMIn functions (Barton,

2002), we used the Akaike information criterion for small sample sizes (AIC_c) in conjunction with model averaging ("zero" method) to rank all submodels within four AIC_c of the best model (Burnham & Anderson, 2002). Model-averaged parameter estimates, standard errors, 95% confidence intervals and relative importance of each moderator variable are reported for the final model.

## 2.4 | Publication bias and robustness of results

Publication bias was evaluated by constructing funnel plots to visually assess the relationship between effect size and sample size (Figure 2). Decreasing effect sizes with increasing sample size indicate the absence of publication bias (Sterne, Becker, & Egger, 2005). The Spearman rank correlation was also used to statistically assess the relationship between effect size and sample size. We examined the robustness of each significant result using Rosenberg's (2005) fail-safe number, which indicates the potential number of additional studies with no effect needed to push the significance level above $\alpha = 0.05$. The results were considered robust if this number was larger than $5N + 10$, with N being the number of studies.

3 | RESULTS

Overall, 183 sets of effect sizes for interactive effects from 12 fish species from seven orders were included in the meta-analysis, with the majority of studies involving Salmoniformes (Supporting Information Table S2). The data included five species of Salmoniformes (*Oncorhynchus tshawytscha*, *Oncorhynchus mykiss*, *Salmo salar*, *S. trutta* and *Thymallus thymallus*), two Perciformes (*Lepomis macrochirus*, *Oreochromis niloticus*) and one each of Cypriniformes (*Pimephales promelas*), Esociformes (*Esox lucius*), Characiformes (*Colossoma macropomum*), Clupeiformes (*Dorosoma cepedianum*) and Osmeriformes (*Plecoglossus altivelis*). Temperature (19 experiments), pH (12) and toxicants or toxins (7) were the stressors most frequently manipulated (Supporting Information Table S2). The overall direction of interactive effects was antagonistic (−0.54 ± 0.11 [all results reported as means ± standard errors]; Figure 3). Vote counting revealed that the majority of interactive effect sizes (183) indicated additive stressor interactions (confidence intervals included zero), followed by 38 antagonisms and seven synergisms. Low I^2 values of the overall model (5.4%) and the moderator variables (<6.5%) indicated a high degree of consistency among studies (Table 1).

3.1 | Investigating effects of moderator variables separately

Of the four fish orders tested for the effects of moderator variables (three orders were removed due to small sample sizes), Cypriniformes, Esociformes and Salmoniformes showed significant antagonistic responses (Figure 3), with Cypriniformes showing greater antagonisms than Esociformes and Salmoniformes (Supporting Information Table S3) and Characiformes revealing a
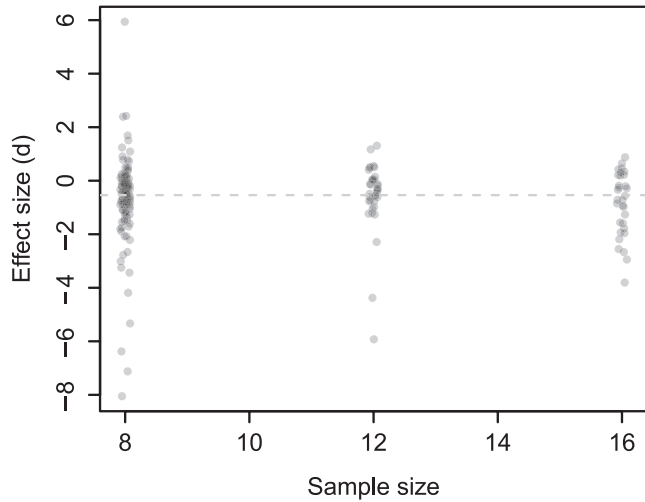
FIGURE 2 Funnel plots of effect sizes (*d*) vs. sample size (*N*).

high variation in interaction outcomes despite having a similar sample size as Cypriniformes and Esociformes. Antagonistic responses did not differ between Esociformes and Salmoniformes, nor between Characiformes and all other taxa (Supporting Information Table S3). All life stages showed antagonisms (Figure 3, Table 1); larval fish did so with a greater magnitude than juveniles (Supporting Information Table S3), whereas adults showed a large variation in interaction outcomes. Consistent antagonistic responses were demonstrated only for survival and physiological response types (Figure 3, Table 1), with survival demonstrating

greater antagonisms than biomass (Supporting Information Table S3). Although antagonisms were shown only for lentic habitats (Figure 3, Table 1), the difference between lentic and lotic habitats was not significant (Supporting Information Table S3). All stressor intensities showed antagonisms (Table 1), and effect sizes did not differ significantly between intensities. Experimental duration (slope −0.33 ± 0.23) and volume of experimental units (0.04 ± 0.85) both showed interactive effect sizes that were additive (confidence intervals of both slopes included zero).

