Science of the Total Environment 573 (2016) 1079–1088

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Untangling the effects of multiple human stressors and their impacts on fish assemblages in European running waters

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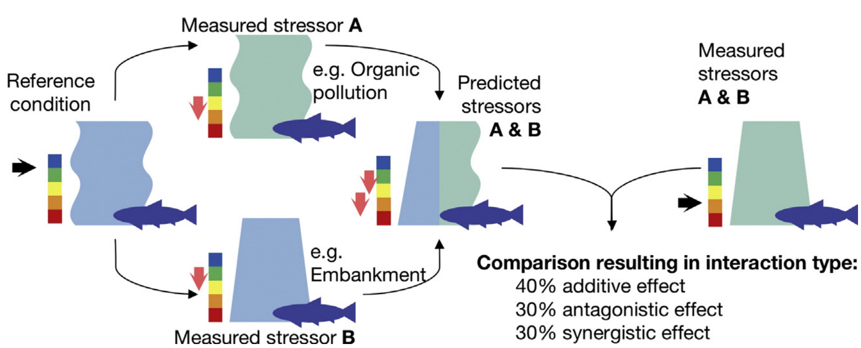
HIGHLIGHTS

- Impact of multiple stressors on fish ecological status was investigated in European rivers.
- Across 3105 sampling sites, we found 15 different stressor categories.
- Of all sites, 28% were unimpacted.
- Impaired sites were affected by single stressors (30%) or in combination (42%).
- Interactions of stressors were additive (40%), synergistic (30%) and antagonistic (30%).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 19 August 2016
Accepted 19 August 2016
Available online xxxx

Editor: Dr. D. Barcelo

Keywords:
Riverine ecosystems
Fish metrics
Multiple human stressors
Ecological status
Water Framework Directive

ABSTRACT

This work addresses human stressors and their impacts on fish assemblages at pan-European scale by analysing single and multiple stressors and their interactions. Based on an extensive dataset with 3105 fish sampling sites, patterns of stressors, their combination and nature of interactions, i.e. synergistic, antagonistic and additive were investigated.

Geographical distribution and patterns of seven human stressor variables, belonging to four stressor groups (hydrological-, morphological-, water quality- and connectivity stressors), were examined, considering both single and multiple stressor combinations. To quantify the stressors' ecological impact, a set of 22 fish metrics for various fish assemblage types (headwaters, medium gradient rivers, lowland rivers and Mediterranean streams) was analysed by comparing their observed and expected response to different stressors, both acting individually and in combination. Overall, investigated fish sampling sites are affected by 15 different stressor combinations, including 4 stressors acting individually and 11 combinations of two or more stressors; up to 4 stressor groups per fish sampling site occur. Stressor-response analysis shows divergent results among different stressor categories, even though a general trend of decreasing ecological integrity with increasing stressor quantity can be observed. Fish metrics based on density of species 'intolerant to water quality degradation' and 'intolerant to oxygen depletion" responded best to single and multiple stressors and their interactions. Interactions of stressors were additive (40%), synergistic (30%) or antagonistic (30%), emphasizing the importance to consider interactions in multi-stressor analyses. While antagonistic effects are only observed in headwaters and medium-gradient rivers, synergistic effects increase from headwaters over medium gradient rivers and Mediterranean streams

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http://dx.doi.org/10.1016/j.scitotenv.2016.08.143

to large lowland rivers. The knowledge gained in this work provides a basis for advanced investigations in European river basins and helps prioritizing further restoration and management actions.

1. Introduction

Across Europe, human stressors impact aquatic ecosystems and their inhabiting communities, especially in rivers and streams. In the past, strong single stressors such as organic pollution or flood protection were prevalent. Today, a complex mixture composed of e.g. hydrological-, morphological-, connectivity- and chemical stressors impacts the functioning of aquatic ecosystems and related ecosystem services, resulting from urban and agricultural land use, hydropower generation, climate change and other factors (Schinegger et al., 2012; Hering et al., 2014). In Europe, EU and member state legislation have been established to manage and protect running waters, first and foremost under the EU Water Framework Directive (WFD, European Commission, 2000), which demands the “good ecological status” of all water bodies (i.e. the related management units). This is addressed in 6-year planning- and management phases and by use of multiple Biological Quality Elements (BQEs) for status assessment. Europe's first River Basin Management Plans (RBMPs) from 2009 indicate that 56% of European rivers fail to achieve good ecological status, as they are affected by a complex set of stressors (European Environment Agency, 2012).

Fish are especially sensitive BQEs for riverine ecosystems, as they react significantly to almost all kinds of human stressors, including eutrophication, acidification, chemical pollution, flow regulation, physical habitat alteration and fragmentation (Ormerod, 2003). Fish also can be classified by a series of metrics based on assemblage structure and functions to reflect the ecological health of the assemblage (Pont et al., 2006). Further, they tend to be better indicators of stressors acting at wider spatial and temporal scales, such as hydromorphological disturbances and connectivity loss (Sindilariu et al., 2006; Birk et al., 2012; Marzin et al., 2012).

A first pan-European study investigated the response of fish assemblages to single and multiple human stressors across broad river types (Schinegger et al., 2013; Trautwein et al., 2013) with 17 fish metrics describing biological and ecological functional traits. Especially density and biomass metrics were found useful for impact assessment in distinct river types. Moreover, fish responded to multiple stressors in all river types in this study, indicating that it is no longer sufficient to explain the relationships between human stressors and BQEs assuming simple dose-response reactions.

Stressors often interact, implying that an addition of the individual stressor effects may underestimate the joint effect (Brown et al., 2013). Additive effects on biota equal the sum of stressor's individual effects, synergistic interactions are present when multiple stressor effects exceed those of additive ones and antagonistic effects are lower than the sum of individual stressors (Crain et al., 2008; Folt et al., 1999). This calls for a wider perspective on stressors and their interactions, to better understand the mechanistic principles behind stressor-response relationships and to provide an overview on patterns and trends. However, studies addressing interactive effects of multiple stressors have been essentially based on mesocosm experiments (e.g. Townsend et al., 2008; Matthaei et al., 2010; Piggott et al., 2012; Piggott et al., 2015a), with limited transferability to the reality of field conditions due to the restricted spatial and temporal scales of most studies (Piggott et al., 2015a). Furthermore, the few studies using empirical data and taking stressor interactions and related response of fish and macroinvertebrates into account typically were conducted at a regional scale or in a single river basin (Wenger et al., 2011; Roberts et al., 2013; Walters et al., 2013; Lange et al., 2014). So far, the complex nature of multiple stressors with special emphasis on their interactive effects on biota has never been addressed at a wider continental scale.

