Synthesizing the impacts of multiple stressors on freshwater biota across scales and ecosystems

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75

76   **Abstract**

77   Climate and land-use change drive a suite of stressors that shape ecosystems and interact to

78   yield complex ecological responses, *i.e.* additive, antagonistic and synergistic effects.

79   Currently we know little about the spatial extent of such interactions and about effect sizes.

80   This knowledge gap needs to be filled to underpin future land management decisions or

81   climate mitigation interventions, for protecting and restoring freshwater ecosystems. The

82   study combines data across scales from 33 mesocosm experiments with those from 14 river

83   basins and 22 cross-basin studies producing 180 combinations of paired-stressor effects on a

84   biological response variable. Generalised linear models showed that only one of the two

85   stressors had a significant effect in 40% of the analysed cases, 27% of the paired-stressor

86   combinations resulted in additive and 33% in interactive (antagonistic, synergistic, opposing

87   or reversal) effects. While for lakes the frequency of additive and interactive effects did not

88   differ significantly between scales, for rivers this frequency increased with scale. Nutrient

89   enrichment was the overriding stressor for lakes, generally exceeding those of secondary

90   stressors. For rivers, the effect sizes of nutrient enrichment were dependent on the specific

91   stressor combination and biological response variable. These results vindicate the traditional

92   focus of lake restoration and management on nutrient stress, while highlighting that river

93   management requires more bespoke management solutions.

94

Introduction

Multiple stressors are increasingly recognized as a major concern for aquatic ecosystems and for those organisations in charge of their management. Stressors commonly interact in affecting freshwater species, communities and functions, but the questions remain to which degree this evidence from experiments can be transferred to field conditions and how relevant stressor interactions are for ecosystem management. Critically, no study has been conducted to systematically confirm the frequency of occurrence of multiple stressor interactions across spatial scales (*i.e.* from waterbody to continental scales) and ecosystem types (*i.e.* for rivers and lakes). Using the most comprehensive large-scale assessment of multiple stressor interactions to date, we show that dominance of a single stressor, namely nutrient enrichment, is still common in lakes, while for rivers stressor interactions are much more relevant, demanding for more complex and informed management decisions.

Formerly, single, intense and well characterised stressors, such as organic and nutrient pollution, dominated freshwater ecosystem responses (van Dijk et al., 1994). However, as these formerly dominant stressors are now controlled and others emerge, recent large scale analyses have shown that freshwater ecosystems are exhibiting novel ecological responses to different stressors (Richardson et al., 2018; Schäfer et al., 2016; Schinegger et al., 2016). For the simplest case of two stressors acting simultaneously, three main types of effects can be conceptually distinguished: (i) Only one of the two stressors has notable ecological effects so that the effects of Stressor A outweigh those of Stressor B or vice versa (stressor dominance); (ii) the two stressors act independently such that their joint effect is the sum of the individual effects (additive effects); (iii) a stressor either strengthens or weakens the effects of the other (interaction). However, there is a striking lack of information on the frequency of occurrence of these effect types across spatial scales (*i.e.* from individual waterbodies to a whole continent) and ecosystem types (rivers *vs.* lakes).

Here we use a combined empirical-exploratory approach and a common quantitative framework to analyse a large set of original and compiled data on combinations of stressor pairs (explanatory variables), with each of them related to a biological response variable. We build on conceptual understanding of ecological responses to stressor interactions (Piggott et al., 2015; Jackson et al., 2016; de Laender, 2018) to structure an empirical modelling approach, using generalised linear modelling (GLM) and 180 stressor combinations with single biological responses from more than 18,000 observations. Outputs of the GLMs were interpreted to identify the frequency of cases with stressor dominance, additive stressor

relationships and stressor interactions (synergistic or antagonistic), stratified by ecosystem
type (lake or river) and spatial scale (experiments, basin studies, cross-basin studies).
With this approach we addressed four questions: (1) How frequent are the four different types
of stressor effects in lakes and rivers? We expected a high share of additive, synergistic and
antagonistic relationships in both lakes and rivers, as intense stressors obscuring the effects of
secondary stressors nowadays rarely occur. (2) To what extent do ecosystem type (lake *vs.*
river) and spatial scale influence the combined effects of two stressors? We expected more
frequent stressor interactions in rivers because greater heterogeneity than in lakes increases
the likelihood for two stressors to have an impact. We further expected more frequent stressor
interactions in small-scale studies (*i.e.* in mesocosms), as these are less influenced by
confounding factors (Vinebrooke et al., 2004; Schäfer and Piggott, 2018). (3) What is the
influence of ecosystem type (lake *vs.* river) and spatial scale on the explanatory power of two
stressors and their interaction? We expected the explanatory power to be lower for rivers
because of greater heterogeneity and thus potentially confounding factors in comparison to
lakes. We also expected a decreasing explanatory power of individual stressors and their
interactions with spatial scale, reflecting the increasing importance of confounding factors at
large scales. (4) Is nutrient enrichment the most prominent stressor affecting European aquatic
ecosystems, and does the importance of co-stressors differ between lakes and rivers? We
expected a dominating effect of nutrient stress in lakes due to the dominance of primary
producers and a greater relevance of hydrological and morphological changes in rivers.
Our study pursues a phenomenological approach (*sensu* Griffen et al., 2016) and seeks to
disclose stressor interrelations under "real-world" conditions, contributing to solve some of
the pertinent issues in ecosystem management (Côté et al., 2016).

Results and discussion

Frequency of stressor effect types

We hypothesised that high proportions of both lake and river case studies would indicate
additive or interactive paired-stressor relationships – this was not supported. Among the 180
cases, 40% of models indicated single stressor dominance, 27% indicated additive paired-
stressor effects, and 33% indicated paired stressors interacting significantly (Figure 2; see also
Supplementary Material Table S2). Single stressor dominance was especially common in
lakes, where responses are driven predominantly by nutrients and secondary stressor effects
are not significant.

