



Configuration of multiple human stressors and their impacts on fish assemblages in Alpine river basins of Austria



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HIGHLIGHTS

- Impacts of multiple stressors on river fish assemblages were investigated in Austria.
- Seven stressor categories and up to 4 stressors at the same site were identified.
- Of all sites, only 31% were unimpacted.
- Impacted sites were affected by single stressors (26%) or multiple stressors (30%).
- Decreasing ecological integrity with increasing number of stressors was identified.

GRAPHICAL ABSTRACT



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ABSTRACT

This work addresses multiple human stressors and their impacts on fish assemblages of the Drava and Mura rivers in southern Austria. The impacts of single and multiple human stressors on riverine fish assemblages in these basins were disentangled, based on an extensive dataset. Stressor configuration, i.e. various metrics of multiple stressors belonging to stressor groups hydrology, morphology, connectivity and water quality were investigated for the first time at river basin scale in Austria. As biological response variables, the Fish Index Austria (FIA) and its related single as well as the WFD biological- and total state were investigated. Stressor-response analysis shows divergent results, but a general trend of decreasing ecological integrity with increasing number of stressors and maximum stressor is observed. Fish metrics based on age structure, fish region index and biological status responded best to single stressors and/or their combinations. The knowledge gained in this work provides a basis for advanced investigations in Alpine river basins and beyond, supports WFD implementation and helps prioritizing further actions towards multi-stressor restoration- and management.

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1. Introduction

The water resources of the European Alps are of central importance for their core area as well as surrounding areas, i.e. large parts of Europe, as they are seen as the ‘water towers of Europe’ (Viviroli et al., 2007).

Today, the river ecosystems of the European Alps (from hereon just named “Alps”) are highly under stress through various human activities (Tockner et al., 2009), which are affecting the physico-chemical conditions of running waters and are strongly influencing and impacting their morphological character, hydrological regime and as a consequence, inhabiting aquatic biota and the overall ecological integrity. Hydromorphological alterations due to hydropower production and flood protection measures can be addressed as the key stressors in the

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Alps (Schinegger et al., 2012). Especially in Austria, hydropower is an important renewable energy source, contributing 65.7% to Austria's national electricity generation, but it also is associated with ecosystem degradations, jeopardizing the aims of the EU Water Framework Directive (WFD; European Commission, 2000) and Habitats Directive (HD, European Commission, 1992) (Seliger et al., 2016). Fish are especially sensitive indicators as they respond significantly to almost all kinds of human stressors, including flow regulation, physical habitat alteration, fragmentation, eutrophication, acidification and chemical pollution (Ormerod, 2003). Fishes are used as Biological Quality Elements (BQEs) in the WFD for riverine ecosystems. Effects of hydromorphological changes on fishes are complex and manifold, including impacts on swimming performance, reduced juvenile fish recruitment, -density, -biomass or -abundance (Wolter et al., 2013). Current knowledge on multiple stressors and related response of fish assemblages is limited in most parts of the world, especially in terms of quantifiable understanding on multiple hydromorphological stress effects – such as morphological alterations, residual flow and connectivity disruption, hydropeaking and impoundments – paired with water quality stress. Several studies on local/experimental spatial scale found responses of aquatic organisms (including fish) to multi-stressor situations, including stressors combined with impoundments (Alonso et al., 2015; Marzin et al., 2012; Van Looy et al., 2014), connectivity disruption and thereby evoked habitat fragmentations by dams and barriers (Alonso et al., 2015; Falke et al., 2013; Van Looy et al., 2014; Branco et al., 2016), water abstractions and residual flow conditions (Lange et al., 2014), morphological alterations (Alonso et al., 2015; Marzin et al., 2012; Milly et al., 2008; Rolis et al., 2013; Van Looy et al., 2014) and hydropeaking (Schülting et al., 2016; Auer et al., 2017; Wright et al., 2016). In contrast, on a very general, pan-European scale, Schinegger et al. (2016) investigated the impact of multiple stressors on fish ecological status in European rivers, including hydromorphological-, connectivity- and water quality stressors, with specific fish metrics responding to certain river types. Moreover, Grizzetti et al. (2017) analysed the WFD ecological status in relation with (modelled) human stressors of mainly all European rivers. However, the type and nature of stressor variables investigated so far was mainly based on land use categories/generalized information (as

surrogates) or expert judgement without a standardised background and without taking into account specific configurations of stressors, often due to lack of precise, larger-scale data. Critical stressor configurations may be associated with specific combinations/hierarchy of stressors (e.g. hydrological and morphological stressors (Gieswein et al., 2017; Trautwein et al., 2013); or the response may be simply related to the number of stressors acting on fish communities (Marzin et al., 2014; Schinegger et al., 2012). Also the intensity of stressors may be as important as the combination and number of stressors. Finally, the response of fish to different stressor configurations may be associated with a change of specific aspects of the fish assemblages (e.g. species composition, reproduction, biomass). However, the response of fish assemblages to multiple stressors in Alpine river basins comparing specific stressor configurations has not been investigated so far.

With this work, we therefore aim to overcome knowledge gaps of multiple stressor effects on biota in rivers, lakes, transitional waters and groundwater (Hering et al., 2015). Our analyses address multiple stressor effects on fish assemblages in Austrian Alpine river basins. We hypothesise that i) different stressor configurations, i.e. combination, number of stressors and intensity of stressors are relevant for fish response; ii) that both the entire fish community and specific aspects of the community (metrics) show significant responses to these configurations and iii) that the response follows a linear trend. Increased understanding of the relationships between riverine ecosystem degradation and biological attributes may support the identification of more appropriate restoration and management actions in a multi-stressor context.

2. Methods

The Austrian Drava and Mura river basins are part of the Danube River Basin and comprise about 23,000 km² of size (12,800 km² and 10,300 km² each; Fig. 1). The Mura river drains into the Drava river at the Croatian-Hungarian border near Legrad. In their Austrian territory, both basins are located in the ecoregions Alps and Dinaric Western Balkan (Illies, 1978). The runoff of both river basins is mainly determined by nival and glacial regimes in the Alps and by pluvial and pluvio-nival regimes in the Dinaric western Balkan regions (Fink et al., 2000). In terms of human stressors, both basins are heavily affected by