The project MARS (Managing Aquatic ecosystems and water Resources under multiple Stress) was funded by the European Union to support European water policies (e.g. the WFD and others) and was initiated to overcome knowledge gaps of multiple stressor effects on biota in rivers, lakes, transitional waters and groundwater. The related MARS framework aims to provide required knowledge, understanding and tools (e.g. analytical ‘cookbooks’) on how stressors interfere and impact upon ecological status and ecosystem services (Hering et al., 2014; Feld et al., 2016). Within MARS, Nõges et al. (2016) reviewed 219 papers on ecological evidence of multiple stressor impacts, finding that despite a huge conceptual knowledge base in aquatic ecology, few studies actually provide quantitative evidence of multiple stressor effects on biota, especially over large spatial extents. They also note a lack of data and standardised investigation methods. Thus, we here contribute to the MARS European-wide analyses by addressing multiple stressor effects on fish assemblages at the continental scale. We hypothesise that i) river-type-specific stressor patterns (number and combination of stressors) can be observed across Europe, ii) that river-type specific fish metrics can be identified to show a response to single and multiple stressors and iii) that stressor interactions (additive, antagonistic or synergistic effects) within these river types are reflected differently by these metrics.

2. Methods

2.1. Fish sampling data and metrics

In total, 3105 fish sampling sites located in 14 European countries were available for our analyses, extracted from an extensive European database (EFI+ Consortium, 2009; Schinegger et al., 2016). Sites were sampled by electrofishing (wading) following European standards (CEN, 2003) and were associated with four fish assemblage types (FATs, i.e. headwater streams (HWS), medium gradient rivers (MGR), lowland rivers (LLR) and Mediterranean streams (MES)) based on fish community and predicted by environmental characteristics (Trautwein et al., 2013). The entire dataset includes 125 fish species recorded, whereof 15 are exotic species (12%). In this work, we target structural and functional groups in fish communities rather than the species level. Thus, no special focus was put on exotic species, as both native as well as exotic species can take up the same function in the available form of the data set.

Overall, 22 fish metrics associated with five structural and functional attributes of fish assemblages (biodiversity, habitat, reproduction, trophic level and water quality sensitivity) were selected (Table 1), based on the findings of Schinegger et al. (2013) and EFI+ Consortium (2008) in terms of response to single and multiple stressors.

2.2. Stressor data/stressor combinations

For fish sampling sites, 13 selected and pre-classified human stressor variables (according to Schinegger et al., 2012 and Schinegger et al., 2013) were available, on a scale ranging from 1 (indicating no stress) to 5 (indicating high stress). Due to lack of variation (rare occurrence of stressor, low variation of gradient) in preliminary stressor analyses, six of the selected stressor variables were not considered in further steps. Remaining stressors were used to differentiate unimpacted sites from single- or multiple impacted ones (Table 2).

Table 1
Description and coding of fish metrics available for analyses. Main trait groups are biodiversity (biodiv), habitat sensitivity (hab), trophic level (troph), water quality sensitivity (wq) and reproduction (repro). Variants are number of species (nsp), density (dens; Ind./ha) or biomass (biom; kg/ha). Direction of response can be increasing (incr) or decreasing (decr) under stress. Asterisk in row "coding" indicates metrics that were log-transformed to resemble normal distribution.

Metric group	Trait	Description	Unit	Coding(s)	Direction	Response according to literature
Nsp_all	biodiv	Total number of species	nsp	nsp_all	incr/decr	Generally declines, may show stress-specific increase in species poor river types
HINTOL_150	hab	Species with habitat degradation intolerance of juveniles <150 mm	n/ha	dens_HINTOL150	decr	Reaction of juvenile individuals (<150 mm) of species intolerant to habitat degradation
HTOL_HTOL	hab	Species with habitat degradation tolerance	kg/ha	perc_biom_HTOL_HTOL*	incr	Reaction of species having a large flexibility in terms of habitat degradation
HabSp_RHPAR	hab	Species with preference to spawn in running waters	n/ha nsp	dens_HabSp_RHPAR* nsp_HabSP_RHPAR	decr	Degradation of lotic spawning habitats
Repro_LITH	repro	Species spawning exclusively on gravel, rocks, stones, cobbles or pebbles	n/ha	dens_Repro_LITH	decr	Degradation of gravel spawning habitats, sensitive to siltation
Atroph_INSV	troph	Insectivorous species	nsp	nsp_Atroph_INSV	decr	Surrogate for evaluating the degree that the invertebrate assemblage is degraded by human pressures
Atroph_PISC	troph	Piscivorous species	nsp	perc_nsp_Atroph_PISC*	decr	Top predator, surrogate for prey fishes
Atroph_OMNI	troph	Food of adult consists of >25% plant material and >25% animal material. Generalists	kg/ha nsp	perc_biom_Atroph_OMNI* perc_nsp_Atroph_OMNI*	inc	Degree that the food base is altered to favour species that can digest both plant and animal foods
WQgen_INTOL	wq	Species which are in general intolerant to usual water quality parameters	kg/ha n/ha n/ha nsp	biom_WQgen_INTOL* dens_WQgen_INTOL* perc_dens_WQgen_INTOL perc_nsp_WQgen_INTOL	decr	Reaction of species with narrow flexibility in terms of water quality degradation
WQgen_TOL	wq	Species which are in general tolerant to usual water quality parameters	kg/ha nsp n/ha	biom_WQgen_TOL* nsp_WQgen_TOL perc_dens_WQgen_TOL*	incr	Reaction of species having a wide flexibility in terms of water quality degradation
WQO2_O2INTOL	wq	Species which are tolerant to low oxygen concentration. >6 mg/l in water	kg/ha n/ha nsp n/ha	biom_WQO2_O2INTOL* dens_WQO2_O2INTOL* perc_nsp_WQO2_O2INTOL perc_dens_O2INTOL	decr	Reaction of species with narrow flexibility in terms of oxygen concentration problems
WQO2_O2TOL	wq	Species which are tolerant to low oxygen concentration: 3 mg/l or less	nsp	perc_nsp_WQO2_O2TOL*	incr	Reaction of species having a wide flexibility in terms of oxygen concentration problems

Sites without or only with slight stressors were considered as "unimpacted" (class 1 and 2), while others were categorized as "impacted" (class 3 to 5). The resulting stressor categories (either one or more stressors present at one site, Table 2) were grouped according to four stressor groups, i.e. connectivity (C), hydrology (H), morphology (M) and water quality (W). To account for natural variability in fish metric measurements, we considered the FATs presented in the data set (Trautwein et al., 2013) for identification of frequently reoccurring stressor categories and the subsequent analyses.

Fish metric responses were only compared to stressor categories occurring frequently in the four FATs ($n \geq 20$).