161   The observed pattern on stressor dominance may change if the stressor gradients are

162   modified, *i.e.* the intensity of the dominant stressor is reduced or the intensity of the

163   suppressed stressor is increased (Feld et al., 2011; Sundermann et al., 2013). The pattern of

164   stressor dominance may further relate to the type of biological response variable. This not

165   only holds for the fundamental distinction between autotrophs and heterotrophs regarding

166   energy and nutrient supply, but also for the more specific differences regarding life-history

167   and habitat preferences (Segner et al., 2014; Lange et al., 2018). For instance, the dominance

168   of phytoplankton as a response variable in the lake cases partly explains why nutrient stress is

169   found to be the most relevant stressor.

170

171   ***Impact of ecosystem type and scale on stressor effect types***

172   We expected a higher proportion of river cases to exhibit stressor interactions, compared to

173   lakes, as a result of greater habitat heterogeneity in rivers – this was supported. The

174   proportions of effect types differed between lakes (60% dominance, 17% additive, 22%

175   interactive) and rivers (30% dominance, 32% additive, 38% interactive; see Figure 2) (Chi-

176   squared test, $p < 0.001$). While freshwater ecosystems in general are sinks "collecting"

177   anthropogenic stressors, the dendritic shape of rivers multiplies human activities in the

178   catchment, such as land and water uses. This results in an increased exposure to hydrological

179   and morphological stressors, the latter also being more relevant in rivers due to their primarily

180   benthic habitats and assemblages. In addition, toxic substances can act more directly in

181   (small) rivers, as much lower compound quantities are needed to reach toxic concentrations.

182   Most of the additive and interactive stressor effects observed in rivers are conditioned by

183   oxygen availability, and antagonistic responses underline counter-directional stressor effects

184   on oxygen contents (e.g. increased aeration due to faster flow in channelized rivers

185   dampening eutrophication effects; Schinegger et al., 2018). Various other stressors act

186   through multiple modes of action including chloride inducing osmotic stress, toxic substances

187   obstructing metabolic processes, temperature stress increasing oxygen demand, and

188   morphological alteration affecting physical habitat availability. These stressors are more

189   likely to act in combination with others in rivers where toxicants can reach (sub-)lethal peak

190   concentrations earlier and habitat disturbance is greater, when compared to lakes (Graeber et

191   al., 2017). Notably, within the 59 cases where models included a significant interaction term,

192   the combinations of nutrients with toxic or morphological stress represented the greatest

193   proportion of confirmed interaction effects (ratio of 0.45 or 0.43, respectively; only

combinations with total number of cases > 5; no significant correlation between total number of cases and share of interactive cases).

We expected that the frequency of interactions would increase with scale – this was only partly supported. While for lakes additive and interactive effects did not differ significantly between scales, for rivers the share of additive and interactive cases increased with scale (Chi-squared test, $p < 0.001$). Two contrasting mechanisms may explain this pattern: On the one hand, increasing spatial scale implies an increase in confounding factors (including stressors not addressed in this analysis), limiting the likelihood of detecting additive or interactive effects between the targeted stressors, as they may be masked by other factors not under investigation. Conversely, unknown stressors could co-vary with the tested stressors, thus providing false positive evidence if an unknown stressor is the true actor. On the other hand, increasing spatial scale may imply longer stressor gradients, possibly increasing the likelihood of additive or interactive stressor effects, which may only occur at certain stressor intensities. The latter holds true only if stressors are effective over the whole gradient length, e.g. the biological response does not level off at low or intermediate stressor levels (as in case of nutrient saturation; Price & Carrick, 2016; McCall et al., 2017). In addition, rivers accumulate stressors from their catchments.

As discussed above, the pattern of stressor dominance largely prevailed for lakes, irrespective of the spatial scale. Across the 34 cases of paired nutrient-thermal stress, however, the nutrient effects became more pronounced than the temperature effects with increasing spatial scale. Confounding factors may have further dampened the effects of thermal stress, perhaps connected to different stressor-specific resilience patterns across lake types (Spears et al., 2015; Richardson et al., 2018). In addition, time series data of single water bodies, which generally imply shorter stressor gradients, were frequently used in lake studies at basin scale (28% of lake cases; "single-site, multi-year"; see Table 2).
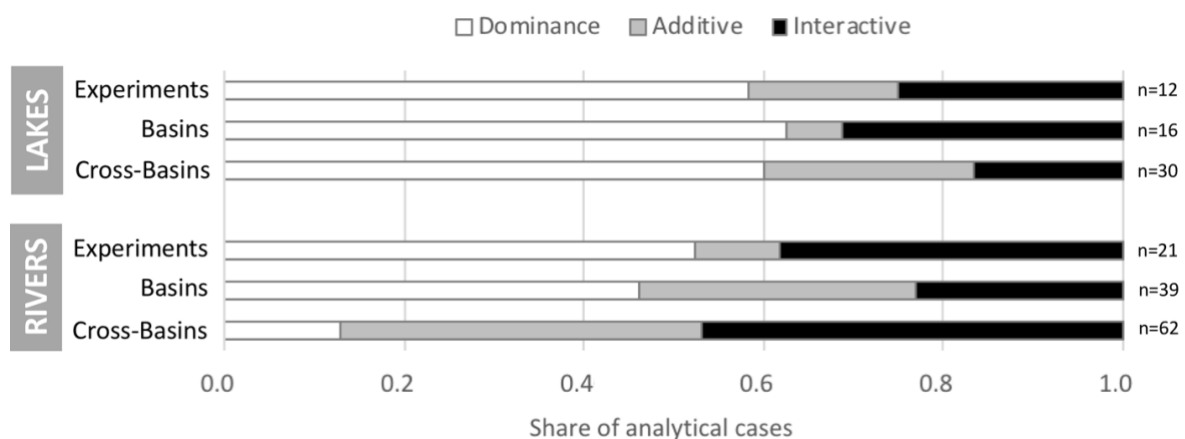
220   Figure 2: Share of analytical cases across experiments, basin studies and cross-basin studies

221   from lakes (n = 58) and rivers (n = 122), for which only a single stressor (dominance), both

222   stressors (additive) or their interaction significantly contributed to the variability of the

223   biological response.