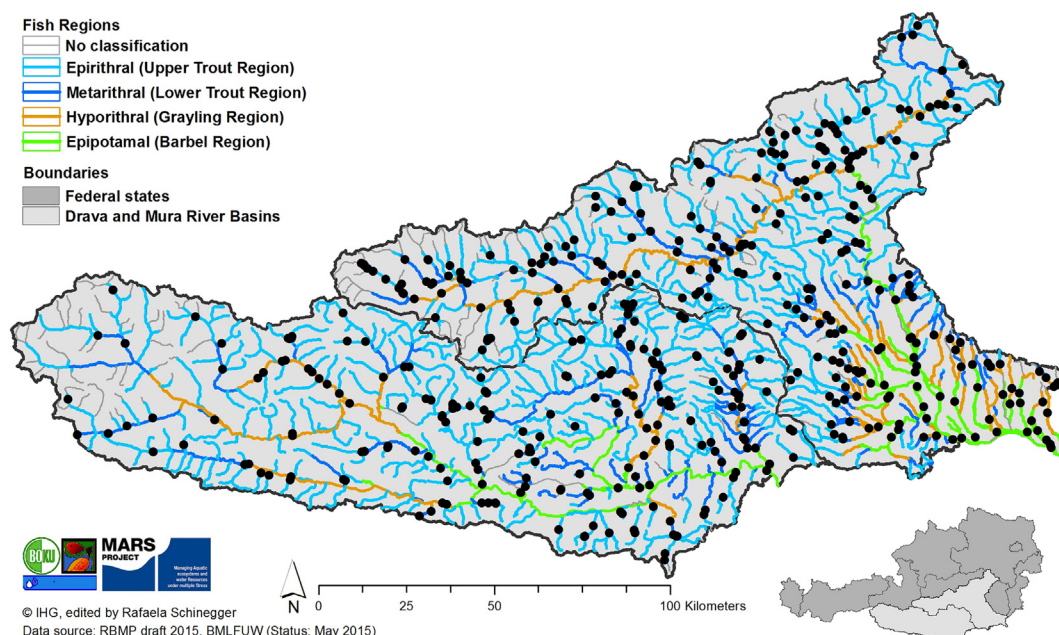


Fig. 1. The 525 investigated fish sampling sites in the Drava & Mura river basins, located at water bodies in various fish regions.

Table 1

Stressor variables considered in MARS Drava & Mura basin analyses.

Stressor intensity classes of the national impact assessment by BMLFUW (in brackets classification used in this stressor analysis)			A: No or very low impact (level 1 – no stress)	B: Low impact (level 2 – not rated)	C: Possible significant impact (level 3 – stress present)	D: Strong significant impact (level 4 – stress present)
Stressors	Impoundment (I)	River basin < 1000 km ²	No I	No I > 500 m & sum I < 10% of surface water body (SWB)	Single I 500–1.000 m or sum of multiple I cover 10–30% of SWB	Single I > 1000 m or sum of multiple I cover >30% of SWB
		River basin > 1000 km ²			Single I 500–2000 m or sum of multiple I cover 10–30% of SWB	Single I > 2000 m or sum of multiple I cover >30% of SWB
	Hydropeaking (H)	Small & medium surface water bodies	No H	<1:3 or designated as “no significant H-impact”*	1:3–1:5 or H amplitude unknown or designated as “significant H – present risk”*	>1:5 or designated as “significant H – present risk”*
		Type “large rivers”		Very slight H or designated as “no significant H-impact”*	Designated as “significant H – present risk”*	>Each distinct flush or designated as “significant H – present risk”*
	Residual flow (R)		No abstraction or abstraction according to QOO Ecology** §12 heel 2	Abstraction with dotation order during full year or with dotation order during authorized abstraction period; according to QOO Ecology** §13 heel 2 values are met or abstraction at facilities authorized 1990–2010 according to specifications of ecological functioning/good status	Abstraction with regulated dotation during the whole year or with regulated dotation within authorized period; values according to QOO Ecology** §13 heel 2 are not met*** or abstracted dotation unknown	No or no dotation order during full year or no continuous dotation order during authorized abstraction period or water body sections, which fall dry due to insufficient dotation during the whole year or during certain periods.
	Connectivity disruption (B)	Within fish habitat	No B or passable without fish migration facility (e.g. ramp)	Limited passability of B or B**** passable due to fish migration facility & no additional non-passable length elements	>= 1 non-passable B	–
	Morphological alteration (M)		All 500 m-sections within SWB = class 1*****	<30% class 3–5*****	30–70% class 3–5 & <30% class 4–5*****	>70% class 3–5 or >30% class 4–5*****
	Chemical state/stress (including toxic substances) (C)*****		1	2	3	–

* According to ‘BOKU Hydropeaking-study’ by Schmutz et al. (2013).

** Quality objective ordinance ecology.

*** Abstractions with MQRW < MJNQInat or NQTRW < NQTnat.

**** Barriers with functioning fish migration facilities and barriers with (possibly) limited passability.

***** Classes according to ‘Guidance on hydromorphological state assessment’ by Mühlmann (2013).

***** Chemical status in intensity classes 1–3 was selected instead of values proposed by impact assessment chemistry.

hydrological stress due to intense hydropower use and morphological alteration due to flood protection measures, etc. (Wagner et al., 2015; Seliger et al., 2016). Also, both basins are of similar natural-environmental nature. Therefore, both basins were jointly analysed here, in order to increase the N of sampling sites/stressor configuration metrics for further analyses. In terms of spatial extent of analyses, we differentiate between two scales for our further analyses: A) the water body scale, which is considered by the Austrian administration for assessment, monitoring and establishment of River Basin Management Plans (RBMP) according to the EU WFD. This scale has a length of several hundred meters to several kilometres, depending on catchment size, extent of stressor etc. This scale was investigated to provide an overview on patterns of stressors in the entire basins, based on descriptive analyses. Further, B) the sampling site scale (fish sampling sites located at water bodies; extent of a few hundred meters per site) was investigated, where detailed fish-ecological information is available from national assessments of WFD ecological status. Analysis of this very detailed information leads to stressor-response relationships, which are important results of basin-wide analyses within the research project MARS (Managing Aquatic ecosystems and water Resources under multiple Stress; <http://www.mars-project.eu>), funded by the European Union to support European water policies and their implementation (e.g. the WFD and other directives).

2.1. Water bodies and available stressor data

The Drava and Mura river basins together consist of 2590 water bodies in total (Fig. 1), according to the national WFD assessment in Austria. For each WFD water body, five hydromorphological stressors, i.e. 'residual flow' (R), 'morphological alteration' (M), 'connectivity disruption/barriers' (B), 'impoundment' (I) and 'hydropeaking' (H) are identified according to the Austrian River Basin Management Plan by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (RBMP database, BMLFUW, 2015; Table 1; for metadata see Schinegger et al., 2017). In addition, the WFD chemical state (C) as well as impacts related to the presence of toxic substances (T) are recorded for the RBMP. Stressors were derived during the risk and impact assessment carried out as part of the Federal Inventory Assessment 2013 for the 2nd Austrian RBMPs. As chemical stress and toxic substances do not occur frequently in high intensities, 'T' was integrated into variable 'C', in order to attain an overall classification for "water quality stress", to then be compared to hydromorphological stress. All seven stressors are recorded in stressor intensity classes from A to D, based on specific criteria derived by BMLFUW. For the further descriptive analyses of stressor patterns, we compared intensity class A (no or very low impact, level 1) with C (possible significant impact, level 3) and D (strong significant impact, level 4) (Table 1). Cases with intensity class B (low impact) were not considered in further analyses to underpin the effects of and deviation between unimpacted and impacted conditions.

2.2. Fish sampling sites

Fish sampling sites cover the biocoenetic regions Epirhithral to Epipotamal (Upper and Lower Trout Region, the Grayling Region and the Barbel Region; Fig. 1). Fish data were obtained from the 'Fish Database Austria' (FDBA) (BAW IGF, 2015; for metadata see Schinegger et al., 2017), which is managed by the Austrian Federal Office of Water Management. Fish sampling was conducted based on a standard sampling protocol by electric fishing (Haunschmid et al., 2010). Overall, 525 samples were available from years 2006 to 2014 (Fig. 1), which fit well to the stressor data, derived from Austrian RBMPs 2009 and 2015.