3.2 | Investigating the relative importance of moderator variables

From the global model, a set of six top models were identified within four AIC_c of the best model (Table 2). Taxon (i.e. fish order) and life stage featured in all top models, with all other moderator variables featuring, in diminishing importance, in a single model each (relative importance: volume of experimental units 0.16, response type 0.14, habitat 0.12, stressor intensity 0.10, duration 0.07). With taxon and life stage included in the model, stressor interactions developed towards synergisms with increasing volume (slope 0.23 ± 0.20) and duration (0.08 ± 0.15).

3.3 | Publication bias

The Spearman rank correlation coefficient suggested that there was no relationship between effect size and sample size (ρ = −0.01,
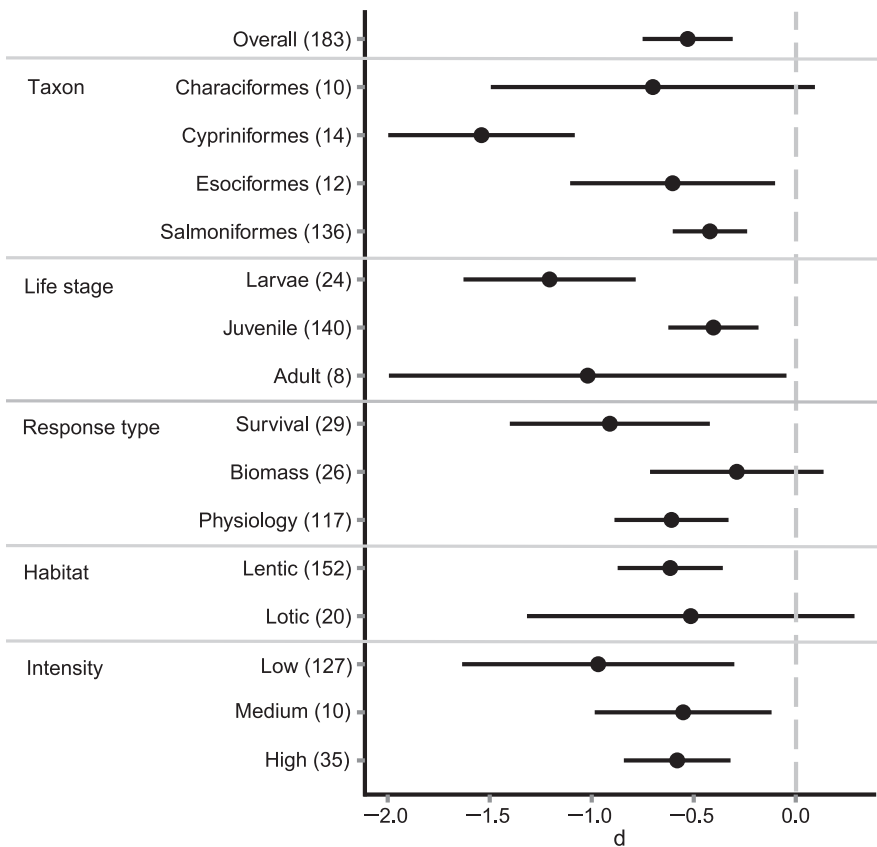
FIGURE 3 Effect sizes and 95% confidence intervals of the overall interaction effect (meta-analytic mean) and levels of the categorical moderator variables (sample sizes in brackets). Negative effect sizes indicate antagonisms and positive effect sizes synergisms.

TABLE 1 Results for the overall and separate mixed-effects meta-analyses of the interactive effects. Shown are the number of effect sizes (k), studies (m) for each variable level, the effect sizes (d), their 95% confidence intervals (CI), Z- and p-values. Statistically significant effect sizes at α = 0.05 are highlighted in bold. The I^2 statistic quantifies the degree of variation among studies (i.e. heterogeneity) with values <25% suggesting a high consistency among studies.