224

225   *Impact of ecosystem type and scale on the models' explanatory power*

226   Contrasting to our expectations, river models performed significantly better than lake models,

227   in spite of a presumably higher number of confounding factors for rivers than for lakes. This

228   better performance can be explained by the specific nature of riverine ecosystems: rivers

229   feature various niche and habitat factors that can be altered by multiple stressors (e.g. water

230   quality, hydrology, benthic habitats), and the riverine fauna is sensitive to the impacted

231   oxygen conditions, which may "collect" the effects of a variety of stressors into a single

232   gradient. Oxygen, however, is rarely measured in a meaningful way in monitoring programs

233   (including the daily maxima and minima) and was thus not considered as a stressor in our

234   analysis. In contrast, lake phytoplankton seems less susceptible to the effects of multiple

235   stressors, as long as nutrients are in the growth-limiting concentration range.

236   We expected a decreasing explanatory power with spatial scale, reflecting the increasing

237   importance of confounding factors at large scales – this was partly supported. The variance in

238   biological response explained by the paired-stressor models (expressed as marginal R^2)

239   ranged between 0.05 and 0.88, with a median value of 0.18. These ranges differed

240   significantly between experiments (median marginal $R^2 = 0.40$) and basin or cross-basin

241   studies (Mann-Whitney U-test, $p < 0.001$), but basin and cross-basin studies did not differ

242   from each other (combined median marginal $R^2 = 0.17$; Figure 3A). The marginal R^2 differed

243   significantly between lakes and rivers, with river cases showing on average slightly higher

244   explanatory power (lakes: $R^2 = 0.14$, rivers: $R^2 = 0.23$; not shown). The importance of the

245   interaction term (expressed as %R^2 change) was significantly higher for lakes than for rivers.

246   For rivers, this importance tended to decrease with increasing scale of investigation, but

247   differences between investigation scales were generally not significant (Figure 3B).

248   For experiments, the high level of control on potentially confounding factors can account for

249   the on average greater explanatory power, when compared to field studies. Furthermore, the

250   experimental studies had lower numbers of observations and less complex biological

251   communities. Compared with this, factors such as temperature variation are already

252   temporally pronounced at basin-scale and the spatial variation across basins is considerable.

253
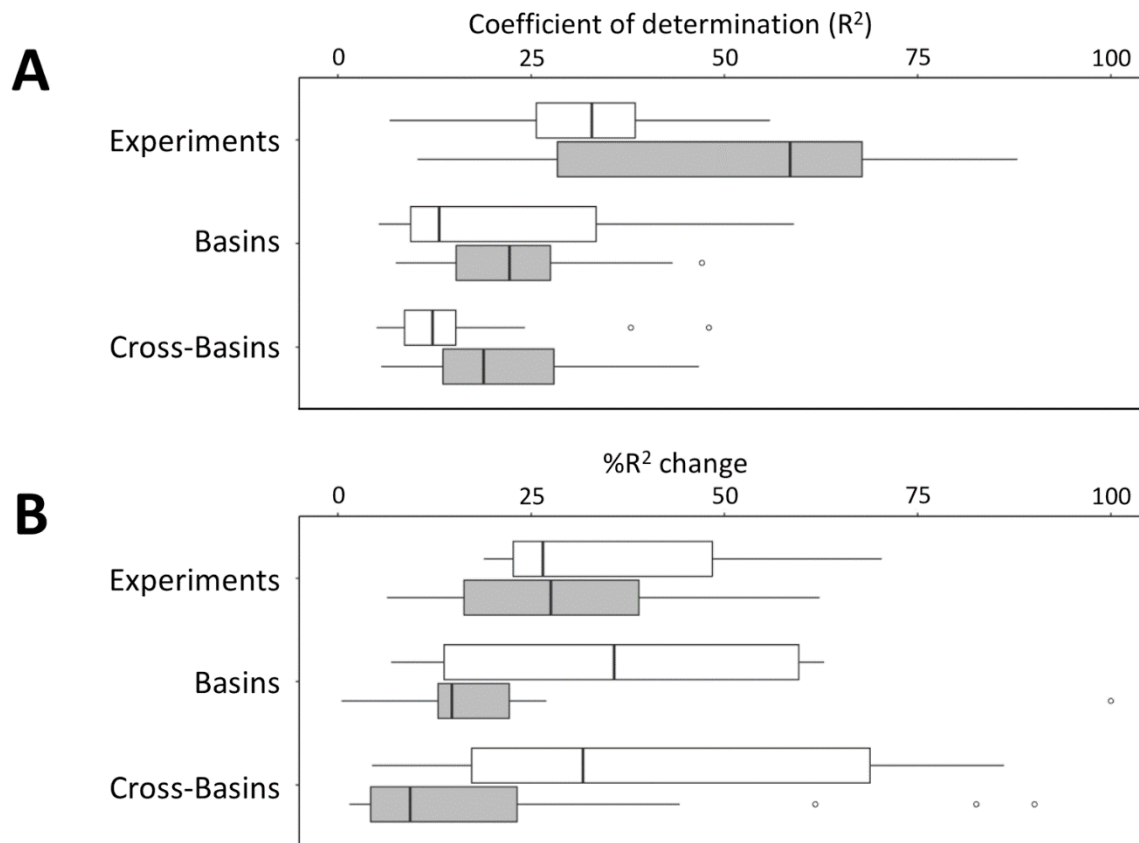
Figure 3: (A) Percent of biological variance explained by the paired stressors including their interaction for the mesocosm experiments ($n = 33$), basin study cases ($n = 55$) and cross-basin study cases ($n = 93$), separately for lakes (white boxes) and rivers (grey boxes). Lakes and rivers differed significantly only for the cross-basin studies (pairwise Bonferroni-corrected Mann-Whitney U-test, $p = 0.001$).

