

1 **Historical distribution of freshwater fishes and the reference**
2 **conditions concept in a large Mediterranean basin**

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4 **Running title:** Long-term dynamics of fishes in a Mediterranean basin

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15

16 **Abstract** (300 words)

17

- 18 1. The EU Water Framework Directive 2000/60/EC (WFD) defines the ecological
19 status of the aquatic systems as the deviation between their current biological state
20 and that which they ought to display in the absence of any major human
21 disturbance, referred to by the WFD as the Reference Condition (RC). It assumes
22 that their biotic composition should remain balanced and constant over time. In this
23 paper we have tested both assumptions against an analysis of the historical
24 distribution of the fish fauna in a large and highly disturbed Mediterranean basin. If
25 fish communities substantially change over time, it will mean that the validity of
26 the RC concept comes into question.
- 27 2. Using presence/absence data for historical native fish fauna from the Guadalquivir
28 basin, distribution changes among sub-basins were quantified by mapping between
29 the 19th century and today.
- 30 3. The range of two native species (*Anguilla anguilla* and *Salmo trutta*) has changed
31 significantly. In addition, diadromous species assemblage has practically become
32 locally extinct, with the exception of the eel. Finally, most Guadalquivir sub-basins
33 (94.7%) have suffered major changes in the composition of their fish communities,
34 either by losing native species or by adding new non-native species.
- 35 4. These results render the definition of any RC unlikely. In Mediterranean areas, the
36 WFD objective of 'good' ecological status recovery based on the integrity of aquatic
37 communities is a theoretical rather than a real goal. Nonetheless, the WFD provides
38 an ecological guiding principle that can also be transferred to the conservation of
39 freshwater ecosystems.
- 40 5. As an alternative to the RC concept in Mediterranean lotic ecosystems, specific
41 multimetric indexes can be used, based on expert criteria, the metrics of which can
42 also relate to the conservation value of water bodies, and not only to their
43 ecological status.

44

45 **Keywords:** river; reservoir; stream; ecological status; distribution; Water Framework
46 Directive; fish; agriculture; alien species; impoundment.

47

48 **1. INTRODUCTION**

49 The European Union (EU) Water Framework Directive 2000/60/EC (WFD) is widely
50 regarded as the most ambitious piece of European environmental legislation to date. At
51 the same time, through different management proposals from a global ecological
52 perspective which set the use of habitat and species as quality goals, the WFD has
53 served as a freshwater conservation tool on a pan-European scale (Boon and Lee, 2005).
54 However, some 20 years since its inception, the WFD has failed to deliver on its
55 objectives, and has gone from showing great promise to suffering implementation
56 problems (Moss, 2008; Voulvoulis et al, 2017; Ramos-Merchante and Prenda, 2017).
57 The Directive defines 'high' ecological status as the state of the system in the absence of
58 any anthropogenic pressures, or only slight biological deviation from what would be
59 expected under undisturbed conditions (Council of the European Commission, 2000).
60 There are two main assumptions implicit in the adoption of this definition: 1) it is still
61 possible to find aquatic European ecosystems undisturbed by human activities, and 2)
62 these ecosystems are static, their composition remaining balanced and constant over
63 time. The good status category is one of the main objectives of the WFD. Another
64 objective is 'no deterioration', so a water body that is at present at high status must not
65 be allowed to fall to good status.

66 Biotic integrity, originally considered an elusive legislative concept by its main
67 promoters (Karr *et al.*, 1986), refers to the biological condition displayed by aquatic
68 ecosystems in which 'composition, structure, and function have not been adversely
69 affected by human activities' (Karr *et al.*, 1986; Moore *et al.*, 1999). The most common
70 assessment of biotic integrity is conducted through the well-known and widely-used
71 multimetric Index of Biotic Integrity (IBI) (Simon and Lyons, 1995; Aparicio *et al.*,
72 2011; Hermoso and Clavero, 2013; Ramos-Merchante and Prenda, 2018). One of the
73 main contributions of this approach relies upon the relative condition of the ecosystem
74 being evaluated with respect to a theoretical 'pristine' status, or 'Reference Conditions'
75 (RC), free from human disturbance. These RC have been codified by the WFD for the
76 protection and improvement of the ecological condition of streams and defined by
77 Stoddard *et al.*, (2006), as 'the biotic integrity or natural state for which long-term
78 variability is acceptable within relatively narrow limits'. The IBI thus measures the
79 deviation observed between the current biological state of the site and that which it
80 ought to display in the absence of any major human disturbance.