Variable	Variable level	k	m	d	Lower 95% CI	Upper 95% CI	Z	p	I^2
Overall		183	26	**−0.54**	**−0.76**	**−0.32**	4.77	**<0.0001**	5.45
Taxon	Characiformes	10	1	−0.71	−1.50	0.09	1.75	0.080	0.00
	Cypriniformes	14	3	**−1.55**	**−2.01**	**−1.09**	6.64	**0.000**	
	Esociformes	12	1	**−0.61**	**−1.11**	**−0.11**	2.39	**0.017**	
	Salmoniformes	136	17	**−0.43**	**−0.61**	**−0.25**	4.61	**<0.0001**	
Life stage	**Larvae**	24	4	**−1.21**	**−1.64**	**−0.79**	5.64	**<0.0001**	1.87
	Juvenile	140	17	**−0.41**	**−0.63**	**−0.19**	3.65	**0.0003**	
	Adult	8	2	**−1.03**	**−2.00**	**−0.05**	2.07	**0.039**	
Response type	Biomass	26	13	−0.30	−0.72	0.13	1.37	0.171	5.88
	Physiology	117	14	**−0.62**	**−0.90**	**−0.34**	4.33	**<0.0001**	
	Survival	29	6	**−0.92**	**−1.41**	**−0.43**	3.68	**0.0002**	
Duration[a]	*Continuous*	172	23	−0.60	−0.84	−0.35	4.73	<0.0001	6.26
Volume[a]	*Continuous*	172	23	−0.61	−0.86	−0.37	4.89	<0.0001	6.25
Habitat	**Lentic**	152	20	**−0.62**	**−0.88**	**−0.37**	4.74	**<0.0001**	6.36
	Lotic	20	4	−0.52	−1.33	0.28	1.28	0.202	
Intensity	**Low**	127	3	**−0.98**	**−1.64**	**−0.31**	2.87	**0.004**	5.05
	Medium	10	5	**−0.56**	**−0.99**	**−0.13**	2.53	**0.011**	
	High	35	23	**−0.59**	**−0.85**	**−0.33**	4.42	**<0.0001**	

[a]These continuous moderator variables were centred and scaled; their slopes are presented in the text.

N = 183, p-value = 0.87), and visual inspection of the funnel plot also suggested the absence of any publication bias (Figure 2). Furthermore, the Rosenberg fail-safe number indicated that an additional 1,617 studies averaging no interactions would be required to push the significance level of the mean effect size above α = 0.05.

4 | DISCUSSION

Our meta-analysis demonstrates an overall antagonistic outcome of multiple-stressor effects on freshwater fish consistent with the only previous meta-analysis on freshwater fish (Matthaei & Lange, 2016), which was partly based on the same data. Importantly, our study expands on this previous meta-analysis by identifying the most important moderator variables influencing direction and magnitude of interactions. Our study thus addresses a key knowledge gap identified in the context of predicting the susceptibility of freshwater fish to multiple stressors (Schinegger, Palt, Segurado, & Schmutz, 2016; Segner et al., 2014). In particular, our results suggest that taxonomic identity and life stage strongly and significantly influence the strength of antagonisms. Once these two variables were accounted for, variation and direction of interactive effects were also influenced by response type (i.e. the biological response measured), habitat, stressor intensity, experimental duration and experimental volume (all are discussed in detail below).

In terms of frequencies, the majority of stressor interaction outcomes in our data set were additive (75%), whereas antagonistic (21%) and synergistic interactions (4%) were less common. However, a meta-analysis holds the advantage of combining individual studies and performing statistical tests based on all data. Hence, the overall result of our meta-analysis, the prevalence of antagonisms, is different to the distribution of frequencies.

The 38 antagonisms reported from individual studies included 13 effect reversals, which represent special cases of antagonisms where interactive effects are not just less than the additive outcome but are opposite to it (Figure 1). For example, warming and acidification individually increased liver protein degradation in juvenile rainbow trout (*O. mykiss*, Salmonidae), whereas both stressors combined decreased degradation rates compared to controls (Reid, Dockray, Linton, McDonald, & Wood, 1997). As a consequence, effect reversals may pose the most challenging ecological consequences for predicting multiple-stressor interactions (Jackson et al., 2016; Piggott, Townsend, & Matthaei, 2015).