2.3. Standardization of metrics

In order to standardize fish metric values to a comparable numerical range and to set a common sign of response to stressors, we used a standardization procedure based on the ecological quality ratio (EQR). This standardization procedure is commonly used with ecological quality indices to express results as a ratio of the monitored to reference, i.e. unimpacted conditions (Van de Bund & Solimini, 2007). All metrics' values were therefore transformed into EQRs by an equation expressing site measurements relative to the mean metric measurement of unimpacted sites within the same FAT. For a more reliable result, outliers were first eliminated from unimpacted sites.

The EQR's calculation was differentiated according to the expected direction of metrics response to increasing human stressors (Trautwein et al., 2013). Some metrics are considered to decrease in value with increasing stressor (e.g. less fish in a guild resulting in reduced abundance and biomass, reduction of number of species) while others are expected to increase (e.g. metrics regarding tolerant species). The EQR was calculated for each site as follows: Formula A is applied for metrics expected to increase under an increasing intensity of stressors. For metrics expected to decrease with an increasing intensity of stressors, the EQR is calculated as indicated in Formula B. For metrics expressed as percentages that increase with an increasing intensity of stressors, the complementary percentage is utilized achieving a proxy

Table 2
Stressor variables considered in subsequent analyses. Stressor groups are hydrological stress (H), morphological stress (M), connectivity stress (C) and water quality stress (W).

Stressor variable	Abbreviation	Stressor group	Explanation and description of intensity classes
Impoundment	H_imp	H	Natural flow velocity reduction on site due to impoundment; 1 = no (no impoundment), 3 = weak, 5 = strong
Water abstraction	H_waterabstr	H	Site affected by water flow alteration/minimum flow; 1 = no (no water abstraction), 3 = weak to medium (less than half of the mean annual flow), 5 = strong (more than half of mean annual flow)
Instream habitat alteration	M_instrhab	M	Alteration of instream habitat conditions; 1 = no, 3 = intermediate, 5 = high
Embankment	M_embank	M	Artificial embankment; 1 = no (natural shoreline), 2 = slight (local presence of artificial material for embankment), 3 = intermediate (continuous embankment but permeable), 5 = high (continuous, no permeability)
Barriers segment downstream	C_B_do	C	Barriers on segment level downstream; 1 = no, 4 = partial, 4 = yes
Eutrophication	W_eutroph	W	Artificial eutrophication; 1 = no, 3 = low, 4 = intermediate (occurrence of green algae), 5 = extreme (oxygen depletion)
Organic pollution	W_opoll	W	Is organic pollution observed; 1 = no, 3 = weak, 5 = strong

metric, which is expected to decrease with an increasing number of stressors (Formula C).

Formula A (for increasing metrics) $EQR_{site} = \frac{mean(Metric_{ref\ sites})+1}{Metric_{site}+1}$.

Formula B (for decreasing metrics) $EQR_{site} = \frac{Metric_{sites}+1}{mean(Metric_{ref\ sites})+1}$.

Formula C (for percent increasing metrics) $EQR_{site} = \frac{101-Metric_{site}}{101-mean(Metric_{ref\ sites})}$.

For the calculation of each site's EQR, only unimpacted sites of the same FAT were used as basis for the reference condition, i.e. the mean metric value of the unimpacted sites (within the same FAT). This step compensates for environmental effects that might influence the sampling site. Out of the set of metric variables, 12 were log-transformed before EQR calculation, to make naturally skewed distributions better comparable and interpretable (Table 1). Commonly, EQRs range from 0 to 1, however, in a slight deviation from the WFD scaling method, e.g. for the 'intercalibration exercise' (Willby et al., 2014), this study uses the mean metric value of the unimpacted sites instead of a maximum value, hence providing a more robust index but also allowing exceedance of the 0 to 1 limits. The resulting indices still express trait metrics, since the EQR standardization is essentially a scalar transformation (i.e. EQRs still have a linear relationship with the original metrics).

2.4. Prediction of joint effects

The dataset consists of sites that are affected only by single stressors and sites that are affected by multiple stressors. First we calculated EQRs for all sites with single stressors. Then, the expected joint effect of multiple stressors occurring at one site was predicted by multiplying the mean EQRs of single stressors occurring at this site (Coors & De Meester, 2008). For interpretation, all stressor categories with metric responses differentiating significantly from the unimpacted sites were considered. Significant negative deviation from the reference was assessed with a one-sample Wilcoxon-Man-Whitney (U-) test. Furthermore, stressor combinations, i.e. stressor categories of multiple stressors, that did not significantly differ from the unimpacted sites but did significantly differ from either their predicted EQR value and/or any of the single stressors they consisted of were included. The mean EQR of a stressor category can be understood as the mean probability of sites affected by this category to achieve the reference metric value. The effect of multiple stressors therefore can be predicted as the joint probability of the involved single stressors, i.e. the product of their EQRs. To determine stressor interaction, the predicted EQR of a stressor combination was tested against actual observed values with the corresponding stressor combination in a one sample Wilcoxon Mann-Whitney test. If the true mean did significantly deviate from the expectation, the stressor combination was considered to be interactive, whereas it was considered additive if expectation and observation aligned. Synergistic interaction between stressors is indicated by a significantly stronger observed effect of the combined stressors than that predicted from the single stressors, whereas an antagonistic interaction is indicated by a significantly weaker effect of the combined stressors than predicted (Coors & De Meester, 2008). Fig. 1 displays the methodological framework, which aims (1) to test a significant difference between reference sites (REF), sites affected by single stress and by multiple stress as well as (2) to identify the "nature of multiple stress", i.e. additive (ADD), synergistic (SYN) or antagonistic (ANT).

All statistical analyses were performed in R version 3.2.5 (R Development Core Team, 2016).

3. Results

3.1. Distribution of stressor categories

In terms of frequent stressor categories (impoundments, water abstraction, eutrophication, organic pollution, barriers downstream, embankment and instream morphological alteration) we found 15 variants, i.e. single stressors or combinations with a frequency of 20 or more sites (Table 3).

Only 27% of all sampling sites were considered as unimpacted (no/slight stress), but 30% of sites were affected by single stress (C, H, M or W). Double stress (CH, CM, CW, HM, HW and MW) occurred at 23% of sites and triple (CHM, CHW, CMW, HMW) at 15%. When considering all stressors in combination, 5% of sites were affected by this category (quadruple stress - CHMW; Table 3).

Within FAT HWS, water quality stress (W) was the most frequent, followed by hydrological stress (H) and connectivity stress (C), along with the combination of hydrological and water quality stress (HW). Other frequent combinations were connectivity and water quality stress coupled (CW) or together with hydrological stress (CHW).