The fish based indicators considered for stressor-response analyses available in the FDBA are based on the Fish Index Austria (FIA), a multi-metric index developed for assessment of the fish-ecological status in Austria to support WFD assessment (Haunschmid et al., 2006). The FIA is composed of a number of core metrics, including information on dominant species, subdominant species, rare species, habitat guilds, reproductive guilds, fish zonation and population age structure, of which metrics referring to species and guild composition as well as population age structure were selected for further analyses (Table 2). The assessment methodology of the FIA is based on the deviation between a predefined expected reference condition ('Leitbild Katalog' BAW IGF, 2015) and the actual values observed (Haunschmid et al., 2006; Appendix Table A2). Reference values for metrics are predefined for all river types and fish zones in Austria and are included in an Excel® spreadsheet for the index calculation provided by the Water Management Office (www.baw-igf.at). The final FIA value is calculated as weighted mean of grouped metrics, ranging from WFD-class one (high status) to class five (bad status). In addition, the biological state (including all biological quality elements) and the total state (ecological state plus main water quality parameters according to WFD) were derived from RBMP-DB (Table 2) and considered for further analyses.

2.3. Stressor configuration and analysis

To express the configuration of stressors on a detailed level, four stressor metrics were calculated in a next step, i.e. 'stressor combination', 'number of stressors', 'maximum stressor' and 'stressor index' for all water bodies and fish sampling sites, to reflect their respective patterns in the entire basins and their specific effects on fish assemblages:

2.3.1. Stressor category (SC)

A site was considered without any stress if all single stressors showed an intensity level 'A'/1 respectively (Table 1). One exception was the chemical status where intensity 'A' was not used in the national classification, as all water bodies in Austria were classified at least with a low impact ('B'/1; Table 1) by BMLFUW. In this case, intensity 'B' was

Table 2
Description and coding of fish metrics/indicators considered for further analyses.

Metric category	Indicator abbreviation	Description	Response direction (with increasing stressor)	Median (Range)
Fish Index Austria (FIA) metrics/index				
Reproductive guild	REPRO_GUILD	Deviation of actual present number of reproductive guilds from reference	Increase	1 (0–5)
Trophic condition	BIOMASS	Biomass in kg/ha of native species and rainbow trout	Decrease	70.8 (0–1664.0)
Age structure	AGE_STRUCTURE	Total evaluation of population age structure of dominant and subdominant species	Increase	3 (1–5)
Biocoenetic region	FISH_REGION_INDEX	Deviation of actual fish zonation index from reference	Increase	0.1 (0–5.7)
Fish Index Austria	FISH_INDEX_AUSTRIA	Multi-metric index, separated in 5 classes	Increase	2.5 (1–5)
WFD status variables				
	BIOL_STATE	Biological status of water body	Increase	3 (1–5)
	TOTAL_STATE	Ecological status of water body	Increase	3 (1–5)

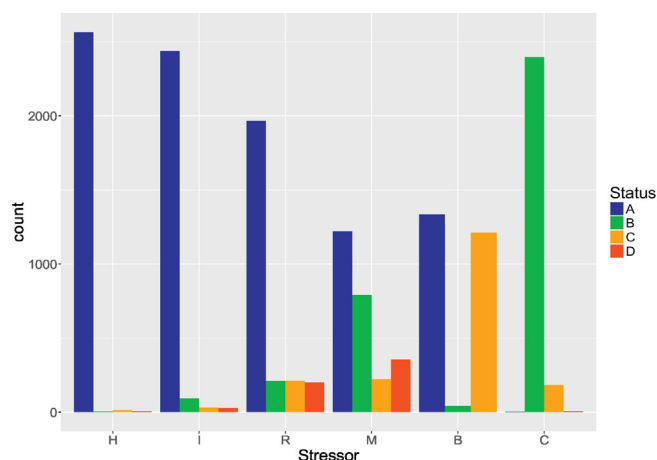


Fig. 2. Distribution of single stressors and their status intensities in Drava/Mura basin water bodies (N = 2590); Stressors: C = chemical alteration/toxic substances; H = hydropeaking; I = impoundment; R = residual flow; M = morphological alteration; B = barriers/connectivity disruption; Status: A: no or very low impact; B: low impact; C: possible significant impact; D: strong significant impact.

considered as no stress. Thus, for all variables, intensities of 'C' or 'D'/3 and 4 were considered as "stress" respectively (Table 1).

2.3.2. Number of stressors (NS)

The stressor metric 'number of stressors' was calculated by counting the stressors with an intensity of 'C' and 'D'/3 or 4 (Table 1).

2.3.3. Maximum stressor (MS)

To derive the maximum stressor value, the level of intensity (i.e. categories 'A'–'D'/1–4 as given in Table 1) was then calculated as the maximum of these values.

$$MS = \max(\text{status})$$

2.3.4. Stressor index (SI)

To evaluate the overall status of stressors in the Drava/Mura basins in terms of individual stressors, stressor combinations and number of stressors in one single value, we calculated a joint stressor index (SI) by summing up the single stressor values with stressor intensity > B (i.e. classes 3 and 4 to avoid that values ≤ 2 compensate for values > 2) for each site.

$$SI = \sum_{\text{status} \geq 3} (\text{status})$$

In a next step, the relationship between stressor metrics and selected fish metrics/WFD status variables was analysed for the 525 fish sampling sites with the use of boxplots. For this analysis, fish metrics were omitted, if at least 50% of values were identical or if they had a variation coefficient below 0.2. Therefore, FIA single metrics 'percentage of dominant species', 'percentage of subdominant species', 'percentage of rare species' and 'habitat guilds' were omitted and not considered in further analyses. In a next step, the collinearity of stressors was investigated by using the Variance Inflation Factor (VIF), where a threshold was set < 3 (Naimi et al., 2014). A test for significance in boxplots against sites with no stressor was conducted using a Mann-Whitney test. The resulting *p*-values were Bonferroni-adjusted. In the boxplot analysis, stressor metrics were only considered/displayed if there were > 10 sites representing a certain category available. The shown curve in boxplots is a LOESS interpolation (Cleveland and Devlin, 1988) of corresponding values. Boxes are coloured according to Fish-Index Austria WFD thresholds (Appendix Table A2). To test linear trends and to identify the most important stressor configuration, we also fitted linear models (LMs) with stressor metrics and biological response metrics (fish metrics/WFD status variables) for the 525 sampling sites. Following the function formula (R package stats (R Development Core Team, 2017)) the model was set up as:

$$\text{Biological response metric} \sim \text{NS} * \text{MS}$$

This included consideration of pairwise interactions of stressor metrics. First, a Pearson correlation analysis was conducted between numeric stressor metrics. Then, a stepwise model selection with the use of AIC was performed. Analysis included calculation of standardised co-