4.1 | Occurrence and strength of antagonisms vary among fish taxa

In our meta-analysis, fish taxonomy strongly influenced the frequency and strength of the observed antagonistic interactions, with both Salmoniformes and Esociformes demonstrating fewer

antagonistic responses than cyprinids. While we acknowledge that this contrast originates from comparisons of a single cyprinid (i.e. fathead minnow, *P. promelas*, Cyprinidae) and Esociformes (northern pike, *E. lucius*, Esocidae) versus multiple salmonid species, both species can be considered as model species of their respective orders. Fathead minnow, like most Cypriniformes, is relatively tolerant to habitat degradation, whereas Esociformes, such as the northern pike, are generally more sensitive to habitat changes (Shields, Knight, & Cooper, 1995). Generalist fish species, i.e. those with morphological, physiological, behavioural and/or life-history traits adapted to wider environmental gradients, are more likely to show tolerance to habitat degradation and co-tolerance to additional stressors than specialists (Shields et al., 1995; Vinebrooke et al., 2004). This rationale may explain the greater prevalence of antagonistic responses for generalist taxa such as Cypriniformes revealed by our meta-analysis. However, more multiple-stressor studies are needed on a wider variety of fish taxa to better understand the role of stressor co-tolerance for interaction outcomes (Segner et al., 2014).

4.2 | Juvenile life stages are most sensitive to stressor interactions

Our study demonstrates a general trend for antagonistic responses to multiple stressors for all fish life stages; nevertheless, the overall response of juveniles was closer to additivity than for larvae and adults. Juveniles generally exhibit a more complex and diverse behaviour (including habitat and resource use) than larvae, which potentially increases their exposure to different stressors, and this may increase the frequency for additive outcomes or synergisms (Segner et al., 2014). Further, juveniles may also be more susceptible to stressors than adults due to a higher body surface to weight ratio and lower body reserves associated with this rapid-growth life stage and, as a consequence, adverse environmental conditions may cause stronger physiological responses to multiple stressors and in turn weaker antagonisms. In contrast to other life stages, adults demonstrated high variability in antagonistic responses. While the minimum required number of effect sizes was reached in our meta-analysis, this high variability highlights the need for more research on adult fish.

TABLE 2 Top models selected for investigating the relative importance of the seven moderator variables for the interactive effects.

Models	df	ΔAIC$_c$	w
Taxon + life stage	9	0.00	0.42
Taxon + life stage + volume	10	1.97	0.16
Taxon + life stage + response type	11	2.14	0.14
Taxon + life stage + habitat	10	2.42	0.12
Taxon + life stage + intensity	11	2.93	0.10
Taxon + life stage + duration	10	3.65	0.07

Note. ΔAIC$_c$: distance to the best model based on the Akaike information criterion for small sample sizes; *df*: degrees of freedom; *w*: relative Akaike weight of the submodel.

4.3 | Effects of extrinsic moderator variables

Our model-selection results suggest that intrinsic variables, i.e. taxonomy and life stage, were more important in determining stressor outcomes than extrinsic variables, i.e. moderator variables related to the experimental design. Nevertheless, several extrinsic variables, namely volume of experimental units, response type, habitat type, stressor intensity and duration, were retained in the set of top models because they explained part of the observed variation in stressor interactions after accounting for differences in taxonomy and life stage (Table 2). We will now focus on how these extrinsic variables shaped interaction outcomes after the intrinsic variables had been accounted for.

The volume of experimental units varied considerably across studies from laboratory beakers to outdoor ponds, from 0.1 to 843,000 L. We found that with increasing volume, the overall stressor interactions shifted from antagonisms towards additive outcomes. This may be a consequence of a positive relationship between ecological complexity and the volume of experimental units. Larger experimental units likely allow for more variable (micro)environmental conditions and the presence of other organisms and trophic levels. With increasing ecological complexity, potential stressor interactions multiply due to the increasing occurrence of indirect stressor effects, which may reduce the probability of antagonistic responses (Bruder et al., 2017; Elliott, 2000; Segner et al., 2014). In real ecosystems, this trend may continue towards a prevalence of synergisms. In line with this hypothesis, recent studies on stressor interactions in a large number of streams and lakes suggest a higher proportion of synergisms and thus a more balanced occurrence of the three interaction types (Nõges et al., 2016; Schinegger et al., 2016) compared to our meta-analysis of experimental studies.

