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Multiple-stressors effects on Iberian freshwaters: A review of current knowledge and future research priorities

Cayetano Gutiérrez-Cánovas[1,*], Rebeca Arias-Real[2], Daniel Bruno[3], Marco J. Cabrerizo[4,5], Juan Manuel González-Olalla[6,7], Félix Picazo[3,7,8], Ferran Romero[9], David Sánchez-Fernández[10] and Susana Pallarés[11]

[1] Departmento de Ecología Integrativa, Estación Biológica de Doñana (EBD – CSIC). Av. Américo Vespucio, 26. Isla de la Cartuja. 41092 Seville, Spain.
[2] Department de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal, 643 08028 Barcelona, Spain.
[3] Departamento de Conservación de la Biodiversidad y Restauración de Ecosistemas, Instituto Pirenaico de Ecología (IPE-CSIC), Avda. Montañana, 1005, 50059 Zaragoza, Spain.
[4] Departamento de Ecología y Biología Animal, Facultad de Ciencias del Mar, Universidad de Vigo, Campus Lagoas Marcosende, s/n, 36310 Vigo, Spain.
[5] Centro de Investigación Mariña da Universidade de Vigo (CIM-UVigo), Illa de Toralla s/n, 36331 Vigo, Spain.
[6] Instituto Universitario de Investigación del Agua, Universidad de Granada, C/Ramón y Cajal, 4, 18071 Granada, Spain.
[7] Departamento de Ecología, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain.
[8] Research Unit Modeling Nature (Mnat), Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain.
[9] Plant-Soil interactions, Agroscope, 8046 Zurich, Switzerland.
[10] Departamento de Ecología e Hidrología, Universidad de Murcia, 30100 Murcia, Spain.
[11] Departamento de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales, CSIC, Calle de José Gutiérrez Abascal, 2, 28006, Madrid, Spain.

[*] Corresponding author: cayetano.gutierrez@ebd.csic.es

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ABSTRACT

Multiple-stressors effects on Iberian freshwaters: A review of current knowledge and future research priorities

Freshwater ecosystems are exposed to an increasing number of stressors, challenging their biomonitoring and management. Despite recent advances in multiple-stressor research, regional-scale assessments in areas with high freshwater biodiversity and increasing anthropogenic pressure are urgently needed. We reviewed 61 studies focused on freshwater individuals, populations and communities from the Iberian Peninsula to (i) quantify the frequency of experimental approaches used (manipulative, observational), aquatic systems, biological organization levels, and types of organisms and modelled responses, (ii) identify key individual stressors and the frequency of significant positive (increase in response magnitude) and negative (decrease) effects and (iii) determine types of interacting stressors and the frequency of their combined effects. Our dataset comprised 409 unique responses to 13 types of individual stressors, 34 stressor pairs and 12 high-order interactions (3- and 4-way). We found a higher prevalence of manipulative (85 %) respect to observational studies, and a greater focus on lotic systems (59 %) and heterotrophic organisms (58 %). The most studied stressors were nutrient (Nutr), thermal (Therm), hydrologic (Hydr), ultraviolet radiation (UVR), toxic (Toxic) and salinity (Sal) stress and land-use pressure. Individual stressors showed a higher proportion of negative (34 %) than positive effects (26 %). Nutr × UVR, Toxic × Toxic, Therm × Toxic, Hydr × Toxic, Sal × Therm, and Nutr × Therm were the most studied stressor pairs. Non-interactive (50 %) and interactive responses (50 %) were balanced. Antagonistic effects (18 %) were slightly more common than synergisms (15 %), reversal or opposing

(13 %) and high-order interactions (4 %). Such proportions varied within experimental approaches, biological organization levels and organism types. Our findings are helpful to manage certain stressor combinations in Iberian freshwaters. Further efforts in Iberian multiple-stressor research should be directed to (i) intensify the study of lentic systems, (ii) explore more observational data, (iii) autotrophic organisms and (vi) biodiversity-ecosystem functioning responses, and (v) cover a wider range of stressors and (vi) more complex interacting stressor scenarios.

Key words: antagonism, biodiversity, cumulative impacts, ecosystem functioning, global change, inland waters, non-additive effects, stressor interactions, synergy

RESUMEN

Efectos de los estresores múltiples en las aguas continentales de la península Ibérica: Una revisión del conocimiento actual y las prioridades de investigación futuras

Los ecosistemas acuáticos continentales están expuestos a un creciente número de factores de estrés, lo que supone un reto para su monitoreo y gestión. A pesar de los recientes avances en el estudio de estresores múltiples a nivel global, resulta también necesario evaluar áreas que concentran una alta biodiversidad acuática y que están expuestas a una elevada presión antrópica. En esta revisión hemos examinado 61 estudios basados en individuos, poblaciones y comunidades de ecosistemas acuáticos continentales ibéricos para (i) cuantificar la frecuencia de la aproximación experimental usada (estudio manipulativo u observacional), el tipo de sistema acuático, niveles de organización biológica, tipos de organismos y de respuestas abarcadas, (ii) identificar estresores clave y la frecuencia de efectos positivos (aumento de magnitud de la respuesta) y negativos (descenso) y (iii) determinar las combinaciones de factores estudiados y la frecuencia de sus efectos combinados. Los estudios revisados incluyen 409 respuestas, 13 estresores individuales, 34 pares de estresores y 12 interacciones de orden superior (3er y 4º orden). Encontramos una mayor frecuencia de estudios manipulativos (85 %), en sistemas lóticos (59 %) y con organismos heterótrofos (58 %). Los estresores más estudiados fueron los nutrientes (Nutr), la temperatura (Therm), la hidrología (Hydr), la radiación ultravioleta (UVR), los tóxicos (Toxic), la salinidad (Sal) y cambios en los usos del suelo. Los efectos individuales fueron más frecuentemente negativos (34 %) que positivos (26 %). Los efectos combinados no interactivos (50 %) e interactivos (50 %) tuvieron una frecuencia similar. Los antagonismos (18 %) fueron ligeramente más frecuentes que los sinergismos (15 %), efectos reversos u opuestos (13 %) y las interacciones de orden superior (4 %). Sin embargo, estas proporciones varían notablemente entre tipos de estudios, niveles de organización biológica y tipos de organismos. Nuestros resultados son útiles para predecir y gestionar los efectos de ciertas combinaciones de estresores en los ecosistemas acuáticos ibéricos. Las investigaciones futuras sobre estresores múltiples en el contexto Ibérico deberían: (i) intensificar el estudio de los sistemas lénticos, (ii) explorar más datos observacionales, (iii) organismos autótrofos y (iv) respuestas de la relación biodiversidad-funcionamiento del ecosistema y (v) cubrir un rango más amplio de factores estresantes y (vi) escenarios de estresores interactivos más complejos.