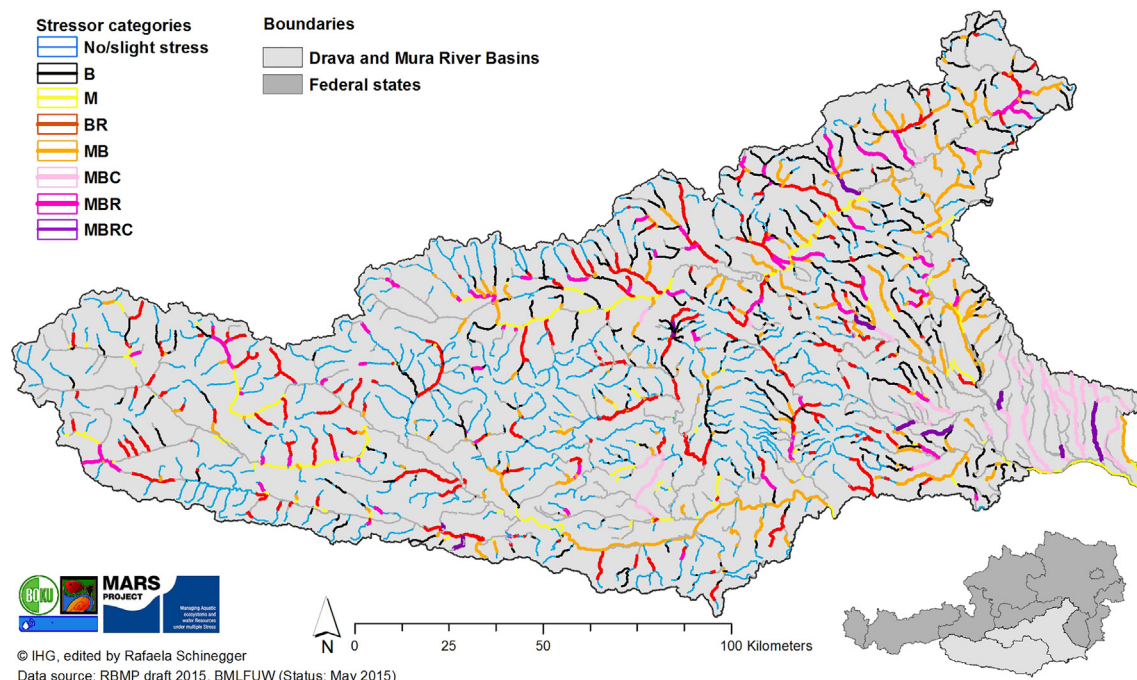


Fig. 3. Water bodies (N = 2590) affected by different stressor categories (single stressors and multiple stressor combinations) in the Drava and Mura river basins of Austria.

efficients (Vittinghoff et al., 2011) to make coefficients comparable without variable scaling.

3. Results

The Austrian RBMP-DB includes information on single stressor intensities for all water bodies (Fig. 2), aggregated into categories 'less impacted' (intensities A and B) and 'more impacted' (intensities C and D; according to Table 1 and as described before). In both, the Drava and Mura river basins, 1782 water bodies (69%) overall were affected by stressors (Fig. 2). Connectivity disruptions (B) were present in 1213 water bodies (47%). Morphological alterations (M) were detected in 578 water bodies (22%) and water abstractions (leading to residual flow sections, R) in 413 water bodies (16%). Moreover, 20 water bodies (0.7%) were affected by hydropowering (H), 59 water bodies (2%) by impoundment (I) and 190 water bodies (7%) by chemical stress (C) (Fig. 2).

Overall, 667 water bodies (26%) in the Drava and Mura river basins were impacted by single and 783 (30%) by multiple stressors and only 808 (31%) face no or very low human stress (noS) (Appendix Table A1).

3.1. Distribution of stressor metrics

In both river basins, overall 33 stressor categories (SC; single stressors and multiple stressor combinations) are observed at investigated water bodies (Appendix Table A1), but there were only seven categories of single and multiple stressors, which occur in at least 10 water bodies (Fig. 3). Most frequently, the single stressors 'connectivity disruption' (B) occurred (at 475 water bodies). Then, 'morphological alteration' (M; 110 water bodies) and the multiple stressor categories 'morphological alteration' combined with 'connectivity disruption' (MB; 251 water bodies), 'connectivity disruption' combined with 'residual flow' (BR; 237 water bodies); 'morphological alteration' in combination with 'connectivity disruption' and 'chemical stress' (MBC; 43 water bodies), 'morphological alteration' combined with 'connectivity disruption' and 'residual flow' (MBR; 90 water bodies) and finally, a combination of all, 'morphological alteration' coupled with 'connectivity disruption', 'chemical stress' and 'residual flow' was found (MBCR; 11 water bodies). Distribution of stressor categories in the water bodies across the Drava and Mura basins is shown in Fig. 3, as well as in

Appendix Table A1 (including percentage values and a distribution for sampling sites and across fish regions and catchment size classes).

Beside 'stressor category', metrics 'number of stressors' (NS) and 'maximum stressor' (MS) were investigated per water body (Fig. 4). A clear trend of increasing stressor metrics from Epirhithral to Hyporhithral was observed for both, NS and MS; however, no clear distinction could be made between sites in Hyporhithral and Epipotamal.

Moreover, the stressor index (SI) basically shows an increase from Epirhithral to Epipotamal in many water bodies (Fig. 5).

3.2. Biological response to stressor configuration

In terms of fish assemblage response to single and multiple stressors, Figs. 6–10 show boxplots with the response of selected fish metrics/WFD status variables towards stressor metrics 'stressor category' (SC), 'number of stressors' (NS), 'maximum stressor' (MS) and 'stressor index' (SI) at the 525 sampling sites. Five of the seven fish metrics/indices and both WFD status variables (Table 2) showed several significant responses (see also Table 3). All remaining biological indicators respond in a similar way to SI and NS. For fish metric 'AGE_STRUCTURE', strong significant responses can be observed with SC, NS and SI (Fig. 6).

The response of metric 'FISH_REGION_INDEX' shows a similar picture, i.e. responses can be observed with SC, NS and SI, but almost no significant response to MS (Fig. 7). The overall 'FISH_INDEX_AUSTRIA' shows different responses, mainly to NS and SI (Fig. 8). Finally, the WFD status variables 'BIOL_STATE' and 'TOTAL_STATE' performed best for all the four stressor metrics (Figs. 9 and 10).

3.3. Results of linear models (stressor configuration)

In terms of performance of stressor metrics, the conducted analysis of the three numeric stressor variables NS, MS and SI at sampling sites showed a high correlation of 0.97 between SI and NS. Furthermore, SI and MS showed a slight higher correlation of 0.67 compared to 0.53 between SI and NS. As a consequence, SI was removed from the LM (Table 3).