81 These RC, often considered in bioassessment methods (Bailey *et al.*, 1998), may serve
82 as a useful theoretical framework for the determination of ecological changes. The
83 characterisation of RC, in the case of biotic communities (e.g. fishes), has followed at
84 least three general approaches: (1) predictions from empirical models, (2) minimally
85 impacted sites, and (3) historical data. However, some studies have raised questions
86 about considering least impacted reference sites as 'natural' conditions that arguably no
87 longer exist within contemporary landscapes (Hobbs *et al.*, 2006; Aparicio *et al.*, 2011;
88 Dallas, 2013; Labay *et al.*, 2015; Ramos-Merchante and Prenda, 2018). Studies of

89 bioassessment rely on benchmarks using pristine conditions (Karr, 1981), that are
90 becoming increasingly difficult to find in today's world, particularly in highly-changing
91 environments such as the Mediterranean (Geri *et al.*, 2010; Aparicio *et al.*, 2011).
92 Humans have distorted their supporting ecosystems throughout history, making any
93 definition of undisturbed reference states ambiguous, to say the least (Hobbs *et al.*,
94 2006; Connor *et al.*, 2019; Manzano *et al.*, 2019). Almost every ecosystem experiences
95 some form of human disturbance, sometimes deep and permanent. Consequently,
96 reference status sites are difficult to establish properly, a problem which is particularly
97 pronounced in the case of freshwater fish communities, one of the world's most
98 disturbed and poorly-known biological communities (Abell, 2002; Kottelat and Freyhof,
99 2007; Freyhof and Brooks, 2011). Most catchment landscapes have been greatly altered
100 during the last centuries (e.g. Boivin *et al.*, 2016), directly influenced by multiple
101 anthropogenic factors, the consequences of which are difficult to classify (Hoffmann
102 2005, Dudgeon *et al.*, 2006; Prenda *et al.*, 2006). The Industrial Revolution of the late
103 18th century precipitated this large-scale distortion, resulting in an unprecedented
104 increase in both anthropogenic changes and the use of natural resources (Steffen *et al.*,
105 2007; Kopf *et al.*, 2015). As a result, very few, if any, of the world's rivers, retain their
106 original functional integrity (Jungwirth *et al.*, 2002; Schletterer *et al.* 2014). The
107 definition of targets for the restoration of impacted ecosystems in a changing world is,
108 therefore, extremely challenging, if not impossible (Nijboer *et al.* 2004; Bouleau and
109 Pont, 2015).

110 The RC concept implicitly assumes that the composition of freshwater communities
111 remains stable through time. Does the species composition really persist more or less
112 unaltered for, say, decades or centuries? The use of a fixed list of components at any
113 given time is a futile exercise and does not consider the fundamental importance of
114 ecosystem variability (Moss, 2008). Over extremely long periods, fish communities can
115 vary greatly (e.g. Muñoz and Casadevall, 1997). Therefore, rather than identifying
116 potential reference sites in indiscernibly transformed landscapes, knowledge of major
117 influential factors on freshwater integrity may be achieved by taking data from previous
118 centuries and then building basic structure and function patterns for the species present
119 in those times (Clavero and Villero, 2014).

120 Historical survey data serves as an important source of information for the
121 reconstruction and identification of species distribution changes, facilitating the
122 knowledge of a freshwater fish status baseline prior to the generalised, large-scale
123 environmental changes that have occurred in recent decades (e.g. major land
124 transformation, industrialisation, construction of transversal barriers, and so forth.)
125 (Habit *et al.*, 2010; Haidvogel *et al.*, 2015a; Pont *et al.*, 2015; Chakona *et al.*, 2020).
126 Several studies have taken information from the last few centuries to create least
127 disturbed communities, to estimate RC, or to develop conservation programmes
128 (Aparicio *et al.*, 2000; Filipe *et al.*, 2009; Habit *et al.*, 2010). Applied historical ecology
129 – the use of historical knowledge in the management of ecosystems – provides a

130 baseline, or a picture of ecosystem health (Stoddard *et al.*, 2006; Haidvogel *et al.*,
131 2015a). The historical ecology data must include chronological sequences of
132 observations of sufficient duration to detect significant changes in both populations and
133 dynamical behaviour and shifts in the main distributions patterns (Swetnam *et al.*, 1999)
134 or alternatively by using molecular data (Chakona *et al.*, 2020).

135 The Guadalquivir Basin in the Mediterranean area is one of the most highly-
136 transformed major river systems in the Iberian Peninsula, particularly since 1900, with
137 the commencement of generalised damming (Granado-Lorencio, 1991; Clavero *et al.*,
138 2004; Prenda *et al.*, 2006). Furthermore, the Guadalquivir drainage network has been
139 greatly affected by recent industrialised agricultural practices ('super-intensive' olive
140 groves, in particular) and by the manifold consequences of a dense human population
141 inhabiting a semiarid land (Ramos-Merchante and Prenda, 2018). However, as the
142 damage which has been caused by anthropogenic disturbances is not irreversible, the
143 WFD assumes that the removal of these causes would mean a return to previous
144 conditions (Bouleau and Pont, 2015). As it is currently impossible to define any aquatic
145 location in the Guadalquivir Basin as pristine (see Ramos-Merchante and Prenda, 2017),
146 RC have to be assessed exclusively via the analysis of historical data or from other
147 rivers in the same ecoregion. Historical reference points are critical for the measurement
148 and interpretation of long-term changes, and for the establishment of meaningful targets
149 for management, restoration and recovery, such as the WFD RC concept. However, the
150 application of historical baselines to ecosystem management and conservation activities
151 must take into consideration the impossibility of restoring ecosystems to previous
152 historical ranges of variability (HRV). A contemporary perspective requires the
153 adaptation of conservation values to manage human-dominated ecosystems (Kopf *et al.*,
154 2015). But at the very least, historical trajectories are an essential element in
155 understanding the main systems to be conserved.