Water quality stress was also most frequent in MGR, along with its combination with morphological stress (MW), as well as with morphological and connectivity stress altogether (CMW). In this FAT all other possible single, double, triple and quadruple stressor categories occurred frequently, with a large amount of sites (90) affected by the four-way combination (CHMW).

For LLR, hydrological stress co-occurring with water quality (HW) as well as with water quality- and morphological stress (MW) occurred frequently, followed by each of these individually and then by their four-way combination with connectivity and hydrological stress (CHMW). In summary, issues with water quality were prevalent in all multiple stressor categories in this FAT.

The proportion of sites with single water quality stress in MES (104) was almost as big as the one of unimpacted sites (109) and water quality stress again was involved in all multiple stressor categories, especially in combination with either hydrological stress (HW) alone or with hydrological and connectivity stress together (CHW). No other stressor category occurred with any frequency apart for hydrological stress individually.

Fig. 2 shows the spatial distribution of stressor categories across Europe.

3.2. Response of fish metrics to single and multiple stressors

All metrics selected for analyses (Table 1) showed a significant response to stressors, whether to single or multiple ones (Tables 4–7). In HWS, 21 metrics revealed a significant deterioration from reference condition (18 responding to single stressors and 21 to multiple ones). For MGR, all metrics showed a significant response (19 metrics to single stressors, all 22 to multiple ones). In LLR, 18 metrics showed a significant response (10 to single stressors 17 to multiple ones) and in MES, 16 metrics were responsive (15 metrics to single stressors and 12 to multiple ones). The results for individual metrics and FATs are displayed in Figs. A1–A4 in the Annex.

3.3. Additive, synergistic and antagonistic effects

In terms of interactive effects of stressors, Tables 4–7 and Figs. A1–A4 show the metric behaviour for single and multiple stress categories, i.e. whether the stressors cause significant deterioration from unimpacted sites and whether they do interact in combination. In HWS, most interactions were of additive or antagonistic nature. Additive effects were observed for water quality stress in combination with connectivity stress (CW) as well as with hydrological stress (HW), especially for metrics related to intolerance to water quality degradation, oxygen depletion and habitat alteration (Table 4). Triple stress (CHW) here showed antagonistic responses especially for density and biomass metrics and additive response especially for biomass metrics. For the combination of connectivity and hydrological stressors (CH), only antagonistic effects were observed. Synergistic effects of multiple stressors only were detected for 3 metrics, two related to water quality degradation tolerance/intolerance (biom_WQgenTOL, perc_nsp_WQ_gen_INTOL) and

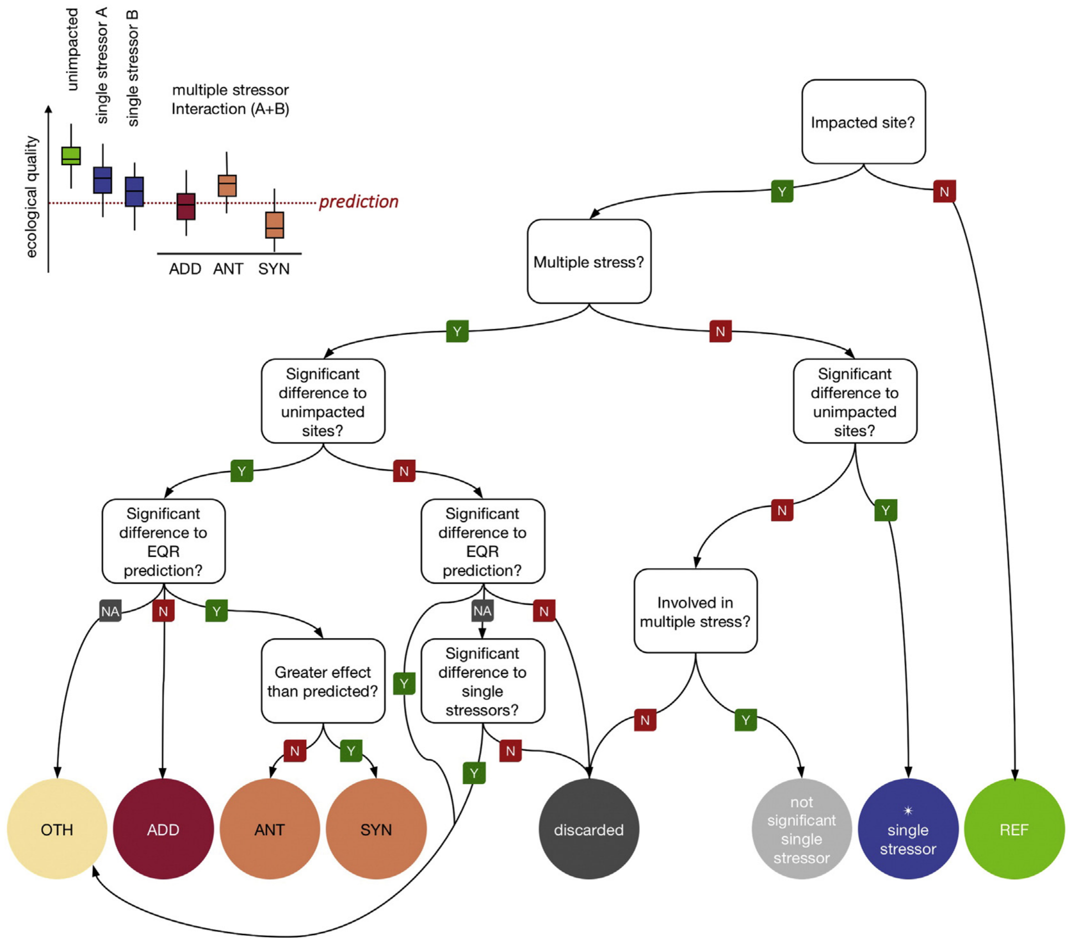

Fig. 1. Methodological framework and theoretical example (top left) indicating analytical steps to test the significant difference between reference sites (REF, green/boxplot), sites affected by single stressors (blue circle/boxplot) and by multiple stressors - including interaction effects as additive (ADD, red circle/boxplot), synergistic or antagonistic (SYN and ANT, orange circles/boxplots). Grey circle (left, "OTH") identifies sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

one related to the total number of species (nsp_all) under double or triple stress.

The results in MGR showed a quite equal distribution among all three interaction types, all 22 metrics showed significant responses to multiple stress effects. Additive and antagonistic interactions were more often detected under double stress conditions as with triple or quadruple stress. Morphological stress combined with water quality stress mainly showed additive responses (MW), whereas the triple combination including connectivity stress (CMW) showed mainly antagonistic responses. By contrast, the combined impact of four-fold stressor (i.e. CHMW) was mainly synergistic.