Our study also provides support for a relationship between interaction outcome and the biological responses assessed (Segner et al., 2014; Townsend et al., 2008). For instance, fish survival displayed significantly stronger antagonisms than responses related to fish biomass, whose overall interaction type was additive. Physiological responses also showed antagonistic interactions; however, these were weaker than those for survival. Synergisms may be promoted by "highly integrative" response types (such as survival), which integrate the effects of various stressor mechanisms. Compensation and acclimatization effects underlying antagonisms are less likely for integrative response types due to the diversity of direct and indirect stressor effects (Jackson et al., 2016; Segner et al., 2014). Based on this rationale, one would expect shifts towards additive outcomes and synergisms with the following order of response types: physiology as the least integrative response type, to biomass and then to survival as the most integrative response type. However, our results did not adhere to this order.

This unexpected result may reflect a potential limitation of additive models when estimating stressor interactions for metrics with a fixed boundary such as survival (Folt et al., 1999). For such metrics, the response to a stressor cannot be larger than the boundary set by the metric; for example, survival cannot be lower than 0%

(corresponding to 100% mortality in an experiment). In these situations, stressor interactions are forced towards antagonisms since each individual stressor effect is constrained to the response space between the effects of the other stressors and the boundary of the metric. Multiplicative models may be able to alleviate such metric-dependent boundary effects on stressor interaction outcomes (Folt et al., 1999). However, a formal comparison of additive versus multiplicative multiple-stressor models was beyond the scope of our study.

Lentic experimental habitats (simulating pond or lake environments) showed a slightly higher prevalence of antagonisms when compared to lotic habitats (simulating streams or rivers) although this difference was not significant. Lotic ecosystems experience an inherently higher variation of environmental conditions at smaller spatial and temporal scales than lentic ecosystems (e.g. flow heterogeneity, diurnal water temperature fluctuations). Further, lotic ecosystems are generally more strongly impacted by their surrounding terrestrial ecosystems and human land-use activities through higher shoreline-to-water volume ratios. This high variation of environmental variables, whether caused by natural variation or anthropogenic stressors, would lead to adaptations of organisms, translating into higher co-tolerance to stressors and consequently an increase in antagonistic outcomes (Vinebrooke et al., 2004). However, our findings did not support this expectation, possibly because of the low realism of most of the experiments analysed in our study (see Matthaei & Lange, 2016).

Experimental duration varied greatly among studies (1–420 days), and increasing experimental duration diminished the prevalence of antagonisms. This corroborates earlier suggestions that synergisms need time to develop, since positive feedbacks between stressors accumulate with time and tend to increase negative physiological responses and population extinction risk (Brook, Sodhi, & Bradshaw, 2008), for example if energetic costs of organisms to tolerate stressors increase with time (Segner et al., 2014). In the only other study that tested temporal effects on multiple-stressor interactions, Darling and Côté (2008) demonstrated that the duration of animal mortality experiments in freshwater, marine and terrestrial environments yielding synergisms tended to be shorter than those producing antagonistic or additive outcomes. However, Darling and Côté (2008) did not account for the influence of taxonomic identity and life stage, both of which we found to be important in determining effects of experimental duration. That stressor interactions may change over time poses a major problem for freshwater fish conservation and management (Côté et al., 2016), since synergisms may develop on timescales longer than those of most experiments, environmental impact assessments and even conservation programs (Brook et al., 2008).

4.4 | Application to biodiversity conservation

Well-designed outdoor mesocosm experiments may represent a good compromise between experimental control and ecological realism (Stewart et al., 2013). Nevertheless, they rarely reflect the spatial and temporal complexity of population and community responses to multiple stressors in real ecosystems. Fish can exploit spatial habitat complexity and respond to unfavourable conditions by moving into refugia (Elliott, 2000; Hillyard & Keeley, 2012), and this behaviour can reduce their susceptibility to additional stressors. For instance, brown trout studied by Elliott (2000) used deeper layers of pools to avoid lethal near-surface water temperatures, which also reduced their susceptibility to low oxygen concentrations probably due to the relationship of standard metabolic rates with temperature.