156 This study uses both historical and current data on the distribution of native fish
157 species to test the validity of the RC concept following the analysis of long-term
158 changes in the fish community of the Guadalquivir Basin.

159

160 2. METHODS

161 2.1 Study area

162 The Guadalquivir River (S. Spain) (Fig. 1) has a drainage basin of 57,530 km². The
163 catchment area comprises three geological units delimited by mountain chains with
164 altitudes ranging from 1,000 m to more than 3,000 m a. s. l. The Guadalquivir Basin is
165 highly asymmetrical: geology, topography and human use vary between the left and
166 right sides of the basin. While the former, delimited by the Baetic System, one of the
167 main Spanish mountain ranges, is comprised of sedimentary flatlands, dominated by
168 intensive agriculture, with high to very high population density and few reservoirs, the
169 latter, delimited/bound by the Sierra Morena mountain range, comprised of old acidic
170 rock, is largely wooded and dominated by extensive *dehesa* (i.e., wooded meadow).
171 Unlike the left side of the basin, the main infrastructure consists of large reservoirs, with
172 relatively low population density, and although historically extensive livestock breeding
173 has been the main activity, agriculture is of minor importance today.

174 The Guadalquivir Basin is a semi-arid region (rainfall ~535 mm year⁻¹; range: 300-1100
175 mm) in which water repartition for environmental and human use is a significant and
176 controversial issue for water management (Martín-Ortega *et al.*, 2008), involving many
177 challenges; (Rodríguez *et al.*, 2007). The high temperatures, combined with a lack of
178 precipitation during summer, result in a marked water deficit (Robles *et al.*, 2002),
179 typical of Mediterranean regions. Furthermore, years of extremely high or low
180 precipitation have tended to cluster together, compounding the effects of drought or
181 flood. Most of the human population (~4.1 million) is concentrated in the lower parts of
182 the basin where irrigated agriculture constitutes one of the most widespread activities
183 and is the main consumer of blue water (192 mm year⁻¹) (Dumont *et al.*, 2013).
184 Agricultural practices demand 87% of the water resources within the catchment area, an
185 amount far exceeding any other use, including urban and industrial. At present, the
186 basin's water resources are highly regulated.

187 Lately, the EU's common agricultural policy has precipitated a rapid evolution in
188 traditional farming methods. The most significant of these changes is the 'super-
189 intensive' cultivation of olive groves, which is highly demanding in terms of water
190 resources, promotes rapid hillside erosion and requires large amounts of 'inputs',
191 (fertilisers, pesticides, etc.). The combination of these processes – irrigation + erosion +
192 external inputs – results in the significant alteration of most aquatic habitats due to a
193 permanent restriction of the water flow, with the scant water remaining containing a
194 high suspended solids load, plus pollution from fertiliser and pesticide use (González-
195 Ortégón *et al.*, 2010 , Hermosín *et al.*, 2013, Carpintero 2015).

196

197 2.2 Historical and current native fish fauna data

198 To assess distribution changes of the most prevalent native fish species, matrices to
199 historical and current period were developed.

200 The historical distribution (‘historical data’) of fishes in the Guadalquivir Basin was
201 obtained using data from the 19th century work by Pascual Madoz (1846-1850), an
202 encyclopaedic dictionary with broad social and environmental information from every
203 Spanish place name structured in a fixed manner across the text (Clavero and Villero,
204 2014). We referred to the dictionary when describing 1) rivers and wetlands, and 2) fish
205 as a food source and game species. Citations of the common names of freshwater fish
206 were found using digitalised copies of the Madoz dictionaries available at
207 www.bibliotecavirtualdeandalucia.es, through the search tool in a pdf document reader
208 (Clavero and Hermoso, 2015). The total historical information included 229 place
209 names (henceforth ‘locations’), across the Guadalquivir Basin, with 472 freshwater fish
210 records, 82 of which referred simply to ‘fish’, 42 to ‘fishing’ and 11 failed to meet the
211 criteria. A presence/absence matrix (fish x locations) of historical fish fauna was
212 developed using data that met the following criteria: 1) fishes were found in enough
213 sites for statistical analysis (usually more than five) and 2) data of freshwater native
214 species were available from both historical and current data. The species that fulfilled
215 the analysis criteria for historical period were European eel (*Anguilla anguilla*), brown
216 trout (*Salmo trutta*), Southern Iberian barbel (*Luciobarbus sclateri*), Iberian nase
217 (*Pseudochondrostoma willkommii*) and Iberian chub (*Squalius pyrenaicus*). So, the final
218 historical data set included data from 176 locations and 337 fish records (out of five
219 species).