Palabras clave: *antagonismo, biodiversidad, impactos acumulativos, funcionamiento del ecosistema, cambio global, aguas continentales, efectos no aditivos, interacciones de factores estresantes, sinergia*

INTRODUCTION

Freshwater ecosystems are exposed to an increasing number of anthropogenic pressures (Birk et al., 2020; Reid et al., 2019) and unprecedented rates of change in natural stressors (Cañedo-Argüelles, 2020; Döll & Schmied, 2012). Currently, 39 % of EU water bodies are affected by two or more anthropogenic pressures (e.g., hydromorphological alteration, diffuse pollution, point-source pollution, invasive species) (Birk et al., 2020; Nõges et al., 2016), causing a widespread degradation of freshwater ecosystems (Grizzetti et al., 2017) and challenging their monitoring and management (Brown, Saunders, Possingham, & Richardson, 2013; Feld et al., 2018; Soria et al., 2020). To reverse this situation and apply successful mitigation actions, it is fundamental to better quantify and understand the combined effects of multiple-stressors and their variation across different types of experimental approaches, aquatic systems, types of organisms and modelled responses.

Multiple-stressor effects can range from individual stressor dominance to more complex situations where two or more stressors need to be considered simultaneously (Côté et al., 2016; Crainet al., 2008; Feld et al., 2016; Piggott et al., 2015). In these cases, combined effects can be additive (i.e., non-interactive), when the combined effect equals the sum of the individual stressor effects, or interactive, when the combined effect is lower (antagonism) or greater (synergism) than the expected additive effect. In such situations, management actions on a stressor-by-stressor basis could be inefficient (Kath et al., 2018).

During the last decade, there has been a growing interest in exploring multiple-stressor effects on freshwaters (Côté et al., 2016; Nõges et al., 2016). Profiting such increase in multiple-stressor literature, recent meta-analyses and syntheses have contributed to identify some consistent patterns across ecosystems and stressor pairs (Birk et al., 2020; Jackson et al., 2016; Lemm et al., 2021; Nõges et al., 2016; Sabater et al., 2018; Velasco et al., 2019; Manuel Villar-Argaiz et al., 2018). For example, a recent pan-European study showed that nutrient stress exerts a dominant effect on lakes, overriding the presence of a second stressor (Birk et al., 2020). However, the context-dependency and high variability of some multiple-stressor effects call for further investigation to provide more robust foundations for freshwater management and restoration (Feld et al., 2018). In addition, multiple-stressor effects also depend on the spatial variation of stressor gradient lengths as well as on the biogeographic, socio-economic and environmental settings of each region (Feld et al., 2016; Newbold et al., 2020). Thus, there is an urgent need to provide regional-scale assessments of multiple-stressor effects to reduce such context-dependency and provide more solid guidance to water managers (Brown et al., 2013; Côté et al., 2016).

The Iberian Peninsula offers multiple features that make it an ideal area to explore multiple-stressor effects on freshwater ecosystems. Iberian ecosystems hold a disproportionate fraction of the European biodiversity as a result of their biogeographical history and the presence of a rich variety of climatic, orographic and lithological conditions respect to central and northern Europe (Múrria et al., 2020; Ribera, 2000; Sánchez-Fernández et al., 2008; Williams et al., 2000). Iberian landscapes combine almost intact areas with others of ancient or extreme alteration due to the widespread presence of agriculture and farms and the uneven distribution of population across the peninsula (Martins et al., 2014). As a consequence, many freshwater systems are threatened by nutrient enrichment, morphological impacts, flow alteration and toxic substances, among others, posing at risk their biodiversity as well as the benefits that these ecosystems provide to people (Grizzetti et al., 2019). In addition, the Iberian Peninsula holds some of the most arid landscapes in Europe, including a disproportionate representation of other natural stressors, like flow intermittence and salinity (Bruno et al., 2016; Gutiérrez-Cánovas et al., 2019), which can intensify under global change (Döll & Schmied, 2012; Estévez, Rodríguez-Castillo et al., 2019) and interact with anthropogenic stressors (Belmar et al., 2019; Soria et al., 2020). The unique combinations of stressors occurring in Iberian freshwaters calls for special management strategies. This particular context may also help to anticipate future effects of multiple-stressors in areas of current wet climate where increasing aridification is predicted.

Here, we provide a systematic review of studies exploring multiple-stressor effects on Iberian freshwaters to (i) describe their variability in experimental approaches, studied aquatic systems, biological organization levels, type of organisms and modelled responses (ii) identify key individual stressors and the frequency of their effects on response variable magnitude (significant positive and negative effects) and (iii) determine types of interacting stressors and the frequency of their combined effects. By synthesising current knowledge on multiple-stressors for the Iberian Peninsula, we will be able to identify existing research gaps, as well as to provide insights directed to improve biomonitoring, management and restoration of Iberian freshwaters.

MATERIALS AND METHODS

Bibliographic search and screening

We searched the literature using Web of Science (WoS, last accessed in November 2020) with the following sequence of field tags and Boolean operators in advanced searches: TS = (Iberia* OR Spain OR Portugal) AND (‘multiple stressor*’ OR ‘multiple pressure*’ OR ‘multiple impact*’ OR ‘multiple environmental pressure*’ OR ‘multiple environmental stressor*’ OR ‘multiple environmental impact*’ OR ‘combined effect*’ OR ‘cumulative effect*’) AND (river* OR stream* OR wetland* OR lake* OR pond* OR riparian OR riverine OR aquatic* OR freshwater*). No restriction was placed on publication year. WoS search retrieved 155 records potentially useful for our analysis (Fig. S1, Supplementary material, available at http://www.limnetica.net/en/limnetica).

We scanned title, abstract and eventually the main text to ensure that the studies met the following three criteria: (i) encompassing and testing individual and interactive effects of two or more stressors on responses measured at any level of ecological organization (from individuals to ecosystems), (ii) focusing on freshwater eco-

Table 1. Classification of stressor types used in this study. *Clasificación de los tipos de estresores utilizada en este estudio.*

Code	Type	Description
BioInv	Biological Invasions	Presence or abundance of invaders
Hydr	Hydrological stress	Flow alteration, intermittence and drying events
Land	Land-use intensification	Increase in land-use pressure (agriculture and urban intensification)
Morph	Morphological alterations	Alteration of freshwater morphology and physical habitat at local or catchment levels
Nutr	Nutrient enrichment	Addition of inorganic nitrogen or phosphorous compounds
Oxyg	Oxygen depletion	Reduction in the concentration or saturation of dissolved oxygen
pH	pH alterations	Modifications in pH or acid-base status
Rip	Riparian degradation	Riparian degradation. Loss or fragmentation of riparian vegetation
Sal	Salinity stress	Changes in osmotic pressure including salinization, osmotic dilution or changes in ionic composition
Therm	Thermal stress	Change in temperature, including warming and cooling
Toxic	Toxic stress	Increase in the concentration of pesticide, nano-particles and other synthetic compounds
UVR	Ultraviolet radiation	Increase in the irradiance of ultraviolet radiation.
Env	Other environmental factors	Factors unaffected by human alterations (e.g., altitude), which influence modelled response in addition to or interacting with anthropogenic drivers

systems (rivers, streams, wetlands, ponds, lakes, riparian habitats) and (iii) observing, collecting or manipulating freshwater organisms, populations and/or communities located in the Iberian Peninsula. Among the manipulative studies, we retained only those that had applied a full-factorial experimental design, including a clearly defined control treatment (or a treatment deemed by the authors to represent non-stressful conditions for any of the stressors), treatments with one or more levels of each individual stressor and combined treatments of all the stressors. We ensured that stress treatments represent challenging conditions for studied organisms. This filtering process resulted in 32 papers.