(B) Percent change in explained biological variance when interaction term is removed from the model (in case of significant interaction term) for the mesocosm experiments ($n = 11$), basin study cases ($n = 14$) and cross-basin study cases ($n = 34$), separately for lakes (white boxes) and rivers (grey boxes). None of the differences within investigation scales are significant.

Definition of box-plot elements: centre line = median; box limits = upper and lower quartiles; whiskers = 1.5x interquartile range; points = outliers.

Role of nutrient stress for lakes vs. rivers

Finally, we supported the hypothesis that responses to nutrient stress is retarded by the presence of secondary stressors in rivers more so than lakes where responses to nutrient enrichment are strongest.

We identified eleven combinations of nutrient stress paired with another stressor, covering morphological, hydrological (including hydropeaking), thermal, toxic and chemical stress (brownification). The number of analytical cases in each stressor combination ranged from four to 33, with the combinations including hydropeaking and brownification stress exclusively comprising data collected at the experimental scale. All other combinations comprised data from up to ten different studies, most of which originated from two or more

spatial scales. Best represented were the combinations of nutrient stress paired with thermal stress affecting autotrophs in lakes, and nutrient stress paired with morphological stress affecting heterotrophs in rivers (Figure 4).
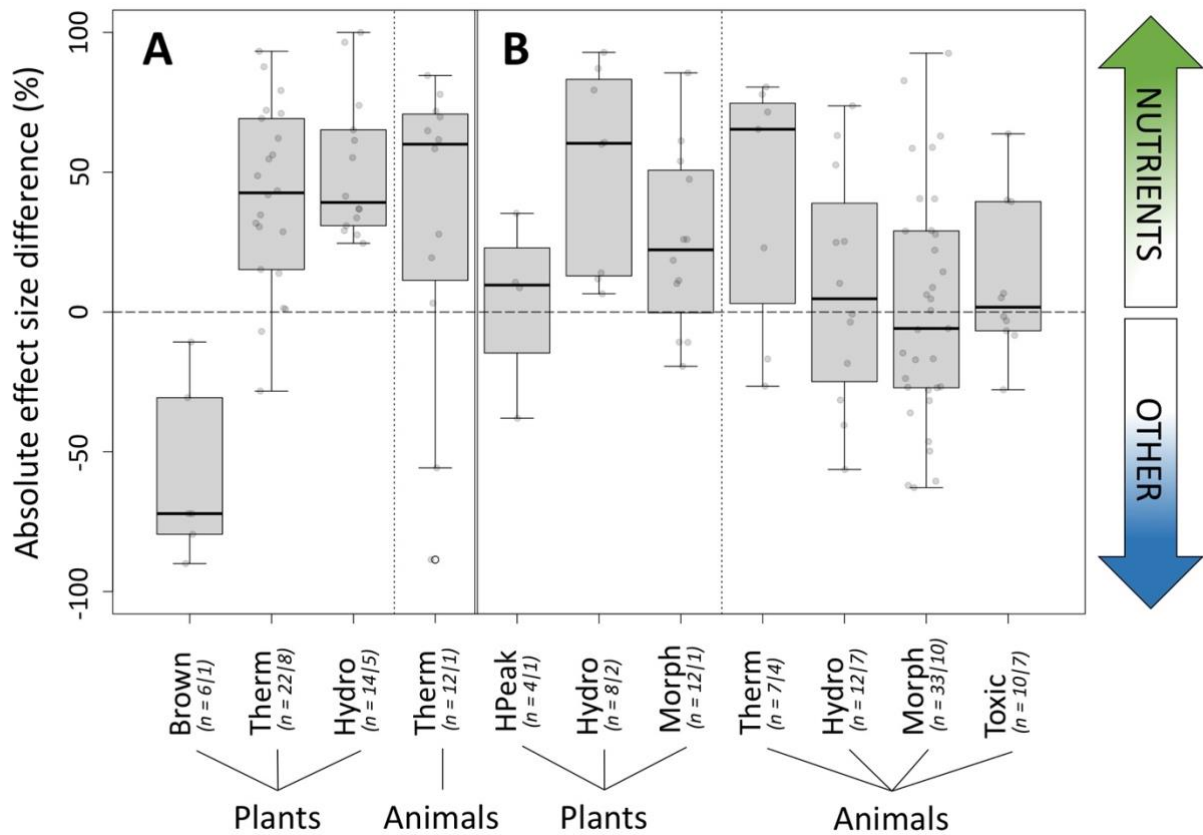
Figure 4: Range of absolute effect size differences (%AES) for nutrient stress and selected other stressors across case-studies from (A) lakes and (B) rivers. Positive %AES indicate stronger effects by nutrient stress, negative %AES indicate stronger effects by the other stressor on the biological response variable (subdivided into plants and animals) in the regression model.
Brown = Brownification, Therm = Thermal stress, HPeak = Hydropeaking, Hydro = Hydrological stress, Morph = Morphological stress, Toxic = Toxic stress; n = Number of analytical cases | case studies.
*Definition of box-plot elements:* centre line = median; box limits = upper and lower quartiles; whiskers = 1.5x interquartile range; points = outliers. Grey dots depict the individual data points.

Nutrient stress often had the stronger effect in the paired-stressor models. Hence, nine of the eleven combinations in lakes and rivers showed a positive %AES median, implying on average stronger effects of nutrients compared to the other stressor. Five combinations even showed a positive 25th percentile %AES, indicating that in three quarters of the cases in these combinations nutrient effects outweighed the other stressors. This was evident for all lake stressor combinations except nutrients and brownification represented by a single case study. The few lake cases, for which the non-nutrient stressor was stronger, included warming affecting cyanobacterial biomass in European lakes, and lithophilous or piscivorous fish abundance in French lakes.