220 Current distributions (‘current data’) were determined through 225 extensive sampling
221 sites in the Guadalquivir Basin, surveyed in 2008. Different sampling methods were
222 used depending on the width and depth of the study site. Fluvial sites with low or
223 moderate salinity ($<1.5 \text{ mS cm}^{-1}$) and shallow depth ($< 1.2 \text{ m}$) were electrofished by
224 wading upstream for an average period of 1 hour. Sites were electrofished for transects
225 of about 100-150 m in length (5-50 m channel widths), which included all geomorphic
226 channel units present in the reach (i.e. riffles, pools and runs), following the CEN - EN
227 14011:2003 standard protocol (UNE, 2003). Where electrofishing was impossible,
228 passive traps were used (two trammel nets, three pairs of fyke nets, fifteen minnow traps
229 and ten pairs of plastic minnow traps were set for a minimum of 8 h). After capture, the
230 fish were identified to species level, counted, measured for total length (TL, mm)
231 weighed (g), and then returned live to the stream. Presence/absence data matrix for all
232 freshwater fish species were generated exclusively from *locations* with catches. The
233 data set consisted of a total of 195 sampling locations in rivers (156) and reservoirs (39)
234 (Fig. 1). A total of ten native species were captured at the current period (Table 1).
235 Finally, only five species were selected for carrying out the analyses, the same as for the
236 historical analysis.

237 Historical and current fish distribution data were synthesised using sub-basins as
238 sampling units, as these represent a more appropriate natural body for the study of the
239 spatial distribution patterns of freshwater fauna and for the more accurate detection of
240 any changes (Abell *et al.*, 2007; Hermoso *et al.*, 2015). Presence/absence data from the
241 locations for both periods of time were transformed into sub-basins separately using
242 ArcMap software, ArcGis 10.1 (ESRI, Redlands, CA, USA). ArcHydro (Maidment,
243 2002) was used to divide the whole Guadalquivir Basin (total area) into 389 sub-basin
244 units from a 100-m Digital Elevation Model (DEM) of 1:25.000 scale. The distribution
245 of each of the five fish species selected was transposed from *locations* (historical place
246 names or current sampling sites) to *sub-basins* through overlapping polygons (using the
247 *Select by* tool in ArcMap). Historical names may refer to a specific location, that is just
248 one point in a sub-basin, but in some instances the name may designate a larger area
249 such as a municipality, which may imply more than one sub-basin. Finally, a total of
250 338 sub-basins out of 389 (86.2%) were selected from historical bibliographic sources.
251 Of these, 207 sub-basins contained a location with a reference to one or more of the five
252 fish species selected. Similarly, current data resulted in a total of 212 sub-basins (67.4%
253 of total sub-basins), of which 191 had fish catches.

254 To evaluate changes in the distribution range of the five native fish species selected,
255 only *sub-basins* with data available for both historical and current distributions were
256 incorporated into the analysis, so those with historical records which were not surveyed
257 in the current period were excluded. Current and historical data overlapped in 187 sub-
258 basins (60.9% of the total area). The aggregate distribution in the Guadalquivir basin for
259 each species among periods was calculated as the sum of all sub-basins occupied by
260 each species in the historical or current period individually.

261 To detect a general species distribution changes between the two periods, the
262 Guadalquivir Basin was divided into 6 evenly-sized sectors, 3 on the left side of the
263 basin and 3 on the right (see Fig. 1). A comparative approach of use/availability of
264 sectors in each period was tested through a statistical chi-square (χ^2) analysis, with the
265 null hypothesis stating there were no differences in the use of the different sectors by a
266 given species between the historical and the current period (see Grossman & Freeman,
267 1987 and Prenda *et al.*, 1997). A matrix of presence-absence with a level of significance
268 of $p \leq 0.05$ was analysed using the R project software version 3.2.3 (R Development
269 Core Team, 2016),

270 To examine the changes in the fish community composition for each sub-basin
271 between historical data and current data a gross fish community integrity index (FCI)
272 was developed. FCI was calculated as the native species lost (i.e. cited in the historical
273 data set, but not in current data) plus non-native species gained (number of added non-
274 native species captured during the current period). Native species lost was calculated
275 only on sub-basins with positive results (0-5 species) and using only sub-basins with
276 data from historical and current periods (see Eq. 1),

277

$$FCi = (NnSp_h - NnSp_c) + (NnonSp_c) \quad \text{Eq. 1,}$$

279

280 where i is the sub-basin from 1 to 137; nSp = native species; $nonSp$ = non-native
281 species; h = historical data; c = current data; N = number.

282

283 Finally, in order to develop a complete framework for the historical fish community
284 from the Guadalquivir Basin, additional diadromous fish species (excluding the eel)
285 were checked-out through a literature review. Sixteen bibliographic sources, including
286 the Madoz dictionary (1846-1850), as well as scientific journals and historical
287 documents with information on these species from the mid-19th (historical data) to the
288 21st (current data) century were consulted (Supplementary Data Table A and
289 Supplementary References).

290

291 3. RESULTS

292 3.1 Historical changes in species distribution

293 Most sub-basins within the Guadalquivir basin have suffered major changes in the
294 integrity of the fish community, from the 19th century until present: 30 (21.9%), gained
295 and/or lost one species, 44 (32.1%), two species and 56 (40.9%), three to seven species
296 (Table 2). Local extirpations or species loss had a greater impact on these results than
297 species gain (Table 2). From here, it can be said that the Guadalquivir fish community
298 integrity is severely impaired at the long term. From the 137 sub-basins analysed, only 7
299 (5.1%), neither lost nor gained any fish species (Table 2).