We also reviewed grey literature, including MSc and PhD theses, by searching in TESEO (Spanish PhD thesis archive; https://www.educacion.gob.es/teseo), RENATES (Portuguese MSc and PhD thesis archive; https://renates2.dgeec.mec.pt/) and Google Scholar using the following keywords (in singular and plural forms): *multiple stressor*, *multiple pressure*, *multiple impact*, *multiple environmental pressure*, *multiple environmental stressor*, *multiple environmental impact*, *combined effect* and *cumulative effect*. This search was less restrictive (terms specifying types of freshwater systems or organisms were not included) to ensure we did not miss any relevant record and because TESEO and RENATES search engines were less flexible than WoS. We searched the Spanish and Portuguese translations of these terms in the corresponding archives and in Scholar Google search engine. In Scholar Google searches, for each term, we only scanned the first two pages that included the top 20 records, which is a cost-effective solution to identify studies that potentially meet our criteria. Next, we searched the published articles associated with the chapters of such theses and other articles produced in the research group where they were conducted. This process resulted in additional 19 papers that met our requirements (Fig. S1). Furthermore, we included 32 additional studies from other sources (four from own databases, 14 from previous multiple-stressor meta-analysis and 14 from cross reference cited literature of the selected papers) that met all these criteria but did not appear in our previous literature searches (Fig. S1). Finally, 22 papers were excluded after reading their whole content to ensure that the final selection met our criteria; our final dataset comprised 61 papers (Fig. S1).

From each article, we retrieved information describing relevant aspects of the study such as the publication year, experimental approach (manipulative or observational), aquatic system (lentic or lotic) and the watershed where organisms and samples were collected or observed. From each modelled response, we gathered information on the level of biological organization (individual, population or community), organism trophic type (autotrophs, heterotrophs or mixed), organism individual size (microorganisms and macroorganisms) and type of response variables (biological or ecosystem responses). We considered as macroorganisms those organisms that are large enough to be seen with the naked eye ($\geq$ 1 mm length), encompassing macroinvertebrates, macroalgae, vertebrates and plants. Microorganisms were represented by smaller organisms (< 1mm length), including bacteria, fungi, microalgae, phytoplankton and zooplankton. Biological responses included measurements at individual, population, assemblage or community levels (biodiversity, biomass, enzymatic activity, molecular biomarkers, nutrient content, survival and tolerance limits), while ecosystem responses comprised properties and processes at the whole ecosystem level (organic matter consumption and release, primary and secondary production, and respiration). For more details, see Table S1, Supplementary material, available at http://www.limnetica.net/en/limnetica.

Stressors were classified as into 13 stress types (Table 1): pH alterations, biological invasion, hydrological stress, land-use pressure, morphological alterations, nutrient enrichment, oxygen depletion, riparian degradation, salinity, thermal stress, toxic stress, ultraviolet radiation and a final category of other environmental factors. The category "other environmental factors" included variables typically unaffected by anthropogenic activity (e.g., altitude, lithology, slope), which are used in some studies as co-variates to better predict biological or ecosystem responses. For simplicity, we used the term stressor in a wide context including also overarching anthropogenic

Table 2. List of the studies used in this review. Study system (LE: lentic, LO: lotic, B: both), experimental approach (M: manipulative, O: observational) and percentage of combined effects are also shown. Combined effect labels: dom: dominance, add: additive, ant: antagonistic, syn: synergistic, oth: other (opposing, reversal), hig: high-order (3- & 4-way), ns: non-significant. *Lista de los estudios usados en esta revisión. También se muestra el sistema de estudio (LE: léntico, LO: lótico), el enfoque experimental (M: manipulativo, O: observacional) y los porcentajes de cada efecto combinado. Tipos de efectos combinados: dom: dominancia, add: aditivo, ant: antagonista, syn: sinérgico, oth: otros (opuestos, reversos), hig: interacciones de 3er o 4º orden, ns: no significativo.*

Study	System	Approach	Combinedeffects (%)						
			dom	add	ant	syn	oth	hig	ns
Arenas-Sánchez et al. (2019)	LE	M	14	43	14	0	0	0	29
Arribas et al. (2012)	B	M	0	67	33	0	0	0	0
Batista et al. (2012)	LO	M	38	25	0	0	38	0	0
Batista et al. (2017)	LO	M	20	0	20	0	60	0	0
Batista et al. (2020)	LO	M	0	10	40	10	20	0	20
Belmar et al. (2019)	LO	O	40	0	40	0	0	0	20
Birk et al. (2020)	LO	O	100	0	0	0	0	0	0
Botella-Cruz et al. (2016)	LO	M	0	50	0	25	25	0	0
Bruno et al. (2016a)	LO	O	25	13	50	13	0	0	0
Bruno et al. (2016b)	LO	O	0	0	67	33	0	0	0
Cabrerizo et al. (2017)	LE	M	0	14	29	57	0	0	0
Cabrerizo et al. (2020)	LE	M	0	0	100	0	0	0	0
Calapez et al. (2017)	LO	M	0	0	0	0	100	0	0
Calapez et al. (2018)	LO	M	0	100	0	0	0	0	0
Calapez et al. (2020)	LO	M	0	0	47	0	39	14	0
Camacho et al. (2003)	LE	M	0	0	0	29	0	0	71
Carbonell et al. (2012)	LO	M	11	67	0	11	11	0	0
Carbonell et al. (2017)	LE	M	50	50	0	0	0	0	0
Carrillo et al. (2008)	LE	M	42	8	25	8	8	0	8
Carrillo et al. (2015)	LE	M	0	0	0	100	0	0	0
Carrillo et al. (2017)	LE	M	11	11	11	44	22	0	0
Céspedes et al. (2013)	B	M	0	25	25	50	0	0	0
Coccia et al. (2013)	LE	M	0	88	0	0	13	0	0
Correa-Araneda et al. (2015)	LO	M	50	0	0	0	0	0	50
Delgado-Molina et al. (2009)	LE	M	0	29	29	43	0	0	0
Di Lorenzo et al. (2019)	LO	M	0	50	0	50	0	0	0
Duarte et al. (2008)	LO	M	0	50	50	0	0	0	0
Duarte et al. (2009)	LO	M	0	50	0	25	25	0	0
Durán et al. (2014)	LE	M	8	4	56	10	0	22	0
Durán et al. (2016)	LE	M	10	23	0	7	20	30	10
Durán-Romero et al. (2020)	LE	M	19	14	0	31	22	14	0
Fernandes et al. (2009)	LO	M	33	67	0	0	0	0	0
Fernandes et al. (2014)	LO	M	0	75	25	0	0	0	0
Garreta-Lara et al. (2018)	NA	M	50	0	0	0	0	50	0
Helbling et al. (2013)	LE	M	0	0	22	14	28	36	0
Herrero et al. (2018)	LO	O	0	100	0	0	0	0	0
Jesus et al. (2017)	LO	M	11	2	4	11	18	0	55
Jesus et al. (2018)	LO	M	14	0	14	14	0	0	57
Korbee et al. (2012)	LE	M	0	27	73	0	0	0	0
Maceda-Veiga et al. (2018)	LO	O	0	50	0	0	50	0	0
Macías et al. (2007)	LE	M	0	0	0	0	100	0	0
Moreirinha et al. (2011)	LO	M	0	33	67	0	0	0	0

Cont.