For the linear model, the stepwise model selection by AIC revealed that WFD status variables 'BIOL_STATE' and 'TOTAL_STATE' showed the best R^2 with 0.20 and 0.24 respectively among all tested models

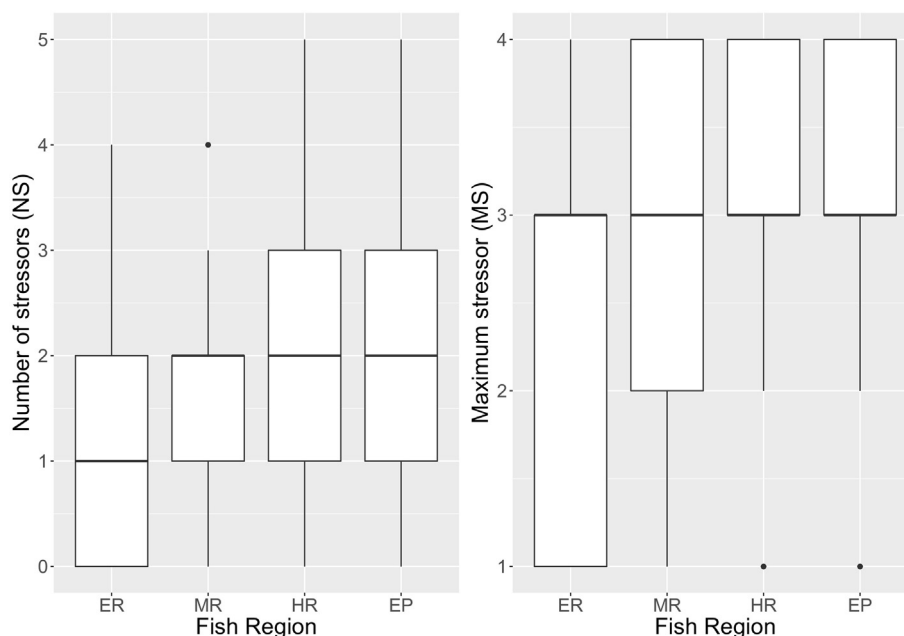


Fig. 4. Distribution of stressor metrics NS and MS along various fish regions (ER = Epirhithral; MR = Metarhithral; HR = Hyporhithral; EP = Epipotamal) at water bodies (N = 2590) in the Drava and Mura river basins.

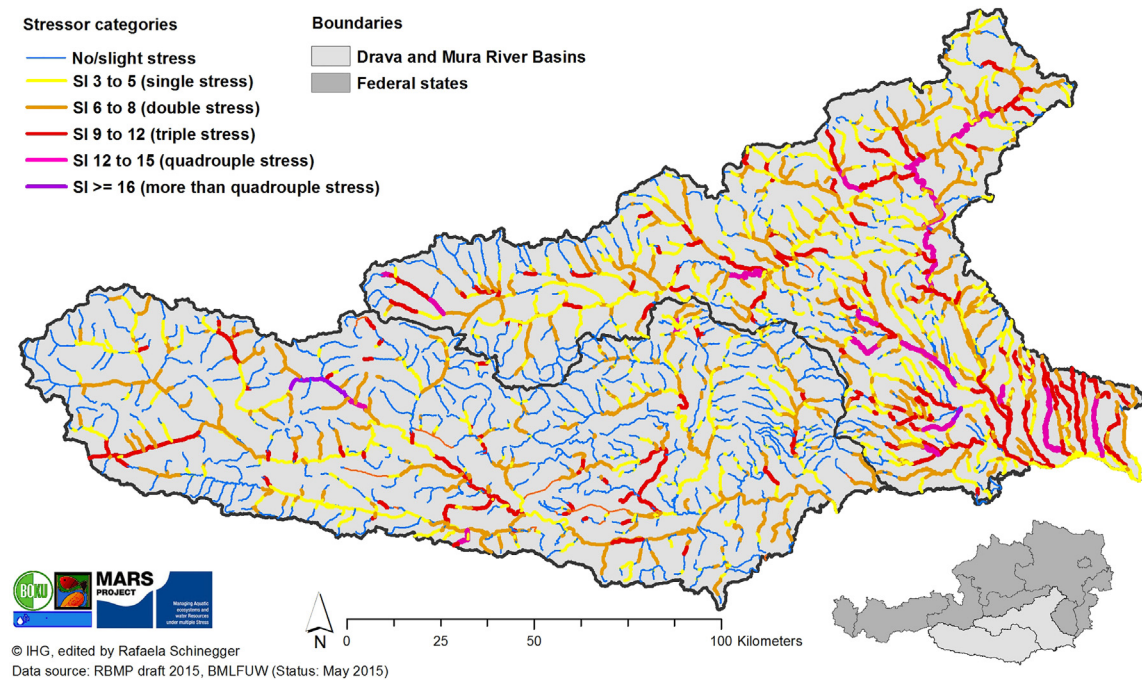


Fig. 5. Distribution of stressor index (SI) in water bodies ($N = 2950$) across the Drava and Mura river basins.

(Table 4). All other models with fish metrics as dependent variable revealed a R^2 below 0.15 and, thus, no further model details are presented.

A comparison of R^2 values in Table 4 shows an improvement by combining stressor metrics MS and NS for both status variables from 0.19 to 0.24 for the BIOL_STATE and from 0.16 to 0.20 for the TOTAL_STATE, which makes them the preferable models. Interaction terms were removed again by AIC model selection, leaving all single variables with a highly significant influence ($p < 0.001$). The models'

constants (intercepts) are around 1, which makes sense, as 1 is the value of sites with no or very slight stress (high status).

4. Discussion

This work investigates the configuration of single and multiple human stressors (expressed by four stressor metrics specifying stressor configuration) and their related impacts on fish indicators

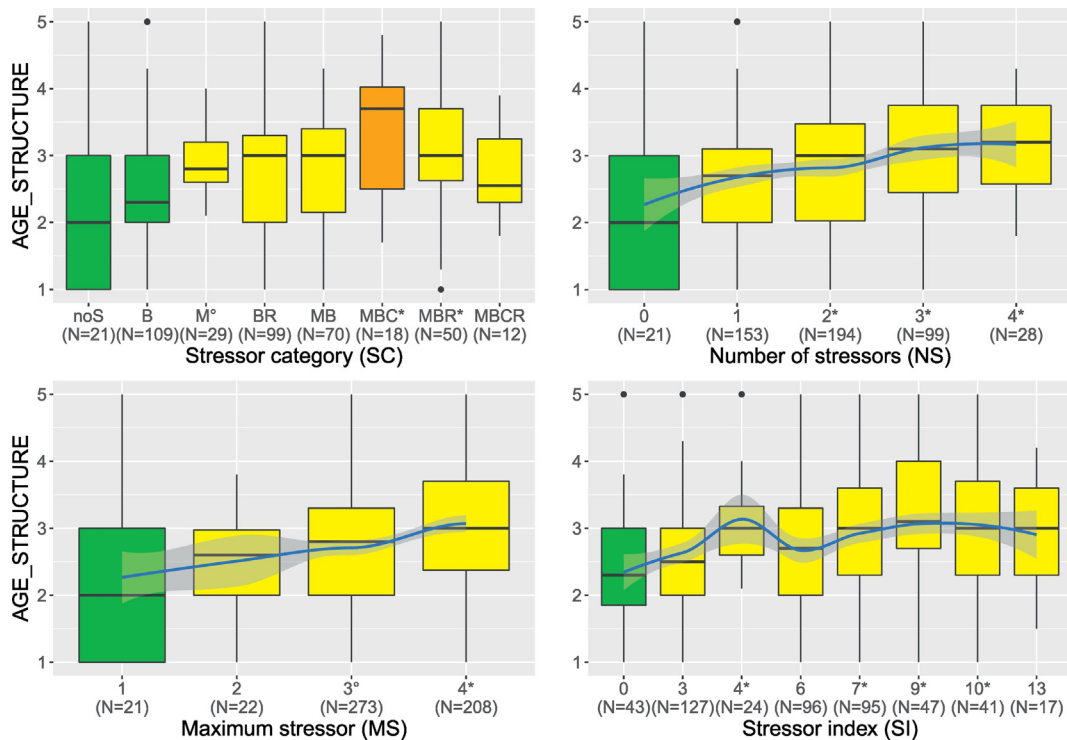


Fig. 6. Response of fish metric "AGE_STRUCTURE" to stressor metrics SC, NS, MS and SI at fish sampling sites ($N = 525$). Significant difference according to Mann-Whitney test indicated by * (p -level 0.05) and ° (p -level 0.1). For each stressor metric, categories with < 10 sampling sites are not shown (therefore sum of N does not match). The shown curve is a LOESS interpolation.