300 European eel (*A. anguilla*)

301 The historical range of the European eel encompassed most of the Guadalquivir Basin
302 (74.8%), the species being present in approximately 86% of the total area analysed.
303 However, according to both general and overlapping data (Table 3), the species has
304 disappeared from 97% of its original area. There were significant differences in the use
305 of large sectors of the basin (Table 3). The eel has been extirpated from those sectors
306 most distant from the mouth of the river. Today the eel is confined to the lower parts of
307 the basin, the mouth of the main river and the Guadiamar river (Fig. 2; Fig. 3), the only
308 sites free from impoundments.

309 Brown trout (*S. trutta*)

310 The brown trout originally presented a scattered distribution pattern throughout the
311 Guadalquivir Basin. However, nowadays it has disappeared from some historically
312 occupied areas, particularly the lowest sites, indicating a significant change in the use of
313 sectors (Table 3). The historical distribution range of the brown trout was approximately
314 half that of the eel (around 43% for total and overlapping sub-basins), and was reduced
315 by 84% (from 15,393 to 2,538 km²) between the 19th and 21st centuries (Table 3).
316 However, the current brown trout distribution area is approximately double that of the
317 eel, whose original area has suffered a much larger reduction. The historical distribution
318 of trout occurred mostly in sectors D, E and F, in the upper Guadalquivir, with some
319 presence in sectors A and C, too (Fig. 2; Fig. 3). Today, the species is present only in
320 sectors D, E and F, most notably the latter, where approximately 60 % of its current
321 total area can be found.

322 Southern Iberian barbel (*L. sclateri*)

323 Both historical and current data show this barbel species to be widely, but unevenly
324 distributed, suggesting a similar distribution pattern between sectors and time periods
325 (Fig. 2; Fig. 3). Historically, over 75% of the total basin area was occupied by the
326 species, while, considering only overlapped sub-basins, species was present in 78.8% of
327 its distribution range and was apparently extirpated from 16% of it, in comparison with
328 current data (Table 3).

329 Southern straight-mouth nase (*P. willkommii*)

330 The prevalence of nase across different large basin sectors has remained fairly
331 constant (Fig. 2, Fig. 3) and the test for distribution changes over time was non-
332 significant (Table 3). In the historical period this species occupied around 61.6%
333 (30,553 km²) of the total area surveyed, or 63.7% of the overlapping basins (Table 3).
334 Although comparison of historical and current overlapping basins reveal current species
335 occupation in only 25.7% of the former area (Table 3) which means a strong reduction
336 in total potential distribution range between the 19th and 21st centuries.

337 Iberian chub (*Squalius pyrenaicus*)

338 This native species, formerly present in those sub-basins located in the upper reaches
339 of the Guadalquivir Basin (mostly sectors C, E and F) (Fig. 2), represented the lowest
340 registered historical distribution from all those studied here (11.8 %; 5,865 km²) (Table
341 3). In fact, there were relatively few historical references to this small non-edible
342 species, and in most of the sub-basins where it was sampled in current period (Fig. 3) it
343 was absent from Madoz (1846-1850). The comparison of historical and current
344 distribution showed an apparent increase of 40% (from 3,725 to 5,171 km²) (Table 3).
345 However, no significant differences in sector use between the 19th and 21st centuries
346 were found (Table 3).

347 **3.2 Diadromous species assemblage**

348 The bibliographic review on diadromous fish fauna highlighted a total of five species
349 in the Guadalquivir Basin, four anadromous: Atlantic sturgeon (*Acipenser sturio*), sea
350 lamprey (*Petromyzon marinus*), allis shad (*Alosa alosa*), twaite shad (*Alosa fallax*); and
351 one catadromous: eel (Supplementary data Table A and Fig. 4). Therefore, the historical
352 assemblage of diadromous species in this basin included 54.5% of all Iberian
353 diadromous fish (see Carta Piscícola Española, 2018).

354 A total of 55 records were found, ranging from 3 to 28 for twaite shad and Atlantic
355 sturgeon, respectively (see Supplementary data Table A), the majority of which (n=24)
356 came from Madoz (1846-1850). Only one of the five species (eel) with historical
357 presence in the basin was captured in the 21st century period, with sea lamprey and
358 twaite shad being extremely scarce, and Atlantic sturgeon and allis shad, regionally
359 extinct (Arias-García 2010; IUCN, 2015) (Fig. 4). In the case of twaite shad, during
360 early autumn and spring, very few individuals are dispersed in the lowest reaches of the
361 basin (Arias-García, 2010) (Fig. 4). Some studies have considered the sea lamprey to be
362 extinct (Arias-García, 2010). We are, however, aware of the sporadic, but periodic
363 capture of this species in the estuary (authors' unpublished data).