Table 2. (cont.)

Study	System	Approach	Combinedeffects (%)						
			dom	add	ant	syn	oth	hig	ns
Ortiz-Santaliestra et al. (2010)	LE	M	0	50	0	25	0	0	25
Pallarés et al. (2012)	B	M	25	58	8	0	0	0	8
Palmero et al. (2009)	LO	M	0	0	0	0	100	0	0
Proia et al. (2012)	LO	O	0	0	25	0	0	0	75
Proia et al. (2013)	LO	M	0	0	17	0	0	0	83
Rodrigues et al. (2018)	LO	M	17	25	17	8	17	17	0
Rojo et al. (2017)	LE	M	0	17	33	50	0	0	0
Romero et al. (2018)	LO	M	17	6	17	17	0	33	11
Romero et al. (2019a)	LO	M	0	0	25	0	0	0	75
Romero et al. (2019b)	LO	M	26	0	43	0	0	9	22
Romero et al. (2020)	LO	M	0	0	0	0	0	0	100
Rosa et al. (2013)	LO	M	0	0	0	100	0	0	0
Sánchez-Fernández et al. (2010)	B	M	0	83	17	0	0	0	0
Segurado et al. (2018)	LO	O	50	38	13	0	0	0	0
Soria et al. (2020)	LO	O	57	21	21	0	0	0	0
Velasco Ayuso et al. (2017)	LE	M	50	0	0	0	50	0	0
Villar-Argáiz et al. (2009)	LE	M	0	63	13	25	0	0	0
Villar-Argáiz et al. (2012)	LE	M	0	0	29	71	0	0	0
Villar-Argáiz et al. (2016)	LE	M	17	17	0	50	0	0	17

pressures (e.g., land-use), although we acknowledge that their impacts act at different temporal and spatial scales (Feld et al., 2016; Jackson et al., 2021). In all cases, we defined stressors as abiotic or biotic environmental conditions exceeding the normal range experienced by organisms, which cause potential injurious changes to biological systems (Bijlsma & Loeschcke, 2005).

For each modelled response in regression-type models, we retrieved the occurrence and direction of significant effects of individual and interactive model coefficients (non-significant when $p \geq 0.05$; positive or negative when $p \leq 0.05$). For individual stressors, significant positive or negative effects reflect an increase or decrease, respectively, in the response variable magnitude; note that these effects were classified based on statistical responses irrespectively to their implications in biomonitoring, management or conservation. In analysis of the variance (ANOVA-type) models, we obtained the direction of individual and combined effects on each response variable by comparing values in control *vs* highest stress treatments and looking at the *post-hoc* tests (when available) and/or plots. In multilevel manipulative experiments (i.e., those with more than two levels of each stressor), for simplicity, we focused on the highest level observed before the event of total mortality or performance decrease of individuals. We are aware that these procedures ignore potential non-linear responses to stressors. However, synthesising data across multiple studies requires the use of simple indicators that are easy to retrieve and compare across studies. To harmonize the direction of stressor effects on organism performance in biological responses measured at the individual level, we ensured that the direction of individual effects on response variables correlates positively with performance (e.g., using survival instead of mortality), changing the sign of the effect when necessary. This ensures that performance effects found across studies are comparable.

Based on the significance and sign of model coefficients (effect sizes), and assisted by classifications found in the examined studies, we typified the combined effects found for each modelled response as described in the following lines (Feld et al., 2016; Jackson et al., 2016; Piggott et al., 2015). We assigned single-stressor dominance effects when only one stressor had significant effects on the response variable. This effect is mutually exclusive respect to additive or interactive effect types. Additive effects were assigned when two or more stressors had a significant effect on the response variable as individual terms (their interactions were non-significantly different from zero). In models with significant interaction terms and more than two stressors, we also quantified additive effects when other terms not included in such interactions had a significant individual effect. We assigned interactive effects when one or more interaction terms were significantly different from zero ($p < 0.05$). The type of interactive effect (antagonistic, synergistic, opposing and reversal) was classified according to the signs and magnitudes of the individual and interactive stressor coefficients, as described in the following lines. Synergistic effects were assigned when the absolute interaction coefficient was greater than the sum of their absolute individual coefficients, having individual and interactive coefficients the same sign. Antagonistic or reversal effects were assigned when both individual coefficients had the same sign, but the interaction coefficient showed a different sign. Besides this condition, antagonistic effects were assigned when the absolute value of the interaction coefficient was lower than the sum of the absolute individual coefficients. Reversal effects were assigned in the opposite case (i.e., the absolute interaction coefficient was greater than the sum of the absolute individual coefficients; Jackson et al., 2016). We assigned interactions as opposing when the signs of the individual coefficients differed (Feld et al., 2016). To summarize, we pooled opposing and reversal interactions together into "other" interactions category. We also counted the number of significant high-order interactions (3-way or 4-way interactions).

Data analysis

Based on the collected information, we provided general descriptors of the studied locations, experimental approaches, aquatic systems, level of biological organization, organism trophic type and size, response types, individual stressor effects and combined effects. Before calculating descriptive statistics among studies (e.g., means, medians, standard deviations), we averaged values of cases belonging to the same study to avoid overrepresenting studies with more cases. For the analysis of the key individual stressors and interaction pairs, we focused on those being analysed at least in five different studies to ensure a minimum degree of generality and replication. Descriptive statistics were conducted using R version 4.0.3 (R Development Core Team, 2020).

RESULTS

Study aspects, experimental approaches, organism types and modelled responses

We found 61 studies that met our criteria (Table 2), including 409 biological and ecosystem responses to multiple-stressors in Iberian freshwaters (Table S2, Supplementary material, available at http://www.limnetica.net/en/limnetica). We observed a median of six cases (modelled responses) per study, showing a high variability across studies (range: 1 to 56). The number of published papers increased over time, reaching a maximum in 2017 and 2018, when eight papers were published (Fig. 1a). Studies were more frequently conducted in Spain (66 %) than in Portugal (34 %). Studied organisms and ecosystems belonged to a total of 17 Iberian watersheds, with the Mediterranean catchments of Andalusia (22 %), Segura (16 %) and Interior Catalan Catchments (9 %) being the most frequently addressed for Spain, and the Cávado (8 %), Mondego (6 %) and Tagus (6 %), the most covered in Portugal. Lotic systems (59 %) were more represented than lentic systems (41 %) (Fig. 1b). Studies mostly carried out manipulative approaches (85 %), while observational studies were less frequent (15 %) (Fig. 1c). Responses at community level were more usual (70 %) than those at individual and population levels (30 %) (Fig. 1d). Among organism trophic types, heterotrophs were the most represented group (58 %), being autotrophs (30 %) and mixtures of both (12 %) less studied (Fig. 1e). Microorganisms (58 %) were more frequently covered than macroorganisms (42 %) (Fig. 1e). Studies showed a mean of 63% of the cases exploring biological responses to multiple-stressors, being smaller the share of ecosystem responses addressed (37 %) (Fig. 1f). Biomass (14 %) and biodiver-