302 The dominance of nutrients over secondary stressors in lakes applies, surprisingly, also to

303 temperature stress, which is often considered to interact in a synergistic way with

304 eutrophication in rivers and lakes (Moss et al., 2011). One mesocosm experiment even

305 demonstrated an antagonistic relationship at high nutrient stress (Richardson et al., 2019).

306 Water temperature may affect lake communities by modifying the food-web structure, e.g. by

307 supporting planktivorous fish (Jeppesen et al., 2010); the two temperature-driven functional

308 fish-trait responses perhaps indicate the emergence of such modification.

309 Brownification is a remarkable exception from this general pattern, but observed here only in

310 a single case study. It strongly superimposes the effects of nutrient stress, in particular by

311 decreasing light transmission in the pelagic zone, which inhibits productivity despite excess

312 nutrient concentrations (opposing interaction) and favours mixotrophic phytoplankton

313 species. Brownification is triggered by global warming and wetter climate, and becomes

314 increasingly relevant in boreal regions, as it originates from dissolved organic carbon in

315 leachates of bogs and permafrost soils mineralising due to increasing temperatures and

316 flushing, and the recovery from acidification (Monteith et al., 2007; Graneli, 2012).

317 Rivers generally showed a more heterogeneous pattern: nutrients clearly affected autotrophs

318 more strongly when paired with hydrological or morphological stress, and heterotrophs when

319 paired with thermal stress. The few river cases in these combinations, for which the non-

320 nutrient stressor was stronger, included fine sediment influx affecting macrophyte and

321 diatoms in UK rivers, and temperature increase affecting sensitive invertebrate taxa in Greek

322 rivers. All other combinations were more ambiguous, with the %AES median being almost

323 zero, indicating stressor effects of roughly equal size.

324 The pattern of nutrient stress outweighing the effects of hydrological or morphological stress

325 for river autotrophs is similar to lakes. Again, "the response variable matters" (Segner et al.,

326 2014) – while river autotrophs have shown to be responsive to hydrological or morphological

327 stress elsewhere (e.g. Baattrup-Pedersen & Riis, 1999; Schneider et al., 2018), their effect size

328 was overruled by the nutrient signal in our study. In one case, however, hydropeaking

329 outweighed the nutrient signal on river autotrophs. The immediate mechanical effect of flush

330 flows is very pervasive, but presumably limited to short river stretches downstream of a

331 hydropower dam.

332 By contrast, river heterotrophs were equally affected by paired stressors when nutrient

333 enrichment was paired with either hydrological, morphological or (to a lesser degree) thermal

334 stress. This indicates that these paired stressors co-act on oxygen contents or habitat

335 availability. In our study, we found small but consistent antagonistic interactions, in particular

336 for channelized rivers, probably due to increased current velocities facilitating the oxygen

337 availability. In the case of toxic stress our conjectures on mechanistic pathways remain

338 speculative. The diversity of compound-specific modes of action across xenobiotics in each

339 mixture renders toxic stress a multi-stressor issue in itself (de Zwart & Posthuma, 2005).

340 Notably, the toxic effects of ambient mixtures were clearly discernible in all respective

341 paired-stressor case studies (n = 17), despite the likely different stressor modes of action

342 (Busch et al., 2016). Given the lack of adequate monitoring of xenobiotics, our findings

343 support that toxic effects in the multiply-stressed freshwaters of Europe are largely

344 underestimated (Malaj et al., 2014).

345

346 **Conclusions**

347 Our study supports the conjecture that eutrophication is still the most relevant stressor

348 affecting many lakes, irrespective of the spatial scale considered. Other stressors are

349 subordinate but may reveal notable effects if interacting with nutrients. These deserve special

350 attention if antagonistic (e.g. lake brownification) and synergistic interactions (e.g. climate

351 warming) can be expected that control the overall nutrient effect on phytoplankton. Relevant

352 stressors and stressor combinations are more variable in rivers and more strongly affected by

353 spatial scales. While river autotrophs are mainly impacted by nutrients, heterotrophs seem to

354 be mainly influenced by oxygen availability that is impaired by a range of stressors (pollution,

355 warming, flow reduction and fine sediment entry) on top of nutrient enrichment. While

356 reduction of nutrient stress is most relevant for lakes, in particular under the conditions of

357 climate warming, rivers require mitigation measures addressing several stressors

358 simultaneously. Options include the establishment of woody riparian buffer strips that address

359 several stressors (eutrophication, hydromorphological degradation) simultaneously.

360

361 **Author contributions**
362 Study conceptualisation: DC, LC, BMS., SB, LB, SJT, DH; data curation: DC, SB; funding acquisition and
363 project administration: DH, LC, SB; data provision and/or formal analysis: AB, AG, AS, BMS, CA, CG-C, CP,
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365 RS, SA, SB, SCS, SJM, SL, SP, SJT, TB, UI, UM; experimental investigations: AB-P, ALS, DG, EB-K, EJ, HF,
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367 reviewed the manuscript and included necessary amendments.

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529

Methods

Case studies

The 45 studies analysed here covered selected European lakes and rivers (including one

estuary) and addressed three spatial scales of investigation: manipulative multi-stressor

experiments in mesocosms and flumes, river basin studies and cross-basin studies (Figure 1,

Supplementary Material Table S1). Several studies contributed to multiple analytical cases,

depending on the available combinations of stressors and responses. The number of cases

totalled 180.

The manipulative experiments were conducted within the framework of the European MARS

project (Hering et al., 2015), involving three lake mesocosm facilities in Denmark, Germany

and United Kingdom, and four artificial flume facilities in Norway, Denmark, Austria and

Portugal. The experiments applied controlled pairs of stressors to study the effects on selected

biological response variables. Overall, 33 analytical cases and 1,448 sample replicates were

considered in our analysis, with a median number of 79 sample replicates per study (range: 20

to 768).