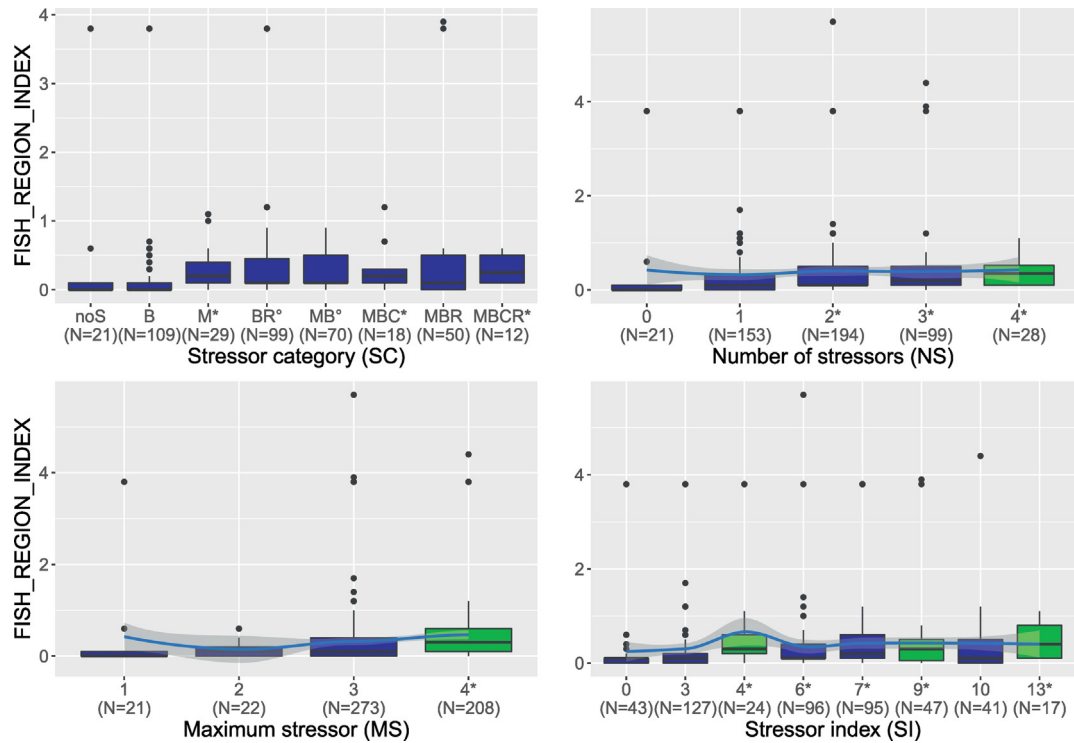


Fig. 7. Response of fish metric “FISH_REGION_INDEX” to stressor metrics SC, NS, MS and SI at fish sampling sites ($N = 525$). Significant difference according to Mann-Whitney test indicated by * (p -level 0.05) and ° (p -level 0.1). For each stressor metric, categories with <10 sampling sites are not shown (therefore sum of N does not match). The shown curve is a LOESS interpolation.

and WFD status variables in Alpine rivers with focus on basin-wide WFD assessment data. Similar studies investigated fish-ecological responses to very general stressor indices (e.g. Pont et al., 2006; Logez and Pont, 2011) or more generic stressor groups such as

water quality vs. hydromorphological alterations (Schinegger et al., 2013; Trautwein et al., 2013), thus often facing limitations in terms of traceability of mechanistic principles of multi-stressor effects on fish. In contrast, our work is based on seven different stressor

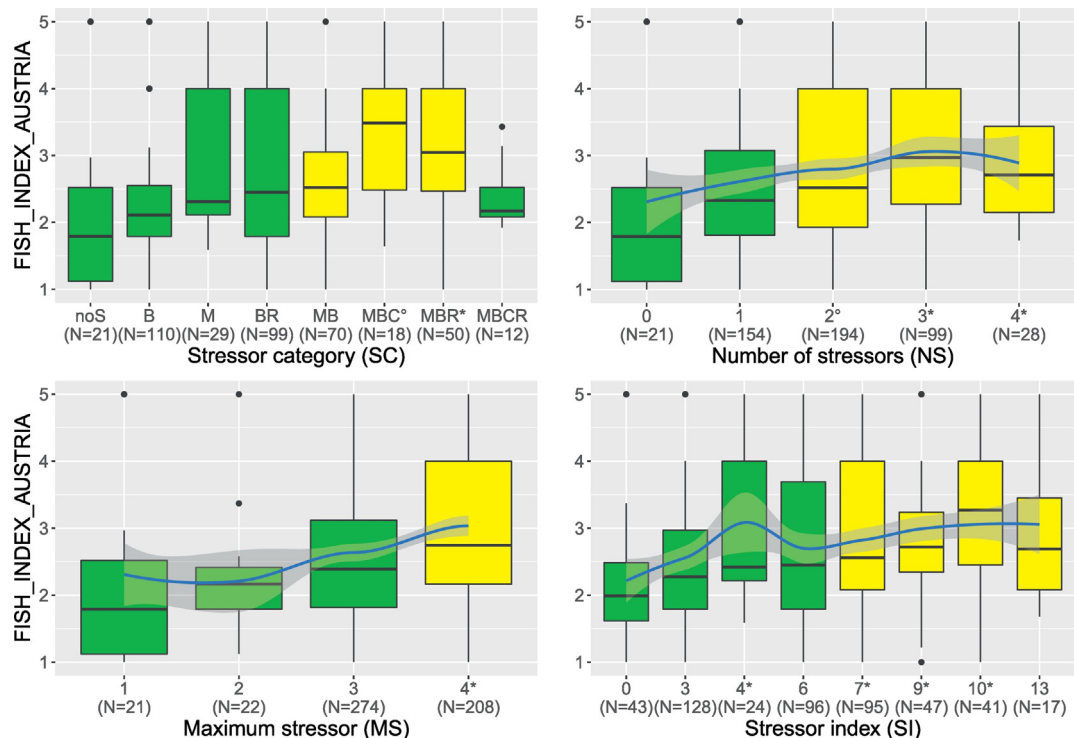


Fig. 8. Response of “FISH_INDEX_AUSTRIA” to stressor metrics SC, NS, MS and SI at fish sampling sites ($N = 525$). Significant difference according to Mann-Whitney test indicated by * (p -level 0.05) and ° (p -level 0.1). For each stressor metric, categories with <10 sampling sites are not shown (therefore sum of N does not match). The shown curve is a LOESS interpolation.