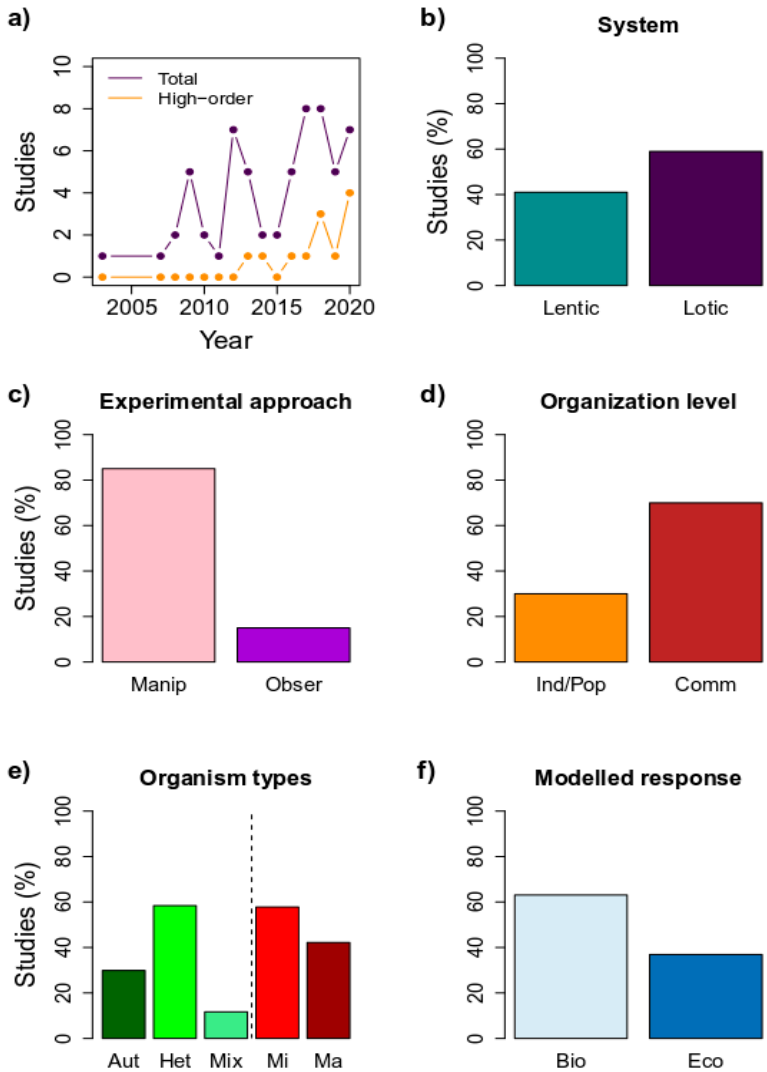

Figure 1. General descriptors of multiple-stressor studies in Iberian freshwater ecosystems: (a) Number of published articles per year (specifying also those exploring high-order interactions, 3- and 4-way), (b) study system (lotic or lentic), (c) experimental approach (manipulative or observational), (d) level of biological organization (individuals/populations or communities), (e) organism trophic strategy (auto-,heterotrophs or mixed) and organism size (micro- or macroorganism) and (f) type of modelled response (biological or ecosystem). *Descriptores generales de los estudios de múltiples estresores en los ecosistemas acuáticos ibéricos: (a) Número de artículos publicados por año (especificando aquellos que exploran interacciones de 3^er^ o 4º orden), (b) sistema estudiado (lótico o lenítico), (c) tipo de aproximación experimental (manipulativo vs observacional), (d) nivel de organización (individuo/población o comunidad), (e) estrategia trófica (organismos autótrofos, heterótrofos o de estrategia mixta) y tamaño (micro o macroorganismos) y (f) tipo de respuesta modelada (biológica o ecosistémica).*

sity (12 %) responses were the most represented biological responses, while the most frequent ecosystem responses were primary production (11 %) and organic matter decomposition (10 %). When comparing between experimental designs, 38 % of the manipulative studies explored both biological and ecosystem responses, while observational studies only addressed biological responses.

Key individual stressors and their overall effects on Iberian freshwaters

The reviewed studies covered 13 types of individual stressors, with a median of two stressor types per study and ranging from studies focused on a single type of stressor (e.g., two different pesticides, Toxic x Toxic) to others addressing up to six different stressor types (Table S3, Supplementary material, available at http://www.limnetica.net/en/limnetica). The seven individual stressor types most frequently explored were nutrient enrichment (Nutr: 28 studies), thermal stress (Therm: 25), hydrological stress (Hydr: 20), ultraviolet radiation stress (UVR: 18), toxic stress (Toxic: 14), salinity stress (Sal: 13) and land-use pressure (Land: 5) (Table S4, Supplementary material, available at http://www.limnetica.net/en/limnetica). Overall, we found a higher proportion of negative responses (34 %) compared to the share of positive effects (26 %), while 40 % of responses were non-significant. Nutrient enrichment (43 %) and thermal stress (35 %) were the stressors that showed a higher proportion of significant positive effects (i.e., magnitude increase) on biological and ecosystem responses, yet with a remarkable share of significant negative effects (nutrient enrichment: 22 %, thermal stress: 23 %) (Fig. 2). Conversely, hydrological stress, UVR, toxic stress, salinity stress and land-use pressure were the stressors that had a higher proportion of negative effects (ranging from 35 to 88 % of the evaluated effects). These overall patterns varied depending on the studied aquatic system, organism trophic strategy or size. Significant negative effects of thermal stress were more prevalent than positive ones on lentic systems (negative: 54 %, positive: 32 %), whereas significant positive effects were more frequent than negative responses in lotic systems (negative: 13 %, positive: 47 %). Hydrological stress tended to have a higher share of significant negative effects on autotrophic organisms (negative: 53 %, positive: 4 %), whereas the share of significant positive effects of nutrients was more evident on autotrophs (negative: 18 %, positive: 44 %) and mixed strategy (negative: 0 %, positive: 70 %). For macroorganisms, we found a prevalence of significant negative effects of nutrients (negative: 39 %, positive: 18 %), while for microorganisms we found the opposite pattern (negative: 19 %, positive: 45 %). Thermal stress tended to have a higher share of significant positive responses in microorganisms (42 %) relative to macroorganisms (33 %).