The MARS project also contributed data on 14 river basin studies selected to cover the main

European regions and their representative stressor combinations (Hering et al., 2015). Based

on harmonised analytical protocols (Feld et al., 2016) the multi-stressor effects were analysed

using comprehensive datasets derived from regional monitoring programmes. For this study

we chose the most relevant paired-stressor response combinations from four lake catchments

550 and ten river catchments that together provided 55 analytical cases with an overall number of

551 2,086 samples (median number of samples per basin: 124, range: 19 to 525).

552
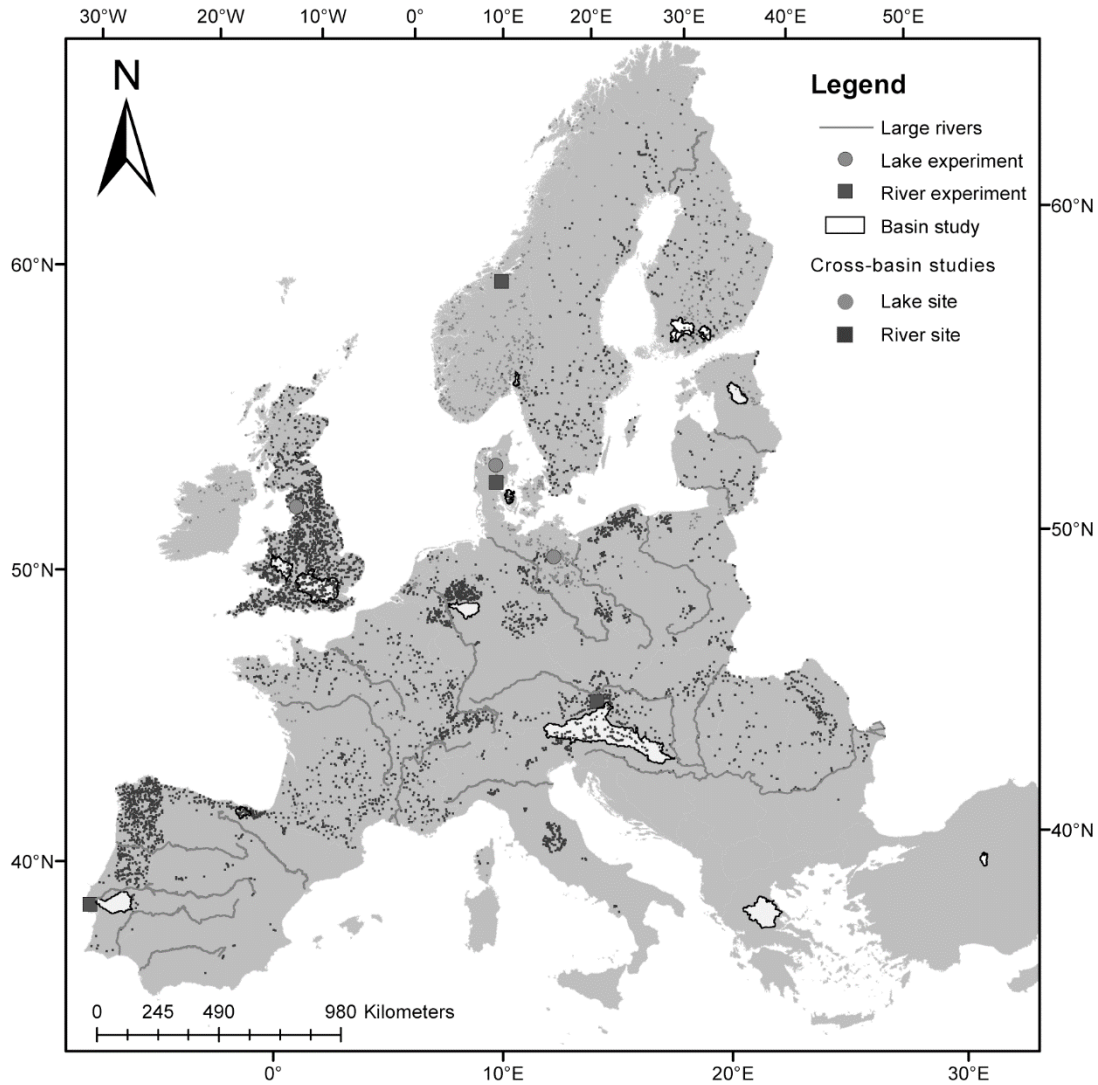
553

554 Figure 1: Location of the seven experimental facilities, 14 basin studies and sampling sites

555 (small dots) for the 22 cross-basin studies of lakes and rivers across Europe (see

556 *Supplementary Material Table S1* for details).

557

558 The 22 cross-basin studies included in this analysis mostly originated from research activities,

559 in which aquatic monitoring data was collated at regional, national or international scale to

560 investigate biological effects of various stressors (e.g. Moe et al., 2008; 2013). The spatial

561 coverage of these studies exceeded a single river basin, and commonly spanned large numbers

562 of lakes and rivers. The number of analytical cases amounted to 92, comprising 14,486

563 samples (median number of samples per study: 374, range: 40 to 3,706).

564

Stressor variables

Within this study we considered a "stressor" as any external factor modified by human intervention, which potentially moves a receptor (*i.e.* response variable) out of its normal operating range (Sabater et al., 2019). The analysed stressor variables belonged to six stress categories (see also Birk, 2019): (1) nutrient stress (145 cases), including experimental addition or field sampling of phosphorus or nitrogen compounds in the water; (2) hydrological stress (63 cases), including experimental manipulation or field measurement of high flow (e.g. high flow pulse duration), low flow (e.g. residual flow), water level change, non-specific flow alteration (e.g. mean summer precipitation as proxy) and hydropeaking; (3) morphological stress (61 cases), including experimental treatment or field survey of river channel, bank and floodplain modification, and river connectivity disruption; (4) thermal stress (57 cases), including experimental heating or field measurement of water temperature (or air temperature as a proxy); (5) toxic stress of mixtures of xenobiotic compounds (18 cases), expressed as the multi-substance Potentially Affected Fraction (de Zwart & Posthuma, 2005), Toxic Units (Liess & von der Ohe, 2005) or runoff potential (von der Ohe & Goedkoop, 2013); and (6) other chemical stress (16 cases), including experimental application of humic substances and field samples of water quality determinants (e.g. dissolved oxygen, chloride, biological oxygen demand).