Similarly, biotic interactions among individuals and trophic interactions in real ecosystems allow for additional mechanisms that may modify the outcome of stressor interactions, including population and community dynamics but also food-web effects (Bruder et al., 2017; Pilati et al., 2009; Power, 1997; Segner et al., 2014). For example, fish affected by indirect stressors effects, e.g. from reduced flows diminishing prey availability, may be particularly susceptible to direct stressors such as increasing water temperatures (Bruder et al., 2017).

However, some studies have demonstrated that findings from experimental research can be transferred to natural real ecosystems. For example, responses of stream invertebrate communities to multiple stressors generally followed similar patterns when comparing outcomes of a field survey with a reach-scale field experiment (Townsend et al., 2008).

A recent large-scale analysis of multiple-stressor effects on river fish communities gives some indication on the prevalence of interaction outcomes in real freshwater ecosystems. Based on a data set comprising 3,105 European river fish communities, Schinegger et al. (2016) found that antagonisms and synergisms were equally likely, whereas in our meta-analysis stressor interactions were on average antagonistic. This difference in interaction outcomes between experimental and real freshwater ecosystems could be a consequence of differences in spatial and temporal scales, which would support our observation that synergisms tend to develop in larger experimental volumes and over longer timescales aligned with real ecosystems as opposed to mesocosm experiments. Alternatively, this difference could also be due to ecological complexity influencing stressor interactions (Segner et al., 2014).

Compared to the limited findings from studies of fish communities in real freshwater ecosystems, our meta-analysis holds the advantages of allowing comparisons based on findings of controlled factorial experiments and testing gradients of moderator variables such as volume of experimental units. Until sufficient controlled factorial experiments from real ecosystems become available, our approach seems the most promising by integrating knowledge from existing studies to (a) extrapolate findings to real ecosystems and (b) identify future research needs.

5 | CONCLUSIONS AND RESEARCH NEEDS

We have shown that the prevalence of antagonistic interactions differed among freshwater fish taxa and life stages. This key finding suggests that a wide range of species as well as different life stages

should be considered in the conservation planning for freshwater fish. We demonstrate that these intrinsic variables, and to a lesser degree also extrinsic variables, modified multiple-stressor interaction outcomes. Strength of antagonisms diminished with increasing experimental size and duration. Conducting more realistic experiments by increasing experimental duration and the volume of experimental units, and by including other compartments of the aquatic food web, such as invertebrates, algae, bacteria and basal resources, will likely result in greater ecological complexity and allow for indirect stressor effects to be tested.

Multiple-stressor situations seem to be generally more common for streams than for lakes (Nõges et al., 2016), and this is at odds with their representation in experimental studies involving freshwater fish (i.e. our meta-analysis included just 20 interactive effect sizes from experiments mimicking streams compared to 152 from experiments mimicking lakes). This contrast might be caused by differences in traditions between the two research fields or methodological feasibility. In any case, this discrepancy suggests more manipulative experiments mimicking stream ecosystems are needed to provide a more solid mechanistic understanding for management and restoration of stream fish populations under the influence of multiple stressors.

Overall, more effort should go into manipulative multiple-stressor experiments on freshwater fish, which will allow assessing the role of extrinsic moderator variables in greater depth. Once more experimental and survey-based data are available, the next exciting step would be to assess stressor-specific impacts on fish by also accounting for stressor identity, for example physical, chemical, resource and predation stress. Further, our study revealed that just four fish orders (represented by seven species) currently provide sufficient data to satisfy the requirements of meta-analytic approaches. This number is very small compared to the approximately 15,750 fish species known globally (Darwall & Freyhoff, 2016) and indicates a major research need. The data available for our analysis are also biased towards cold-water and economically important species. While it is easier to get permits to study fish species that are abundant and easily reared under artificial conditions, we urgently need more studies on rare and endangered fish species.

To conclude, in future multiple-stressor research on freshwater fish, we recommend investigating: (a) tolerance and co-tolerance in a wide variety of fish species, including rare and endangered species where feasible; (b) all fish life stages, but especially adults; (c) impacts on stream and river ecosystems simulated in realistic experiments; and (d) impacts determined in experiments of long durations that involve different stressor intensities.

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