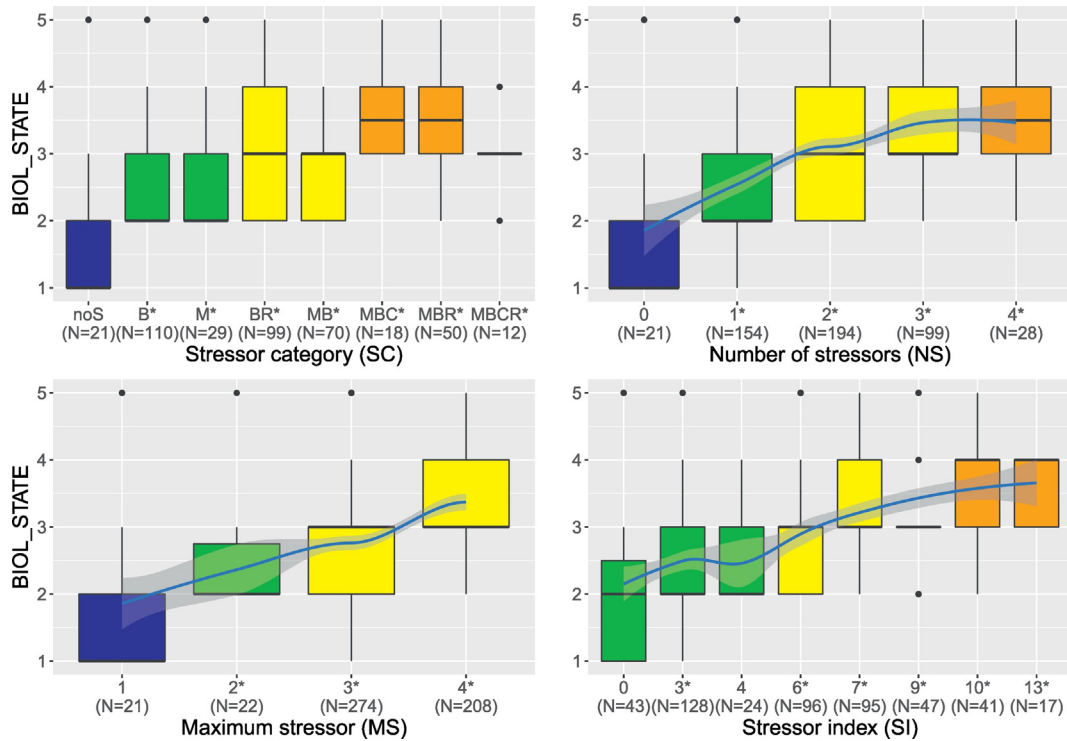


Fig. 9. Response of WFD status variable “BIOL_STATE” to stressor metrics SC, NS, MS and SI at fish sampling sites ($N = 525$). Significant difference according to Mann-Whitney test indicated by * (p -level 0.05) and ° (p -level 0.1). For each stressor metric, categories with <10 sampling sites are not shown (therefore sum of N does not match). The shown curve is a LOESS interpolation.

categories with up to four different stressors occurring at the same time. Therefore, biological responses to both single and multiple stressors can be investigated more thoroughly. Moreover, the

biological response to maximum stress and a combination of number of stressors and maximum stress (integrated into a stressor index and GLM) was investigated.

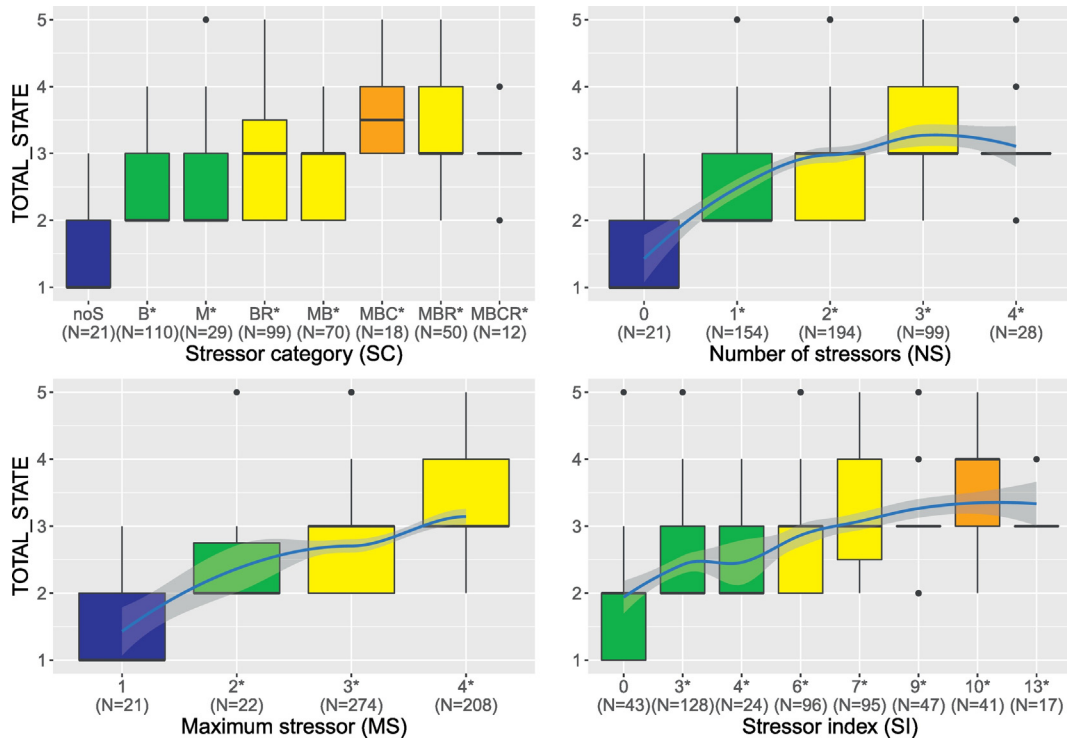


Fig. 10. Response of WFD status variable “TOTAL_STATE” to stressor metrics SC, NS, MS and SI at fish sampling sites ($N = 525$). Significant difference according to Mann-Whitney test indicated by * (p -level 0.05) and ° (p -level 0.1). For each stressor metric, categories with <10 sampling sites are not shown (therefore sum of N does not match). The shown curve is a LOESS interpolation.

Table 3

Pearson correlation coefficient between numeric stressor metrics 'number of stressors' (NS), 'maximum stressor' (MS) and 'stressor index' (SI).