Table 3
Number of sites related to no/slight stress and various stressor categories respectively and across FATs: HWS = headwater streams; MGR = medium gradient rivers; LLR = lowland rivers; MES = Mediterranean streams.

Stressor category		Fish assemblage types				
		HWS	MGR	LLR	MES	Total
No/slight stress		230	380	137	109	856
Single stress	C	51	56	11	17	135
	H	89	65	13	35	202
	M	9	71	53	10	143
	W	120	185	47	104	456
Double stress	CH	21	33	7	13	74
	CM	1	23	4	4	32
	CW	21	82	11	16	130
	HM	12	33	5	16	66
	HW	51	64	22	48	185
	MW	13	136	59	19	227
Triple stress	CHM	4	28	2	4	38
	CHW	31	70	14	28	143
	CMW	10	121	15	2	148
	HMW	13	64	28	18	123
Quadruple stress	CHMW	7	90	41	9	147

For LLR, synergistic effects occurred more frequently than additive effects, whereas antagonistic effects were not detected. Significant interactive effects only could be investigated for morphological stress coupled with water quality stress, where synergistic effects dominated (about 59% of metrics indicated a response), followed by additive effects (41%). One metric (perc_dens_WQgen_INTOL) showed significant negative effects for both single stressors and the combination, i.e. for combined morphological and water quality stressors, which means the situation in this stressor combination is still worse than the expectation based on the composing single stressors (Table 6).

For MES, again only additive and synergistic significant effects of double stress were detected, when hydrological and water quality stressors co-occurred (HW; Table 7). To this stressor combination, 58% of metrics responded in an additive way and the remaining 42% in a synergistic way. These were six water quality metrics, one habitat metric and one biodiversity metric. For MES, no significant antagonistic effects were observed.

4. Discussion

This work constitutes the first assessment of multiple human stressors and their interacting effects and related responses of fish metrics across broad fish assemblage types in Europe based on detailed, semi-quantitative data. Previous studies often were investigating fish-

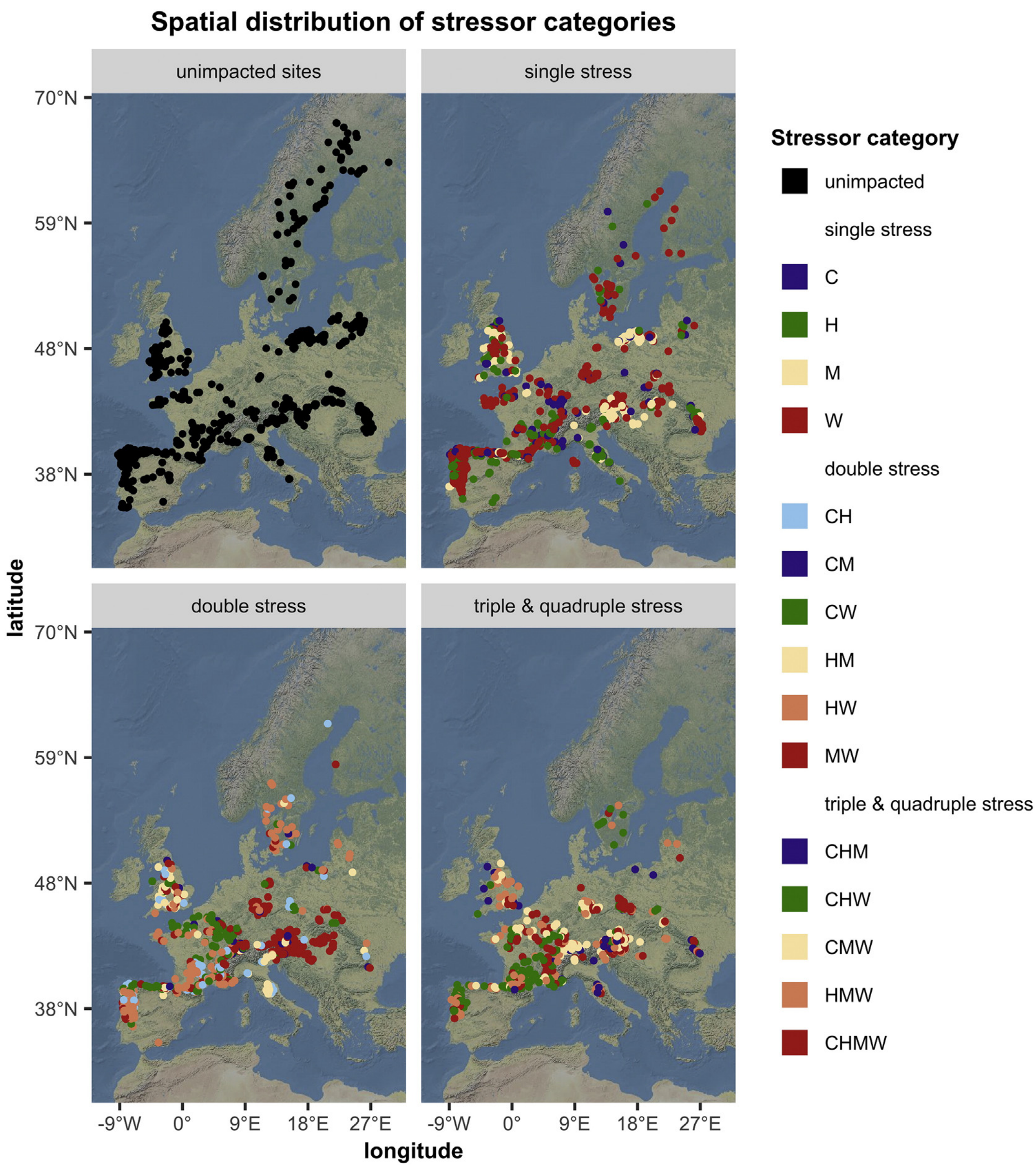

Fig. 2. Spatial distribution of unimpacted sites (top left) and stressor categories separated into single stressors (top right), double stressors (bottom left) and triple & quadruple stressors (bottom right) across Europe.

ecological responses to generalized pressure indices (e.g. Pont et al., 2006; Logez & Pont, 2011) or more generic stressor categories such as water quality vs. hydromorphological alterations (Schinegger et al., 2013; Trautwein et al., 2013), thus often facing limitations in terms of traceability of multi-stressor effects on fish. In contrast, our work is based on 15 different stressor categories, i.e. 4 single stressors and 11 combinations, where fish metric responses to both single and multiple stressors can be investigated more thoroughly.