364

365 4. DISCUSSION

366 4.1 Long-term shifts in fish community composition

367 Ecological communities are highly dynamic, subject to continual change, particularly
368 over the long-term (Swetnam *et al.*, 1999). Examples of this can be seen in many
369 different ecosystems and biocoenoses, most notably, plant communities and landscapes
370 (Connor *et al.*, 2019, Manzano *et al.*, 2019). Indeed, community composition seems to
371 retain a certain equilibrium only when viewed over relatively brief human timescales of
372 a few years at most. Thus, long-term comparisons using historical and current data
373 provide a real opportunity to understand basic questions related to degradation patterns
374 of freshwater fishes in the present day and the quantification of long-term shifts in the
375 fish distribution of large catchments (Blanchet *et al.*, 2009; Haidvogel *et al.*, 2015b;
376 Schletterer *et al.* 2018). Here, historical quotations from reference texts about the
377 geography of the Guadalquivir Basin were used to establish a baseline of the original
378 distribution in the 19th century of five native fish species (eel, trout, barbel, nase and
379 chub), most of nutritional value to humans. The main results point to a strong decline in
380 the extent of the distribution of most of these five native freshwater fish species between
381 historical and current period, a fact which is borne out by studies of other Mediterranean
382 rivers where similar extinction processes were detected (Aparicio *et al.*, 2000; Habit *et*
383 *al.*, 2010, Clavero and Hermoso, 2015).

384 While Aparicio *et al.*, (2000) observed a narrowing of European eel distribution and
385 an expansion of brown trout, our results revealed an overall average decline of 50% in
386 the occupied area across all the species analysed. It seems that some species,
387 particularly eel, trout and Iberian nase, have been extirpated from multiple sub-basins in
388 the past. However, although these species have suffered major reductions in their
389 historical range, the respective narrowing distribution patterns across the Guadalquivir
390 basin differed strongly. In those sub-basins farthest from the mouth of the basin, in a
391 process clearly linked to reservoir building, eels have become locally extinct (Clavero
392 and Hermoso, 2015). On the other hand, the trout area has been limited to the highest
393 river reaches, close to the headwaters, in a process likely driven by alterations in the
394 aquatic habitat, especially water quality and climate change (Clavero *et al.*, 2017;
395 Almodóvar *et al.*, 2012). This is an intensely managed species due to its value in
396 recreational fishing, which may have prevented a still larger reduction in distribution
397 range.

398 The situation with respect to the cyprinids is more variable. The distribution range of
399 barbel, the species that has suffered the least reduction in range, has changed little from
400 historical to the current period. Nase distribution, on the other hand, seems to have
401 disappeared from the more degraded lower stretches of the basin and is, nowadays,
402 generally confined to the less impacted upper reaches. Finally, regarding chub, there is a
403 clear difference, most likely due to the fact that it is non-edible, being typically deemed
404 unsuitable for culinary purposes. Containing a large number of bones, it is difficult to
405 eat, and is not particularly palatable. In fact, some cookery books and websites advise

406 against consuming it. Furthermore, as it fails to reach a large size, it is not especially
407 profitable. For these reasons, this species may have escaped notice in the past due to the
408 complete lack of culinary or commercial interest shown in it. It simply was not
409 registered, as interest was likely only bestowed upon those species of potential utility to
410 people, such as barbel, nase, eel and trout, all of which are highly appreciated for human
411 consumption. Therefore, we may conclude that chub, a generalist species with an ample
412 ecological tolerance (Blanco-Garrido *et al.*, 2003), was probably widespread in the
413 Guadalquivir Basin during 19th century, but under-registered in the Madoz dictionaries.
414 Although our data suggest an apparent increase (~40%) in the chub distribution range
415 between historical and current data, this could simply be a spurious result. In all
416 likelihood, this species may have suffered a reduction in distribution similar to eel or
417 trout.

418 The four additional diadromous species considered in this study (sturgeon, lamprey
419 and allis and twaite shads) are extinct or almost extinct today, but were widely
420 distributed in the 19th century, especially in the middle and lower sectors of the basin. A
421 combination of habitat alteration (river fragmentation, pollution and channel
422 modifications), and overfishing have led to this decline (Fernández-Pasquier, 1999;
423 Mateus *et al.*, 2012). The ecological status of the basin in the current period makes the
424 recovery of any of these species virtually impossible (Ramos-Merchante and Prenda,
425 2018). Currently, about 9,193 hm³ of river flow is retained in 29 large reservoirs (> 100
426 hm³) and over 140 smaller ones (< 100 hm³) (Confederación Hidrográfica del
427 Guadalquivir, 2015). In addition, there are also numerous cutoffs, channelling and
428 dredging works to promote river traffic, particularly in the lower reaches of the river
429 (Granado-Lorencio, 1991). As a consequence, the natural flow pattern of the main
430 channel and tributaries has been vastly altered and these species are unable to complete
431 their life cycle (Fernández-Pasquier, 1999). For these reasons, neither of them can be
432 included in the RC as a real conservation goal for the Guadalquivir Basin and at the
433 same time it renders the definition of any RC impossible (*sensu* WFD).

434 Those species native to the Guadalquivir Basin not considered here may have suffered
435 a similar fate to those presented in this paper. Although we have focused on large,
436 valued and probably heavily-exploited species, for which historical declines are likely
437 larger than they would be for a random sample of the Guadalquivir fish community, our
438 results highlight that overall native fish distribution in the Guadalquivir Basin has been
439 seriously altered throughout the last two centuries. The fish distribution pattern from the
440 historical to the current could be established by amalgamating the patterns derived, on
441 the one hand, exclusively from the ecological preferences and non-human mediated
442 interactions of each species, with those attributable to human disturbances and the likely
443 continual translocation of those species of interest to humans (Sánchez-Polaina and
444 Fernandez-Delgado, 1997; Hoffmann 2005; Lopes-Cunha *et al.*, 2012).