	Number of stressors	Maximum stressor	Stressor index
Number of stressors	1.00	0.54	0.98
Maximum stressor	0.54	1.00	0.67
Stressor index	0.98	0.67	1.00

4.1. Configuration of human stressors in Alpine river basins

The intent of the conducted stressor analysis was to describe the configuration of single and multiple stressors occurring in the Austrian Drava and Mura river basins with the most current data available from the Austrian 2nd RBMP inventory assessment (BMLFUW, 2015). Our findings constitute that only seven single and multiple 'stressor categories' (SC) occur at least 10 times in the investigated total basins. Configurations frequently identified with SC are the number of water bodies/sampling sites impacted by connectivity disruption (B) and by connectivity disruption combined with residual flow (BR), decreasing from Epirhithral to Epipotamal (Appendix Table A1). In contrary, the number of water bodies/sampling sites, where morphological alteration (M) or morphological alteration combined with connectivity disruption (MB) occur, do increase from Epirhithral to Epipotamal. This can be explained with the fact that in higher elevated areas of the total basin, multiple barriers were constructed for flood protection, torrent control and hydropower production. Headwater streams are often naturally constrained, therefore, morphological alterations are not as significant in contrast to medium gradient streams and lowland rivers (Hyporhithral and Epipotamal), which naturally are braided or meandering, but have been channelized for agricultural and urban land use.

However, also some differences in distribution of stressor categories between investigated water bodies and sampling sites were observed: In terms of water bodies, many, still unimpacted or very slightly impacted headwaters are located in the Drava and Mura river basins (31% of water bodies of intensity class A according to Table 1), whereas sampling sites investigated showed a different pattern (only 4% categorized as 'NoS'; Appendix Table A1), as WFD monitoring sites often are located in impacted water bodies. Scheikl et al. (2016) found that overall, 37% of headwaters in Austria's river basins (<10 km² catchment size) are still in a very good or good ecological state (after WFD) and that thus, there is a huge protection need in parallel to further development and planning in Austrian river basins. This also can be underpinned with our results, and appropriate protection strategies for the 31% of water bodies in SC 'NoS' will be urgently needed in both investigated basins in the near future. Finally, the metric 'stressor index' (SI) was used to

combine 'number of stressors' (NS) and 'maximum stressor' (MS). The SI can be a valuable tool, as it classifies the status of stress in the investigated catchments with a standardised single value. According to Solimini et al. (2006) such standardised tools are necessary to make profound political decisions and to successfully implement the WFD.

With such findings, our stressor analysis can support river basin managers by helping to identify water bodies, which are degraded by the specific stressor categories (combinations) to apply suitable restoration measures. Moreover, future developments in terms of single and multiple stressors can be compared with today's situation.

4.2. Fish assemblage response to stressor configuration

Along with increasing quantity and intensity 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 92% of impaired sample sites across both Alpine basins, >62% 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. In general, we found that ecosystem integrity decreases with an increase of all four stressor metrics (SC, NS, MS and SI; Table 5), and that all fish metrics/indices and WFD status variables responded to multiple stressors. The 'BIOMASS' metric showed the weakest response to stressor metrics, followed by the 'REPRO_GUILD' (Table 5). In contrast, metrics 'AGE_STRUCTURE' and 'FISH_INDEX_AUSTRIA' performed best, as they showed 27 significant responses to stressor configuration metrics each. Also, WFD status variables 'BIOL_STATE' and 'TOTAL_STATE' showed 26 significant responses to SC, NS, MS and SI (Table 5).

Finally, standardised coefficients of numeric stressor metrics as outputs of linear models show a slightly higher influence of 'maximum stressor' than 'number of stressors' (Table 4). Within our data set, they have a similar potential in decreasing the status of a water body. Coefficients show a deterioration of both, WFD variables by about 0.4 status classes per 'maximum stressor' class and by about 0.25 for each additional stressor. This fact explains that in terms of restoration efforts, both stressor metrics play an important role, and that it will be insufficient to only focus on one of them in further management programs.

4.3. Uncertainties & outlook

Beside the given results, this study contains also some uncertainties and limitations, e.g. the number of water bodies available per stressor category. There are only seven stressor categories occurring at least 10

Table 4

Test statistics and coefficients of linear models with WFD status variables and stressor metrics for best-performing models (BIOL_STATE, TOTAL_STATE) using maximum stressor and number of stressors as well as the combination of both.

Status variable	Model	R ²	Stressor	Coefficient	Standardised coefficient	p (t-statistics)	Significance
BIOL_STATE	MS	0.19	Constant	0.90	0.00	0.000	*
BIOL_STATE	MS		Maximum stressor	0.61	0.44	0.000	*
BIOL_STATE	NS	0.19	Constant	2.14	0.00	0.000	*
BIOL_STATE	NS		Number of stressors	0.40	0.43	0.000	*
BIOL_STATE	MS & NS	0.24	Constant	1.08	0.00	0.000	*
BIOL_STATE	MS & NS		Maximum stressor	0.40	0.29	0.000	*
BIOL_STATE	MS & NS		Number of stressors	0.26	0.28	0.000	*
TOTAL_STATE	MS	0.16	Constant	1.04	0.00	0.000	*
TOTAL_STATE	MS		Maximum stressor	0.53	0.40	0.000	*
TOTAL_STATE	NS	0.15	Constant	2.14	0.00	0.000	*
TOTAL_STATE	NS		Number of stressors	0.34	0.39	0.000	*
TOTAL_STATE	MS & NS	0.20	Constant	1.19	0.00	0.000	*
TOTAL_STATE	MS & NS		Maximum stressor	0.36	0.27	0.000	*
TOTAL_STATE	MS & NS		Number of stressors	0.21	0.24	0.000	*

Table 5

Responsiveness of fish metrics/indices and WFD status variables to configuration of stressors (stressor metrics). Significant response: * indicates p -value < 0.05; ° indicates p -value < 0.1.

Fish metric/WFD status	Stressor metric (configuration)																															
	Stressor category							Number of stressors				Maximum stressor			Stressor index																Count	
B	M	BR	MB	MBC	MBR	MBCR	1	2	3	4	B	C	D	3	4	6	7	8	9	10	11	12	13	14	15	16	*	°				
REPRO_GUILD	*		°			*					°	*	°		*	*								*		°	*	11	4			
BIOMASS						°														°	°		*					4	3			
AGE_STRUCTURE		*	*	*	*	*	°	*	*	*	*	°	*	*		*	°	*	*	*	*	*	*	*	*	*	*	24	3			
FISH_REGION_INDEX		*	*	*	*	*	*		*	*	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	22	0			
FISH_INDEX_AUSTRIA		*	°	*	*	*	°	*	*	*	*		*	*	°	*	*	*	*	*	*	*	*	*	*	*	*	24	3			
BIOL_STATE	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	26	0			
TOTAL_STATE	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	26	0			

times, which poses a challenge for statistical analysis, as a minimum sample size is required. A study by Stockwell and Peterson (2002) showed that the effects of sample size on the accuracy of species distribution models suggests that for machine-learning methods, accuracy was near maximum at 50 data points. For finer surrogate models and logistic regression models, a sample size of about 100 data points would be necessary for the same accuracy. Thus, we investigated stressor configuration by the use of boxplots and statistical tests. Some limits, especially related to data quantity may be resolved by extending the datasets and by using water bodies from comparable regions in entire Austria in the near future. Anyhow, our current results are unique and novel and provide an important step forward in the investigated river basins, as they describe the stressor configuration and related biological response in a very detailed and specific manner, by disentangling mechanistic principles and supporting future management actions. This work therefore represents a valuable contribution to multi-stressor research, which can be considered exemplarily for other river basins in the Alps and in other parts of Europe.

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