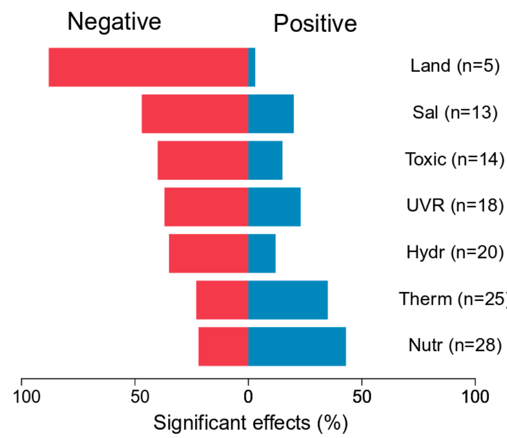

Figure 2. Percentage of significant negative (red) and positive (blue) individual effects found in studies, aggregated by stressor type. Land: land-use pressure, Sal: Salinity stress, Toxic: Toxic stress, UVR: Ultraviolet radiation, Hydr: Hydrologic stress, Therm: Thermal stress, Nutr: Nutrients. *Porcentaje de efectos individuales negativos (rojo) y positivos (azul) encontrados en los estudios, agregados por tipo de estresor. Land: presión por uso del suelo, Sal: salinidad, Toxic: estrés por toxicidad, UVR: radiación ultravioleta, Hydr: estrés hidrológico, Therm: estrés térmico, Nutr: nutrientes.*

Frequency of combined effect types on Iberian freshwaters across study and organism features

The examined studies had a median of one significant combined effect per study but showed a large variability (from 0 to 5). Overall, half of the combined effects were interactive and half were non-interactive (Table S5, Supplementary material, available at http://www.limnetica.net/en/limnetica). Antagonistic effects (18 %) were the most common interactive effects followed by synergistic (15 %), reversal and opposing (13 %) and high-order interactions (4 %). Among non-interactive effects, additive effects (25 %) were more frequent than dominance (13 %) and non-significant responses (12 %). These overall patterns showed differences within experimental approaches, organization level, organism trophic

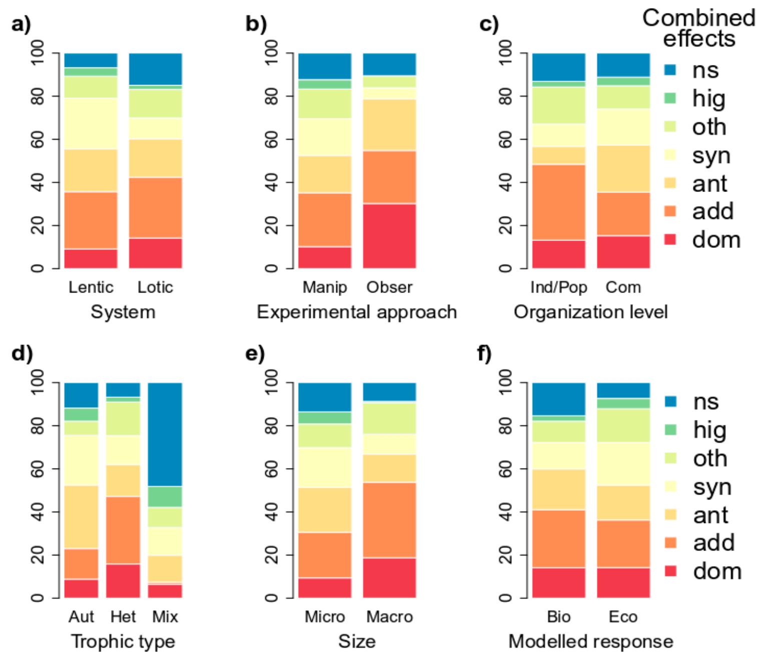

Figure 3. Percentage of combined effects aggregated by: (a) study system, (b) experimental approach (manipulative or observational), (c) level of biological organization (individual/population or community), (d) organism trophic strategy (autotroph, heterotroph or mixed), (e) organism size (microorganism or macroorganism) and (f) type of modelled response (biological or ecosystem). Combined effect labels: dom: dominance, add: additive, ant: antagonistic, syn: synergistic, oth: other (opposing, reversal), hig: high-order (3- & 4-way), ns: non-significant. *Porcentaje de efectos combinados agregados por: (a) sistema estudiado (lótico o lenítico), (b) aproximación experimental (manipulativo u observacional), (c) nivel de organización (individuo/población o comunidad), (d) estrategia trófica (organismos autótrofos, heterótrofos o de estrategia mixta), (e) tamaño (micro o macroorganismos) y (f) tipo de respuesta modelada (biológica o ecosistémica). Tipos de efectos combinados: dom: dominancia, add: aditivo, ant: antagonista, syn: sinérgico, oth: otros (opuestos, reversos), hig: interacciones de 3er o 4º orden, ns: no significativo.*

group and size (Fig. 3). Studies performed in lentic systems had a higher percentage of interactive responses (58 %) than those carried out in lotic systems (44 %). These differences across systems were particularly evident for synergisms (lotic: 10 %; lentic: 24 %) (Fig. 3a). Manipulative studies exhibited a higher share of interactive responses (53 %) than observational ones (34 %) (Fig. 3b). Dominance (30 %) and additive (25 %) responses were the most frequent in observational studies, followed by antagonistic effects (24 %). Synergistic responses were more frequent in manipulative studies (17 %) than in observational ones (5 %). The share of interactive responses was lower for individual and population (39 %) than for the community level (54 %) (Fig. 3c). Interactive responses also showed a higher occurrence on studies on autotrophs (65 %) and microorganisms (56 %) relative to those focused on heterotrophs (46 %) and macroorganisms (37 %) (Fig. 3d, e). We also found a higher frequency of synergistic effects on autotrophs (23 %) and microorganisms (18 %) than in heterotrophs (13 %) and macroorganisms (9 %), respectively. Ecosystem responses tended to exhibit more interactive responses (57 %) than biological ones (44 %) (Fig. 3f).

The majority of studies examined pairwise stressor interactions (80 %). Those addressing 3-way (17 %) and 4-way (3 %) stressor interactions were scarce but increased over time (Fig. 1a). We found a total of 34 stressor pairs, but studies mostly focused on six of these combinations (Nutr × UVR, Sal × Therm, Therm × Toxic, Hydr × Toxic, Nutr × Therm and Toxic × Toxic), which represented 52 % of the total studied pairs over all the studies. The higher proportions of sig-

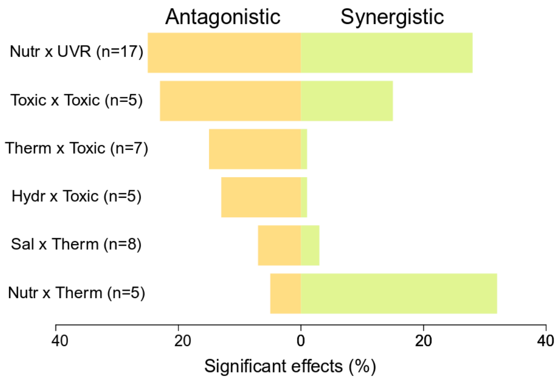

Figure 4. Percentage of significant antagonistic (light orange) and synergistic (light green) effects found in studies, aggregated by stressor pairs. Hydr: Hydrologic stress, Nutr: Nutrients, Sal: Salinity stress, Therm: Thermal stress, Toxic: Toxic stress, UVR: Ultraviolet radiation. *Porcentaje de efectos significativos antagónicos (naranja claro) y sinergísticos (verde claro) encontrados en los estudios, agregados por tipo de estresor. Hydr: estrés hidrológico, Nutr: nutrientes, Sal: estrés por salinidad, Therm: estrés térmico, Toxic: estrés tóxico, UVR: radiación ultravioleta.*

nificant interactive effects were observed for the pairwise combinations Nutr × UVR (65 %) and Nutr × Therm stress (51 %), which also showed a higher proportion of synergisms relative to antagonisms (Fig 4). On the other hand, antagonisms were more frequent for the pairs Toxic × Toxic, Hydr × Toxic, Therm × Toxic and Sal × Therm. When exploring patterns within study aspects, we found some trends that differed from the overall patterns reported. For the pair Sal × Therm, a higher percentage of significant anstagonisms (15 %) and other interactions (25 %) were found in lotic systems relative to those found in lentic ones (antagonistic: 7 %, others: 2 %). For the pair Nutr × UVR, we found a higher percentage of significant synergistic responses for heterotrophs (38 %) relative to autotrophs (22 %), for which antagonistic responses were slightly more frequent (24 %). Autotrophs (15 %) exhibited a higher percentage of significant opposing and reversal interactions (pooled as "others") than heterotrophs (8 %).