We always selected the stressor combinations most relevant for the respective broad lake or river type in the particular river basin or region (Lyche-Solheim et al., 2019; see *Supplementary Material Table S1*). These included stressors prevalent in European freshwaters (EEA, 2018) and addressed in previous multi-stressor studies (Nõges et al., 2016). In the experimental studies, stressor intensities were applied emulating "real-life" conditions of the respective water body type. For instance, flumes mimicking nutrient-poor calcareous highland rivers were enriched by ten-fold phosphorus increase towards mesotrophic conditions – a realistic scenario in case of alpine pasture use in the floodplains. Mesocosms mimicking eutrophic shallow lowland lakes were enriched by five-fold phosphorus increase towards hypertrophic conditions – a realistic scenario in intensively used agricultural lowland landscapes. In the field studies, stressor intensities reflected the existing gradient in the particular river basin or region. Thus, the stressor "forcings" in all study cases represent conditions typical for the specific lake or river type, the river basin (featuring certain land uses) and the European region. In several of the investigated basins or cross-basins, more than two stressors were acting; in these we selected those that were assumed to affect the biota

598　most strongly, either based on their intensity or based on previous studies on the relevance of

599　the stressors in the region.

600　Overall, twelve paired-stressor combinations were investigated, including seven combinations

601　that only covered rivers (Table 1). For rivers, the combination of nutrient and morphological

602　stress was the most frequent, amounting to more than one-third of cases. For lakes, the

603　combination of nutrient and thermal stress was the most frequent, amounting to more than

604　half of the cases.

605　Table 1: Number of paired-stressor cases analysed across lakes and rivers

Paired stressors	Lakes	Rivers
Nutrient | Hydrological	14	24
Nutrient | Morphological	0	46
Nutrient | Thermal	34	9
Nutrient | Toxic	1	10
Nutrient | Chemical	6	1
Hydrological | Morphological	0	6
Hydrological | Thermal	3	11
Hydrological | Chemical	0	5
Morphological | Morphological A	0	1
Morphological | Toxic	0	5
Morphological | Chemical	0	2
Toxic | Chemical	0	2

606　A Connectivity disruption and morphological river alteration

607

608　*Response variables*

609　A variety of organism groups was investigated, including phytoplankton (55 cases); benthic

610　flora, *i.e.* macrophytes or phytobenthos (22); benthic invertebrates (66 cases); and fish (37

611　cases). Within the 180 cases, four categories of biological response variables were used: (1)

612　biodiversity (76 cases), including indices reflecting the proportion of a taxonomic group

613　within the assemblage (e.g. percentage of Chlorophyta in the benthic algal assemblage), taxon

614　richness, Ecological Quality Ratios (as derived from ecological classification tools for the

615　European Water Framework Directive) and taxon-sensitivity indices (e.g. saprobic indices,

616　ASPT); (2) biomass/abundance (54 cases), including biomasses or total abundances of

617　phytoplankton or fish, chlorophyll *a* concentrations or cyanobacterial biomass; (3) functional

618　traits (38 cases), including the absolute or relative abundance of functional groups such as

619　habitat preferences, feeding types or life cycles and trait-based quality indices (e.g. SPEAR;

620　von der Ohe & Goedkoop, 2013); and (4) behaviour (12 cases), exclusively including drift

621　rates of invertebrates and stranding rates of juvenile fish. While the response category

622 "biodiversity" covered all organism groups, the category "biomass/abundance" was limited to

623 phytoplankton (except for two cases each with benthic algae and fish), and both "functional

624 traits" and "behaviour" were limited to animals (invertebrates and fish).

625

626 *Statistical analysis*

627 The relationship between the biological response and the paired stressors was investigated for

628 each individual analytical case by GLM based on the general formula

629     $E(Y) = g_{-1}(a·x_1 + b·x_2 + c·x_1·x_2)$,

630 with $E(Y)$ is the expected value of the biological response variable Y, g is the link function

631 that specifies how the response relates to the linear predictors, x_1 is the standardized

632 measurement of Stressor 1, x_2 is the standardized measurement of Stressor 2 and $x_1·x_2$ is the

633 interaction of the standardized measurements of Stressor 1 and Stressor 2. Parameters *a*, *b* and

634 *c* scale the effects of Stressors 1, 2 and their interaction, respectively.

635

636 *Data processing of stressor and response variables*

637 For large-scale data (multi-site biomonitoring data with no, or very short, temporal

638 component), long-term average measures of stress were used. For multi-year data (single or

639 multiple site), each year provided one stress measurement per site. When data was at higher

640 temporal resolution, it was pre-processed to an annual level. Categorical stressor variables

641 (e.g. experimental flow treatment) had only two levels representing stressed *vs.* unstressed

642 conditions.

643 All continuous variables (responses and stressor variables) were standardized by

644 transformation to approach normal distribution. A version of the Box-Cox transformation was

645 used (Box & Cox, 1964), including an offset to ensure strict positivity (all values > 0).

646 Transformed data was inspected for normality by plotting frequency histograms. If the data

647 exhibited skewness because of extreme outliers, these outliers were excluded from the

648 analysis. Following Box-Cox transformation, each transformed variable was centred and

649 scaled, so they had a mean of zero and a variance of one.