445 **4.2 Reference conditions in highly disturbed river basins?**

446 The existence of a 19th century geographical register, such as that obtained from
447 Madoz, provides a fundamental baseline to discern historical long-term changes in fish
448 community composition. The emerging historical fish distribution pattern may offer
449 new insights into past river ecosystems, as well as providing an essential context for
450 contemporary basin management. Considering the five species widely and reliably
451 registered in Madoz (eel, trout, barbel, nase and chub), we have given an account of
452 those sub-basins with historical data (137) that currently either maintain the same
453 composition or have lost species. At the same time, the non-native species detected in
454 each basin in the current sampling were similarly computed. The combination of non-
455 native species gained and native species lost provides a comprehensive index of
456 community integrity useful for the evaluation of overall changes in these aquatic
457 biocoenoses. Furthermore, it helps to validate the RC concept from a double
458 perspective, if: 1) communities, per se, are stable entities that tend to maintain their
459 composition long-term, irrespective of environmental conditions, and 2) community
460 resilience is high enough to cope with relatively recent human impacts.

461 The detection of a 'pristine' baseline – a true 'reference condition' – can be a rather
462 complicated undertaking. Ecological systems follow environmental dynamics, which
463 are mostly climatically driven, but at the same time they also interact with
464 anthropogenic influences, and the detectable ecological consequences of this interaction
465 go back much further than the modern era (Hobbs *et al.*, 2006; Hoffmann 2005, Lotze
466 and Worm 2009). Here, we report that most of the sub-basins analysed in the
467 Guadalquivir Basin have suffered substantial changes in fish community integrity from
468 the mid-19th century onwards. Soft impacts from human activities, such as those related
469 to low-intensity urbanisation, had dramatic impacts on warm water stream fish
470 assemblages in North America over a 50-year period (Weaver and Garman, 1994), a
471 much shorter time span than that considered here. Over the last 150 years, Andalucía,
472 the territory encompassing most of the Guadalquivir Basin, has seen great change in
473 terms of general human development, which may have profound consequences for
474 landscape and river ecology (Ales *et al.*, 1992, Leyton *et al.*, 2017). In short, the
475 Guadalquivir Basin has gone from a sparsely populated area in the 19th century,
476 unchanged since mediaeval times, to a highly-developed, densely-populated zone in the
477 21st. The changes in urbanisation, agriculture, land cover, infrastructure and industry in
478 a semiarid basin, highly dependent upon very limited water resources, must necessarily
479 translate to the status and biodiversity of the river ecosystems (Riis and Sand-Jensen
480 2001; Dallas, 2103). Damming, wastewater, eutrophication and siltation are major
481 factors that have had an increasing impact since the beginning of the 19th century
482 onward. A similar study of the Schuylkill River Drainage (N America), observed a
483 major reorganisation of the structure of the fish community from the 19th century,
484 affecting both native and introduced species (Fairchild *et al.*, 1998). Furthermore, the
485 authors recognised that fish communities change discernibly not over the course of
486 centuries, but in a matter of decades.

487 Three basic approaches can be followed to establish RC for regional fish
488 communities: (1) use of predictive models, (2) sampling undisturbed sites and (3) use of
489 historical data. The option of constructing basin-wide models predicting species
490 composition is largely ineffective, particularly in highly-disturbed basins, like the
491 majority of those in the Mediterranean area, including the Guadalquivir (Ramos-
492 Merchante and Prenda, 2018). The option of sampling fish communities from nearby
493 sites unaffected by human activity, directly upstream, or from similar adjacent sub-
494 basins, requires the existence of such sites for the whole set of environmental conditions
495 observed in the area of study. Again, in Mediterranean basins greatly affected by
496 humans, such 'pristine' sites are almost non-existent, especially in the lower stream
497 reaches (Dallas, 2013). Finally, there are several limitations to the historical approach
498 which at best hinder and at worst render impossible its application: 1) the question of
499 which moment should be chosen to establish the baseline if fish communities undergo
500 significant changes in a matter of decades, 2) the general absence of adequate historical
501 records, and 3) the fact that, where these records do exist, they provide reference
502 communities of impossible practical application because they are associated with
503 environmental conditions that may have long since disappeared (Chakona *et al.*, 2020).

504 The historical data reported in Madoz (1846-1850) were used to describe a potential
505 'reference situation', but this was far from being an 'undisturbed situation' (Soranno *et*
506 *al.*, 2011). Conceptually, the best approach to the establishment of a 'reference
507 condition' for a freshwater ecosystem is to use information from a time period when the
508 ecosystem was in the 'reference' state, i.e. not adversely affected by human activity
509 (Karr *et al.*, 1986). However, this rarely exists, nor can it be measured in any objectively
510 scientific manner. Iberian landscapes, like most Mediterranean ones, have been subject
511 to continuous, long-term environmental change throughout the Holocene (Magny *et al.*,
512 2003, Jalut *et al.*, 2009) and inhabitation by humans since prehistory has deeply
513 modified the environmental conditions (Carrión *et al.*, 2003; Boivin *et al.*, 2016). Over a
514 shorter time span, from the 19th century onward, the area has been subject to increasing
515 disturbance (e.g. Urbietta *et al.*, 2008; Leblanc *et al.*, 2000; Butzer, 2005), and the main
516 issue continues to be the question of when to set the 'reference condition'. Although it is
517 difficult to obtain reliable information from freshwater ecosystems from a period
518 lacking 'significant' human pressure, this study attempts to supply an adequate temporal
519 source to establish proper RC prior to critical anthropogenic impacts on freshwater
520 ecosystems (Granado-Lorencio, 1991; Haidvogel *et al.*, 2015b).