4.1. Distribution and patterns of single and multiple stressors in water bodies and biological implications

Despite the fact that modern management of water bodies should no longer target one stressor alone, the historically often critical aspect of water quality due to organic pollution as well as eutrophication is still very prevalent and was found in 8 out of 15 multiple stressor categories across all FATs frequently. This fact could especially be related to challenges with diffuse sources of eutrophication, which causes widespread problems of nutrient enrichment in inland and coastal waters across Europe (EEA, 2012). Eutrophication often comes along with oxygen depletion, leading to hypoxia (Teichert et al., 2016) and thus catastrophic events in fish assemblages, as intolerance to low oxygen concentration is a widespread species attribute in small coldwater European streams (Wootton, 1991). As agriculture has substantial impacts on aquatic ecosystems, ranging from streams and rivers to the estuarine and marine environment, nutrient reduction measures, e.g. when implemented in the EU Common Agricultural Policy (CAP) thus should play a key role in addressing diffuse pollution in the next years (Stoate et al., 2009).

Human stressors have affected fish species unevenly: guilds of specialized species that are highly adapted to specifically riverine conditions have declined far more than generalist species (Aarts et al., 2004). It is well known from literature, that fish communities respond to hydrological and morphological alterations (Schmutz et al., 2015), as the diversity of habitat conditions supports various crucial biological functions for marine, freshwater and estuarine resident species (Teichert et al., 2016). Hydromorphological stressors and altered habitats have been identified as a “significant pressure” in a WFD assessment for 48.2 and 42.7% of the rivers of Europe, respectively (Fehér et

Table 4
Responsiveness of fish metrics to single and multiple stressors in headwater streams (HWS). Asterisk in "single stressors" indicates significant response; significant interactive effects in "multiple stressors" are additive (ADD), synergistic (SYN) or antagonistic (ANT).

Metric	Single stressors			Multiple stressors			
	C	H	W	CH	CW	HW	CHW
biom_WQgen_INTOL		*	*			ADD	ANT
biom_WQgen_TOL	*		*			SYN	ANT
biom_WQO2_O2INTOL		*	*			ADD	
dens_HabSp_RHPAR	*	*	*	ANT	ANT	ANT	ANT
dens_WQgen_INTOL	*	*	*	ANT	ANT	ADD	ANT
dens_WQO2_O2INTOL	*	*	*	ANT	ANT	ADD	ANT
nsp_WQgen_TOL	*		*		ANT	ADD	ANT
perc_biom_HTOL_HTOL	*		*			ADD	ANT
perc_dens_WQgen_INTOL	*	*	*	ANT	ADD	ADD	ADD
perc_dens_WQgen_TOL	*		*		ANT	ADD	ANT
perc_nsp_Atroph_PISC							
perc_nsp_WQgen_INTOL	*		*	ANT	ADD	SYN	ADD
perc_nsp_WQO2_O2INTOL			*		ADD	ADD	ADD
perc_nsp_WQO2_O2TOL	*		*		ANT	ADD	ANT
nsp_all	*	*	*	ANT	ADD	SYN	SYN
perc_biom_Atroph_OMNI						ADD	ADD
perc_nsp_Atroph_OMNI			*			ADD	ADD
nsp_Atroph_INSV					ADD		ADD
dens_MetHINTOL150	*	*	*			ANT	ANT
perc_dens_MetO2INTOL			*		ANT	ADD	ADD
dens_MetRHPAR			*		ANT	ADD	
dens_MetLITH						ANT	

al., 2012). In terms of flow alterations, European river ecosystems are under significant threat with about two-thirds at medium or high risk of change (Laizé et al., 2014). In our study, hydrological stress combined with morphological stress was frequently occurring in all 4 FATs and, moreover, density of species intolerant to degradation of lotic spawning habitat and density of juveniles of (<150 mm) of species intolerant to habitat degradation showed significant responses to single hydrological or morphological stressors, as well as to their combinations. Interactions between hydrological and morphological stressors were often of additive nature. As fish respond in a consistent way to hydromorphological restoration measures by an increase of rheophilic and a decrease of eurytopic fish (Schmutz et al., 2015), a combination of morphological restoration measures combined with the implementation of environmental flows and the mitigation of hydrological stressors due to hydropower production (e.g. hydropeaking or impoundments) should be considered in a holistic way in the future.

Fish feature highly mobile consumers at different levels of the aquatic food chain, making them very susceptible to multi-stressor effects (Nõges et al., 2016). Understanding the feeding ecology of fishes is critical to understanding the success of individuals and populations as it influences survival, growth, and reproductive potential (Wootton, 1998). As a result of their feeding- and reproductive requirements, fish will move within and between habitats to improve their opportunities (Cooke et al., 2016). However, the influence of connectivity disruption was detected in a wide range of FATs across Europe in our study, also in combination with hydromorphological- and water quality stress. Although similar in concept to habitat fragmentation in terrestrial ecosystems, disconnections in rivers are particularly damaging because the structure of stream networks restricts movement pathways, making it more difficult or even impossible to avoid barriers (Fagan, 2002; Fullerton et al., 2011). In Europe, most rivers are heavily fragmented, with major consequences for sediment transport, nutrient delivery, and the dispersal and migration of organisms including fish (Nilsson et al., 2005). As a potential solution, optimization models to prioritize the removal of fish passage barriers and to maximize habitat availability for resident fish, coupled with other restoration measures could be applied (e.g. Schmutz & Trautwein, 2009; O'Hanley et al., 2013).

4.2. Untangling of interactive stressor effects

Along with increasing intensity and number of stressors placed on riverine ecosystems, both scientists and water resource managers need greater understanding of relationships between multiple human stressors and related responses of the aquatic community, to understand the consequences for future management of aquatic ecosystems and their services (Allan et al., 2013). In this work we found that, among the 73% of impaired sample sites across Europe, >64% were affected by two or more stressors. This finding highlights the importance to consider effects resulting from the interplay of multiple stressors when seeking for responses of biological indicators.

To improve the management of aquatic systems subjected to multiple stressors, it is necessary to understand the effect of stressor interactions on fish metrics (Nõges et al., 2016). In our study, all fish metrics

Table 5
Responsiveness of fish metrics to single and multiple stressors in medium gradient rivers (MGR). Asterisk in "single stressors" indicates significant response; significant interactive effect in "multiple stressors" is additive (ADD), synergistic (SYN) or antagonistic (ANT).