DISCUSSION

Our review found that multiple-stressor studies carried out so far in the Iberian Peninsula are biased towards lotic systems and heterotrophic organisms, and focused on seven main stressors (nutrient enrichment, thermal, hydrologic, UVR, toxic and salinity stresses and land-use pressure) and six of their potential pairwise combinations. Other stressor types, such as invasive species or morphological alterations, have been scarcely considered in the reviewed papers. Despite their valuable contribution, these studies covered just a portion of the multiple-stressor spectrum. Most of them were based on manipulative experiments (particularly, for ecosystem responses), which limits to some degree the transferability of their findings to real-world ecosystems. The observed biases might in part reflect that research groups exploring multiple-stressor effects on Iberian freshwaters tend to focus on stressor combinations occurring regionally and on taxonomic groups and techniques that match their expertise. Although this is fundamental to explore impacts with immediate relevance for management, it might ignore global stressors gradually intensifying over time, such as climate change or toxic compound accumulation. On the positive side, this situation gives rise to a chance for a more coordinated and collaborative multiple-stressor research agenda, which addresses global change effects in the Iberian freshwaters more comprehensively. Despite the narrow scope, the intense efforts conducted by these 'multiple-stressor clusters' provide an opportunity to synthesise their results and identify consistent patterns with a greater confidence.

For individual stressors, we found an overall negative effect (i.e., decrease in the response variable magnitude) caused by the prevalent negative effects of land-use pressure (Bruno et., 2016), salinity (Gutiérrez-Cánovas et al., 2015), toxicants (Beketov et al., 2013), hydrological stress (Arias-Real et al., 2020) and UVR (Carrillo et al., 2008), which are in line with previous studies. We found that nutrient enrichment and thermal stress had a higher proportion of positive effects (i.e., increasing response magnitude), probably because of the high number of studies performed in cold, oligotrophic lakes (e.g., Carrillo et al., 2008; Durán et al., 2016; González-Olalla et al., 2018). In these particular conditions, moderate additions of inorganic nutrients or warming can boost productivity, diversity and ecosystem functioning (Coveney & Wetzel, 1992; Häder et al., 2014; Woodward et al., 2012). However, the final outcome of nutrient or thermal changes on freshwater ecosystems strongly depends on their magnitude, duration and direction (Barton et al., 2020; Durán-Romero et al., 2020; Padfield et al., 2016). Previous studies have shown that nutrients seem to have overall negative effects only at high concentrations (Birk et al., 2020; Nõges et al., 2016; Woodward et al., 2012), although aspects such as productivity can be still high at eutrophic conditions (Arroita et al., 2019). On the other hand, manipulative changes in temperature give rise to contrasting responses depending on multiple methodological aspects related with stress intensity and duration (Barton et al., 2020; Duffy et al., 2021; Terblanche et al., 2011).

Generally, we found a balanced share of non-interactive and interactive responses. However, the proportions and types of non-interactive and interactive effects varied across methodological aspects and biological features of the organisms. Thus, in observational studies focusing on individual or population-level responses of heterotrophs and microorganisms, we found a prevalence of non-interactive effects, while the opposite pattern was found for manipulative studies. These results are consistent with previous meta-analysis studies synthesising the results of manipulative experiments in marine and freshwater ecosystems, which also found a greater proportion of interactive responses (74 % - 99 %) (Crain et al., 2008; Jackson et al., 2016; Manuel Villar-Argaiz et al., 2018). By contrast, analyses addressing both experimental and observational studies detected a prevalence of non-interactive responses (non-interactive: 67 %, vs. interactive: 33 %) (Birk et al., 2020). The impossibility to control for the multiple factors operating in field conditions might have limited the capacity of observational studies to detect potential interactive responses for specific stressor pairs (Birk et al., 2020; Spears et al., 2021). On the other hand, manipulative studies are typically performed over short periods and may overestimate interactive responses associated to potential acclimation, adaptation, recovery or resilience mechanisms (Collins et al., 2020; Orr et al., 2020). In this vein, although most studies generally assume that multiple-stressors operate simultaneously, this is a rare case in natural systems (Jackson et al., 2021) because the temporal dynamics of stressors can be either discrete (e.g., agricultural nutrient pollution) or continuous (e.g., average temperature). Therefore, short-term manipulative studies might have overlooked the plausible adaptation or legacy effects of past experienced stressors that allow organisms and communities to resist or even recover from multiple-stressor effects (Cañedo-Argüelles et al., 2014; Jackson et al., 2021; Lake, 2000). Despite the lower ecological realism inherent to manipulative studies, they allow us to identify the mechanisms underlying response patterns to multiple-stressors, and together with field-based evidence, they aid to understand the complexities of real-world systems (Birk et al., 2020; Jochum et al., 2020). The level of biological organization of the modelled responses along with organism's trophic strategy can also influence the proportion of interactive effects (Jackson et al., 2016). For example, previous meta-analyses showed that dominant or additive responses tend to be more common in autotrophs (Birk et al., 2020; Jackson et al., 2016; Velasco et al., 2019). However, we found the opposite pattern, potentially due to the prevalence of manipulative experiments. Furthermore, hierarchical dominance of some stressors can determine the combined outcome towards non-interactive prevalence, as occurs with salinity (Velasco et al., 2019).