650

651 *Choice of regression model*

652 The type of statistical model used to fit the paired-stressor response data depended on two

653 major considerations: (1) The type of analytical case, which determined whether a GLM was

654 sufficient or if a generalised linear mixed model (GLMM) with random effects was needed

655 (see Table 2 for the criteria). GLMMs were used when the data structure included grouping

656 factors, such as experimental block, site or year. In most cases the analyses included random

657 effects in the standard way as random intercept terms. However, if considered appropriate

658 (e.g. due to large data volume) models with both random intercepts and slopes were used. (2)

659 The type of response data, which determined the link function and error distribution of the

660 model (Gaussian errors and an identity link for continuous data, Poisson errors and a

661 logarithmic link for count data). GLMs were fitted with the base R libraries and GLMMs

662 were fitted with the *lme4* and *lmerTest* R packages.

663

664 Table 2: Summary of model choice criteria

Analytical case	Is a mixed model (with random effect) needed?
Mesocosm experiment	Choice depending on experimental design. Grouping factors such as block or measurement period were included as random effect.
Single-site, multi-year (temporal)	No.
Multi-site, multi-year (spatio-temporal)	Yes. Random effects of site and year were included.
Multi-site, single-year (spatial)	No.

665

666 *Testing and correcting for residual autocorrelation*

667 Where necessary, we tested whether model residuals showed strong evidence of spatial or

668 temporal autocorrelation, which can cause the statistical significance of model terms to be

669 exaggerated. This was only required when the analysis used GLMs without random effects,

670 since the random effects in the mixed effects models should account for grouping in space

671 and time. Autocorrelation in space or time was identified with Moran's tests on model

672 residuals and, where substantial autocorrelation was detected, the model was re-fitted

673 including a "trend surface" generated using a smoothing spline or polynomial functions

674 (Dormann et al., 2007). This is a simple and generally effective way of reducing the influence

675 of autocorrelation on the model's stressor effects of interest.

676

677 *Model evaluation*

678 To evaluate our models, residuals were examined for correlation to the fitted values and

679 deviation from the normal distribution (Shapiro-Wilk Test). We excluded 28 models where

680 residuals were correlated with fitted values ($R > 0.35$) and non-normally distributed. Model fit

681 was evaluated as the marginal R_2, *i.e.* the proportion of variance explained by the models

682 fixed effects, ignoring the contribution of any random effects (Nakagawa & Schielzeth,

683 2013). We excluded only models with marginal $R_2 < 0.05$. Model fixed effects (main effects

684 of both stressors and their interactions) were evaluated from the standardized partial

685   regression coefficients and their significance (*t* Test), in the following referred to as

686   standardised effect sizes (SES).

687   Several case studies allowed for analysing different response variables within the same

688   organism group or across different organism groups, using datasets from the same river

689   basin(s). To avoid redundancy in paired-stressor responses we checked that model results

690   differed in marginal R_2 and fixed effects.

691

692   *Importance of the interaction term*

693   The importance of the interaction term was estimated by the change in marginal R_2 upon

694   dropping the interaction term, considered in cases with a significant interaction term,

695   expressed as a percentage change relative to the full model's marginal R_2 (%R_2 change).

696

697   *Interaction classification*

698   The type of interaction was characterised from the SES and only considered in case of a

699   significant interaction term. We applied a simple classification scheme to the full model,

700   referring to both stressors' main effects and their interaction. This was based on the direction

701   of the interaction effect, relative to the directions of the main effects of both stressors.

702   Synergistic interaction was assigned when the SES for both stressors and their interaction all

703   had the same sign (*i.e.* all positive or all negative). Antagonistic interaction was assigned

704   when SES for both stressors had the same sign, but their interaction had the opposite sign.

705   Opposing interaction was assigned when the signs of the SES for both stressors differed, and

706   we distinguished between opposing contributing to either Stressor 1 (*i.e.* Stressor 1 and

707   interaction with same sign) or Stressor 2 (*i.e.* Stressor 2 and interaction with same sign).

708   Reversal interaction (*sensu* Piggott et al., 2015; Jackson et al., 2016) was assigned when the

709   SES' sum for both stressors had a value smaller than and a sign different from the

710   interaction's SES.

711

712   **Synthesis analysis**

713   We identified the frequency of analytical cases with a significant interaction term

714   ("interactive"), or where one ("dominance") or both stressors ("additive") were significant but

715   not the interaction term. The importance (share) of these three types of stressor interrelations

716   was compared between ecosystems (from studies of lakes or rivers) and between scales (from

717   experiments, basin and cross-basin studies). These comparisons were tested using the Chi-

718   squared test. The range of marginal R_2 values from full models were compared between study

719   scales, as well as the *%R₂* change for those cases with significant interaction terms. These

720   comparisons were tested for significant differences using pairwise Mann-Whitney U-tests

721   with Bonferroni correction for multiple comparisons.

722

723   To evaluate the relevance of nutrient enrichment in the paired-stressor context, we selected a

724   subset of cases that included both nutrient stress paired with another stressor. The strength of

725   their effect sizes was compared, distinguishing between effects on autotrophs and

726   heterotrophs across lakes and rivers. In this analysis we simply considered the magnitude of

727   the absolute effect sizes of the two stressors (and their interaction) rather than whether they

728   had positive, negative or opposing effects on the response variable.

729

730   We calculated the relative absolute effect sizes per analytical case (%AES) by setting the sum

731   of the absolute SES of Stressor 1, Stressor 2 and their interaction to 100 % (irrespective of

732   their statistical significance in the regression analysis), and expressing the individual SES as a

733   percentage. The difference between %AES of the nutrient stressor and %AES of the other

734   stressor revealed which stressor had the stronger effect on the biological response, with

735   positive values indicating stronger effects of nutrient enrichment, and negative values

736   indicating stronger effects of the other stressors. In the case of an opposing interaction, the

737   %AES of the interaction term was added to the stressor's %AES with which the interaction

738   SES shared the sign (e.g. the %AES of a positive interaction SES was added to the %AES of

739   the nutrient stressor if its SES was also positive). In case of a synergistic or antagonistic

740   interaction, we considered the interaction effect to be equally relevant for both stressors with

741   no implications for the difference in the individual stressor effects.