Metric	Single stressors				Multiple stressors										
	C	H	M	W	CH	CM	CW	HM	HW	MW	CHM	CHW	CMW	HMW	CHMW
biom_WQgen_INTOL							ADD			ADD					
biom_WQgen_TOL				*			SYN		SYN	SYN		SYN	SYN	SYN	SYN
biom_WQO2_O2INTOL							ADD			ADD		ADD			
dens_HabSp_RHPAR			*					ADD		ANT	ADD			ANT	ANT
dens_WQgen_INTOL			*	*	ADD			ADD	ANT	ANT	ADD	ANT		ANT	ADD
dens_WQO2_O2INTOL			*	*		ANT		ADD	ANT	ANT	ADD		ANT	ANT	ANT
nsp_WQgen_TOL				*			SYN		ADD	ADD		SYN	SYN	SYN	SYN
perc_biom_HTOL_HTOL				*			SYN		ADD	ADD		SYN	ADD	SYN	SYN
perc_dens_WQgen_INTOL				*	SYN		SYN		SYN			SYN	ADD	SYN	SYN
perc_dens_WQgen_TOL				*			ADD		ADD	ANT		ADD	ANT	ADD	SYN
perc_nsp_Atroph_PISC	*					SYN	ADD						SYN		
perc_nsp_WQgen_INTOL				*	SYN				SYN		SYN	SYN		SYN	SYN
perc_nsp_WQO2_O2INTOL				*	SYN		ADD		ADD	ADD		SYN		SYN	SYN
perc_nsp_WQO2_O2TOL				*	ADD		ANT	ADD	ANT	ANT		ANT		ANT	ANT
nsp_all				*			SYN		SYN		SYN	SYN		SYN	SYN
perc_biom_Atroph_OMNI				*	ADD		ADD		ANT	ANT		ANT	ANT	ANT	ADD
perc_nsp_Atroph_OMNI				*			SYN		SYN	ANT		SYN	ANT	SYN	SYN
nsp_Atroph_INSV									ADD	ADD					
dens_MetHINTOL150		*	*	*	ADD		ANT	ADD	ANT	ADD		ADD	ANT	ANT	ANT
perc_dens_MetO2INTOL				*	ADD		ANT		ADD	ADD		ADD	ANT	ADD	ADD
dens_MetRHPAR				*	SYN		ANT		ADD					ANT	ADD
dens_MetLITH	*			*	ADD			ADD	ANT			ANT	ANT	ANT	ANT

Table 6
Responsiveness of fish metrics to single and multiple stressors in lowland rivers (LLR). Asterisk in "single stressors" indicates significant response; significant interactive effect in "multiple stressors" is additive (ADD), synergistic (SYN) or antagonistic (ANT).

	Single stressors		Multiple stressors			
Metric	M	W	HW	MW	HMW	CHMW
biom_WQgen_INTOL						
biom_WQgen_TOL		*		SYN		
biom_WQO2_O2INTOL						
dens_HabSp_RHPAR				ADD		
dens_WQgen_INTOL		*		ADD		
dens_WQO2_O2INTOL				ADD		
nsp_WQgen_TOL				SYN		
perc_biom_HTOL_HTOL		*		SYN		
perc_dens_WQgen_INTOL	*	*		SYN		
perc_dens_WQgen_TOL	*			SYN		
perc_nsp_Atroph_PISC				ADD		
perc_nsp_WQgen_INTOL		*		SYN		
perc_nsp_WQO2_O2INTOL				SYN		
perc_nsp_WQO2_O2TOL				SYN		
nsp_all						
perc_biom_Atroph_OMNI		*		SYN		
perc_nsp_Atroph_OMNI				SYN		
nsp_Atroph_INSV	*					
dens_MetHINTOL150						
perc_dens_MetO2INTOL	*	*		ADD		
dens_MetRHPAR				ADD		
dens_MetLITH	*			ADD		

responded to at least one single stressor and showed to be often affected by interacting multiple stressors. For water quality metrics, species intolerant to water quality degradation in general and to O_2 depletion showed the strongest responses (in >50% of cases). Moreover, significant single and interactive stressor effects on two habitat metrics – density of juveniles of species intolerant to habitat degradation in general and density of species intolerant to degradation of lotic spawning habitats - were also shown to occur in >50% of cases. Concerned species for these two categories are especially salmonids as *Salmo trutta fario* and *Thymallus thymallus* but also *Barbus barbus* or *Cottus gobio*, which are key-indicator species in HWS, MGR and MES. *Thymallus thymallus* is a highly threatened species, which has already been eradicated by river alterations (Persat, 1996; Gum et al., 2009).

Table 7
Responsiveness of fish metrics to single and multiple stressors in Mediterranean streams (MES). Asterisk in "single stressors" indicates significant response; significant interactive effect in "multiple stressors" is additive (ADD), synergistic (SYN) or antagonistic (ANT).

	Single stressors		Multiple stressors	
Metric	H	W	HW	CHW
biom_WQgen_INTOL		*		
biom_WQgen_TOL				
biom_WQO2_O2INTOL		*		
dens_HabSp_RHPAR		*	ADD	
dens_WQgen_INTOL	*	*	SYN	
dens_WQO2_O2INTOL	*	*	ADD	
nsp_WQgen_TOL	*		ADD	
perc_biom_HTOL_HTOL				
perc_dens_WQgen_INTOL	*	*	SYN	
perc_dens_WQgen_TOL	*		ADD	
perc_nsp_Atroph_PISC				
perc_nsp_WQgen_INTOL	*	*	ADD	
perc_nsp_WQO2_O2INTOL	*	*	SYN	
perc_nsp_WQO2_O2TOL			ADD	
nsp_all	*		ADD	
perc_biom_Atroph_OMNI		*		
perc_nsp_Atroph_OMNI	*		SYN	
nsp_Atroph_INSV				
dens_MetHINTOL150		*		
perc_dens_MetO2INTOL	*	*	SYN	
dens_MetRHPAR				
dens_MetLITH				

In terms of interaction types, several previously conducted studies highlight that additive effects are not often prevalent when two stressors act in combination (e.g. Piggott et al., 2015b; Teichert et al., 2016). Overall, in our study, different types of combined effects were observed (i.e. additive, synergistic, antagonistic), but synergism and antagonism were more common than additive interaction, i.e. for 60% of tested interactions. Of most significance is our finding that 40% of metrics showed an additive response under multiple stressor situations, whereas 30% indicated a synergistic and 30% an antagonistic response over all river types (Fig. 3). This result is comparable with findings of Nõges et al. (2016), who found additive interactive effects to be present on 39% of stressor pairs, synergistic effects on 35% and antagonistic effects on 26%. However, it contrasts with other studies that found antagonistic effects among stressors to be largely dominant for fish assemblages in estuaries (Teichert et al., 2016).

The observed prevalence of interactive stressor effects reflects a complex scenario for ecosystem management (Brown et al., 2013; Folt et al., 1999; Teichert et al., 2016), because the complete recovery for mitigating one stressor is only expected when other stressors are also removed. In this context, the identification of dominant stressors in riverine systems should be accomplished by taking into account the direction and strength of interactions to improve the assessment accuracy of the stressors impacts (Teichert et al., 2016).