In relation with the interaction types, we found an overall prevalence of antagonisms over synergisms, which was consistent across four out of six of the most studied pairwise stressor combinations. These results are in line with previous freshwater meta-analyses (Birk et al., 2020; Jackson et al., 2016; Teichert et al., 2016; Velasco et al., 2019; Manuel Villar-Argaiz et al., 2018) and contrast with the higher prevalence of synergisms found in marine ecosystems (Crain et al., 2008; Przeslawski et al., 2015; Manuel Villar-Argaiz et al., 2018). Disturbance regime, microevolutionary selection and the evolutionary abiotic context might explain the prevalence of antagonisms in freshwaters. First, freshwater organisms are adapted to frequent disturbance and high abiotic dynamism (e.g., recurrent floods and drying events, daily changes in dissolved oxygen; Lake, 2000), exhibiting adaptations that can provide resistance against some anthropogenic stressors (Petrin et al., 2007; Soria et al., 2020). Second, there is evidence suggesting that long-term exposure to stressors can trigger evolutionary selection of species traits. These processes can favour stress-resistant genotypes or phenotypes (Cothran et al., 2013; Higgins et al., 2014) that could co-tolerate further stressors (Gotcha et al., 2018; Hintz et al., 2019). Third, the evolutionary context of some clades make them resistant to certain stressor combinations, as observed for the increased resistance to metals and acidity in stoneflies (Buchwalter et al., 2008) and the co-tolerance to desiccation and salinity in some water beetles (Pallarés et al., 2017a; Pallarés et al., 2017b). Notably, exceptions to the prevalent antagonistic pattern were the pairs Nutr × Therm and Nutr × UVR, for which we observed a higher frequency of synergisms. The combination of nutrient enrichment and warming can boost autotrophic production (Doyle et al., 2005; González-Olalla et al., 2018; Kosten et al., 2012), whereas nutrient pulses seem to accentuate the harmful UVR effects on autotrophs (Carrillo et al., 2008). From a management perspective, synergistic interactions are expected to cause the most detrimental impacts on ecosystems, while they also offer opportunities to apply cost-effective restoration measures. In this case, even a single stressor abetment is expected to result in a greater improvement of the ecological status than in additive or antagonistic scenarios (Brown et al., 2013; Crain et al., 2008; Piggott et al., 2015; Spears et al., 2021). In cases where stressors show dominance or additive effects, tackling those exerting stronger effects will offer a cost-effective mitigation solution (Velasco et al., 2019), while antagonisms require a tailored approach (Spears et al., 2021).

Our review presents some limitations due the exclusion of a large proportion (70%) of the initially scanned papers that did not meet our selection criteria. Excluded papers either did not evaluate the effects of multiple-stressors simultaneously, did not statistically test interactions between stressors or reported insufficient results to classify stressor combined effects. This reduction of our initial dataset has considerably limited the coverage of different systems, levels of biological organization, organism types and modelled responses. Further research should consider all these methodological aspects to ensure that empirical works analyse data appropriately (Feld et al., 2016; Spears et al., 2021) and provide comparable information that can be used in future syntheses (Birk et al., 2020; Schäfer & Piggott, 2018). Specifically, studies should clearly report stressor coefficients / effect sizes, *p*-values of main effects and *post-hoc* analyses (when apply), and ideally, measures of goodness of fit of the models and the classification on the combined effects.

According to the knowledge gaps identified in our review, we propose six research priorities to guide future studies addressing the effects of multiple-stressors in the Iberian Peninsula:

1. ***Intensifying the research on lentic systems.*** Lentic systems were less represented in Iberian multiple-stressor studies and were focused on a limited number of stressors (nutrient enrichment, UVR and thermal stress) (Cabrerizo et al., 2017; Carrillo et al., 2008; Durán-Romero et al., 2020). Therefore, we urge to investigate more lentic systems and pairwise combinations, including other typical stressors such as biological invasions (Gallardo et al., 2016), toxicants (Hijosa-Valsero et al., 2016) or hydrologic stress (Álvarez-Cobelas et al., 2001).

2. ***Collect more observational data.*** Given the scarcity of field-based multiple-stressor studies in the Iberian Peninsula, there is a need to perform more observational work to confront against manipulative evidence. Observational studies can be useful to identify common stressor pairs in the field that are still unexplored from an experimental perspective (Spears et al., 2021). The growing availability of observational data resulting for intensive Water Framework Directive (WFD) monitoring efforts offers a cost-effective opportunity to fill this gap.

3. ***Investigating more autotrophic responses.*** Although some recent progress has been made (Cabrerizo et al., 2020; Romero et al., 2018), there is still a low representation of autotrophic organisms in the Iberian context. Given that some widely used WFD indicators data include autotrophic organisms (diatoms and macrophytes),

we underscore the need of addressing multiple-stressor effects based on these low-cost, observational sources. Future studies will benefit from the improvement and wider availability of molecular tools that ease the identification of autotrophic microbes and related functions (Picazo et al., 2020; Romero et al., 2020).

4. ***Addressing impacts on biodiversity - ecosystem functioning relationships.*** Reviewed multiple-stressor studies exploring changes in ecosystem properties and functions were all manipulative, which indicates a strong gap for real-world systems. Furthermore, we did not find any study exploring multiple-stressor effects on the biodiversity - ecosystem functioning relationship or ecosystem service provision in the Iberian Peninsula. Investigating such aspects is crucial to understand the ecological mechanisms through which global change and biodiversity loss affect global biogeochemical cycles (Aufdenkampe et al., 2011; Vinebrooke et al., 2004) and the contribution of Iberian freshwaters to people's welfare (Felipe-Lucia et al., 2014; Grizzetti et al., 2019).

5. ***Wider cover of stressor pairs.*** We found a strong focus on just six stressor pairs. Considering that nutrient enrichment is a widespread stressor in agricultural and urban areas (Birk et al., 2020; Monteagudo et al., 2016), the study of its combinations along with other common Iberian stressors (hydrological, salinity or toxic stresses) (Estévez et al., 2019; Kuzmanovic et al., 2017; Mellado-Díaz et al., 2019) can help to better target mitigation measures and to improve the ecological status of Iberian waterbodies. Since the Iberian Peninsula holds some of the most arid landscapes in Europe and climate change is predicted to intensify this situation (de Luis et al., 2010), it is of paramount importance to understand how the combination of thermal × hydrological stress can alter Iberian freshwater ecosystems. A greater focus should be also put on biological invasions, which represent a major threat to freshwater biodiversity (Gallardo et al., 2016), and whose ecological effects are strongly driven by interactions with other stressors (Rolls et al., 2017).

6. ***Considering more high-order stressor interactions.*** Most of the reviewed studies focused on pairwise interactions, whereas global change can yield more complex interactions (Cabrerizo et al., 2020; Rillig et al., 2019; Romero et al., 2019). Factorial designs including three or four stressors will require more resources (time, sampling effort, materials and personnel) and are often difficult to interpret and communicate to managers. However, recent research indicates that high-order interactions can help to build more accurate and realistic predictions of global change effects and unveil multiple-stressor effects that are acting in a hidden way (Boyd et al., 2016).

Because of its geographic situation, climate and economic model, the Iberian Peninsula is expected to suffer the impacts of climate change more strongly than other areas where water resources are more abundant and food production and tourism have a lower weight on economy (Döll & Schmied, 2012). Climatic models predict an aridification of the Iberian Peninsula (Drobinski et al., 2020), which combined with increasing demands for food production and urban areas (Roser & Ritchie, 2013), would exert an extreme pressure on Iberian aquatic ecosystems, people and economy – all strongly dependent on clean water availability. To tackle this multiple-stressor scenario, we need to implement mitigation actions that simultaneously preserve ecosystem health and ensure food and water supply. These actions must be supported by an extensive and solid empirical knowledge. Although we are still far from reaching this goal, our synthesis can establish a starting point to better understand multiple-stressor effects and to guide future research and management actions in the Iberian Peninsula.

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