4.3. Uncertainties & limitations

Significant progress in biomonitoring science and earth observational technologies as well as standardization processes (e.g. for sampling and mapping) are now providing new opportunities to address

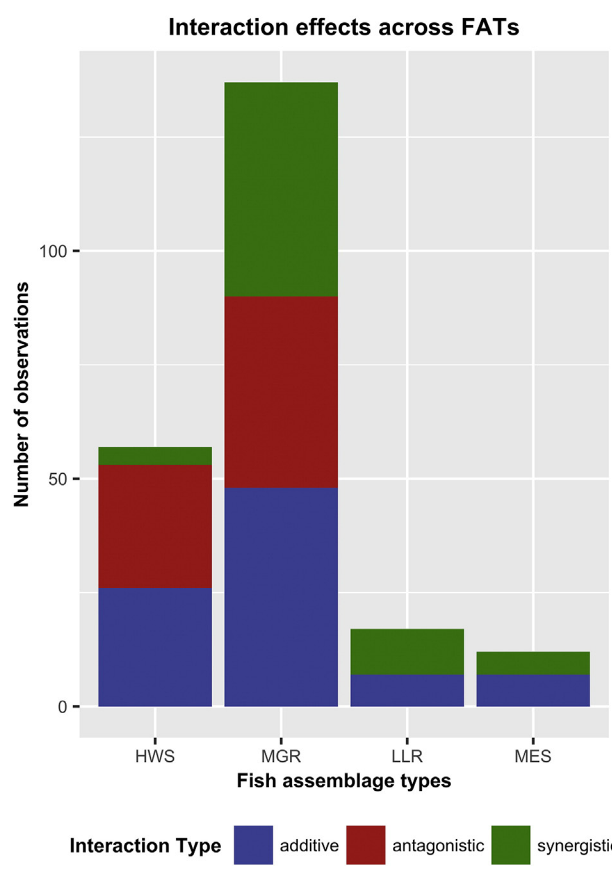

Fig. 3. Distribution of interaction effects across fish assemblage types (FATs) indicating number of observed interactions in fish metric responses. Headwater streams (HWS), medium gradient rivers (MGR), lowland rivers (LLR) and Mediterranean streams (MES) after Schinegger et al. (2013). Interaction effects are antagonistic (ANT), additive (ADD) or synergistic (SYN).

problems across large spatial and temporal scales, which were previously impossible (Dafforn et al., 2015). In Europe, the implementation of the EU Water Framework Directive (European Commission, 2000) has led to harmonized assessment methods (Birk et al., 2012), resulting in extensive, publicly available freshwater databases (e.g. the Water Information System of Europe WISE, http://water.europa.eu), including biological information on ecological status and on various human stressors (e.g. hydromorphological alterations, water quality problems, barriers etc.) at the waterbody scale.

Even though our study was based on an extensive, pan- European dataset, the number of sites represented in each stressor category was restricted (Table 3). A main limitation concerns stressor data, as for this study, monitoring- and field-mapping data from national databases compiled for the WFD and expert judgement were only available on a semi-quantitative basis (Schinegger et al., 2012). Moreover, most often the samples were not evenly distributed along the whole stressor gradients, which might explain the often week and vague response of fish metrics to single and multiple stressors. A central reason for this is that harmonized data for large-scale investigations were lacking in the past, especially on national or wider spatial scales. However, there is a need for quantitative evidence of biotic responses to multiple stressors, so that it can serve as the basis for risk assessment and appropriate management actions (Nõges et al., 2016).

As planning and implementation of sustainable conservation and restoration solutions requires detailed knowledge on complex system relationships (Hipsey et al., 2015), new attempts are needed to investigate these with a special focus on restoration of river ecosystems and conservation of fish assemblages in Europe by using emerging sources of 'big picture data', interdisciplinary tools and by the incorporation of results obtained from previous projects in the future. Although constrained by the available biomonitoring data, this research represents a first step into this direction. New and better harmonized stressor data from most recent works are expected to improve this picture, e.g. compiled by EU member states within the 2nd River Basin Management Plans (supporting the implementation of the EU WFD). Related assessments will provide the opportunity to directly link drivers, stressors and ecological state, with much better detection and diagnosis of human-induced changes for a broad spectrum of scenarios and heterogeneous landscapes across Europe (Dafforn et al., 2015). Moreover, the consideration of spatial ecology in future studies is fundamental to design, implement, and interpret biological assessment data, as well as to develop models (e.g., habitat and environmental models) that inform and evaluate alternative management and conservation strategies (Cooke et al., 2016).

5. Conclusions

Our study highlights that only 40% of combined stressors in European running waters were of additive nature, whereas the other 60% were synergistic or antagonistic. Thus, future management plans should consider the type and the strength of interactions (Halpern et al., 2008), in order to improve their outcomes and avoid potential disappointments (Teichert et al., 2016). Regardless of the successful identification of fish metrics, which were responsive to interactive effects of multiple human stressors in some FATs in our study, further work is needed to provide new scientific perspectives for effective restoration of running waters affected by multiple stressors in Europe. In the context of multiple stressor assessment, our results therefore highlight the following research and management needs:

1. Development of river-type-specific restoration strategies is needed, including the interactive effects of stressors.
2. Consideration of the "full gradient" of quantifiable stressors for future stressor compilations, i.e. to have a broad range of sites available including reference conditions, slight/medium stressors and strong single stressors.
3. A more robust sample design for future biomonitoring programs, specifically directed to disentangle single and joint effects, i.e. to select a priori sets of sample sites representative of the different stressor combinations: from single stressors to combinations of two or more stressors making it possible to assess interactions among a larger number of stressor combinations.
4. Determination of restoration priorities by focusing on the mitigation of stressors providing the maximum ecological benefit in a multi-stress context.
5. Conducting further investigations on how global, regional and local stressors interact in terms of their impact on fish assemblages in Europe's running waters.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.08.143.

Acknowledgments

This work was partially funded by the EU projects MARS (Managing Aquatic Ecosystems and Water Resources under Multiple Stress, Contract No. 603378, http://www.mars-project.eu) and AQUACROSS (Knowledge, Assessment and Management for AQUAtic Biodiversity and Ecosystem Services aCROSS EU policies, Grant Agreement no. 642317; http://aquacross.eu). We are grateful to two anonymous referees for their constructive reviews improving the manuscript. Special thanks are due to all partners and contributing institutions of the EFI+ project in Europe for providing fish data. Sincere thanks are also given to Maria Teresa Ferreira for comments in the early state of the manuscript.

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