521 This study compares two settings taken at two specific periods. However, to anticipate
522 further changes in the distribution of native fish species, it would be necessary to
523 construct new future settings and to develop predictive models. To improve the
524 knowledge on the decline of native fishes in the Guadalquivir Basin, future research
525 should refresh the information on sub-basins and rivers where fish species were
526 historically found. A more detailed analysis of the intensity of human pressures and
527 climate variability is necessary to better understand the general degradation process

528 under analysis. In concurrence with previous authors, we assert that historical data are
529 essential for the documentation of changes in fish distribution patterns, before any
530 increase in critical human pressures on the environment and climate (Tingley and
531 Beissinger, 2009; Pont *et al.*, 2015, Haidvogel *et al.*, 2015b).

532 Our case study of the Guadalquivir Basin demonstrates the long-term impact of
533 human pressures on the fish community in the Mediterranean. The strength of the
534 historical sources describing the occurrence and relative abundance of fish species
535 within this river network between the historical and current period, has been crucial to
536 the understanding of the dynamics of natural systems under intense critical
537 anthropogenic impact and, furthermore, may serve to establish reliable conservation
538 goals based on the reality that contemporary humans are imposing on freshwater
539 ecosystems. The disappearance of many species and most of their original habitats,
540 render the definition of any RC unlikely (*sensu* WFD). However, if a water mass is
541 highly disturbed, WFD allows it to be classified as a Heavily Modified Water Body
542 (HMWB), whose reference conditions are established by its Maximum Ecological
543 Potential (MEP). The MEP is the state in which the biological status reflects, as far as
544 possible, that of the closest comparable surface water body, taking into account the
545 modified characteristics of the water body. But this is only useful for bodies of water
546 which are substantially changed in character as a result of physical alterations by human
547 activity. That is, the classification cannot usually be applied to water bodies affected by
548 other common impacts that imperil Mediterranean lotic ecosystems, such as diffuse
549 pollution, eutrophication, invasive species, water abstraction, etc. (Dallas, 2013).
550 Nonetheless, the main WFD procedure, including the RC approach, provides an
551 ecological guiding principle that can also be transferred to the conservation of
552 freshwater ecosystems.

553 **4.3 River conservation, WFD and alternatives to reference conditions**

554 The WFD ultimately tries to maintain or to restore the integrity of European
555 freshwater ecosystems. Thus, it has more to do with European river conservation than
556 any other European legislation, for three main reasons (Boon and Lee, 2005; Boon,
557 2012): 1) it covers all surface waters throughout most of Europe; 2) it requires river
558 basin management plans to ultimately achieve 'good' ecological status for all European
559 water bodies; and 3) it uses a theoretically true ecological perspective to address the
560 integrity of freshwater ecosystems, much more than any other previous law, especially
561 in countries like Spain. Although the WFD, as opposed to the Habitats Directive (HD),
562 is not a nature conservation directive per se and the concept of "ecological status" is not
563 intrinsically synonymous with "conservation value" (Boon and Lee, 2005), a gradient in
564 ecological status may run parallel to a gradient in conservation value. And this positive
565 correlation may increase latitudinally from north to south as the ecological status of the
566 water bodies diminishes, as happens in Mediterranean areas (Gasith and Resh, 1999;
567 Prenda *et al.*, 2006; López-Doval *et al.*, 2013).

568 If the RC concept is not easily applicable to fish communities, at least in
569 Mediterranean areas, what should be done then? How can we assess the ecological
570 status of water bodies based on fish metrics? Aparicio *et al.* (2011) developed a fish
571 multimetric index (IBI-Júcar) for the Júcar basin (E Spain) based on five metrics that
572 did not need any RC. Ramos-Merchante and Prenda (2018) adapted this new index to
573 another Mediterranean basin in S Spain. In these cases the alternative to RC was to
574 create a set of metrics based on expert knowledge that took into account recognized and
575 accepted interpretations of ecological conditions and the health of the ecosystem. The
576 metrics employed in these indexes measured five/six aspects at three levels of fish
577 health: individual (percentage of individuals with anomalies, and fish condition),
578 population (age structure of native fish populations, and abundance of native fishes) and
579 community (loss of native species, and alien fish pressure). In both cases, Guadalquivir
580 and Júcar, the results were fully satisfactory and the ecological status of the sampling
581 sites was adequately characterized, as the high correlation with many other
582 environmental indexes demonstrated (Aparicio *et al.*, 2011; Ramos-Merchante and
583 Prenda, 2018).

584 But are these IBIs useful for determining the conservation status of river stretches, in
585 addition to the ecological status of water bodies, in accordance with the WFD, by using
586 abundance, composition, and age structure of fish assemblages? It could be argued that
587 both IBIs are also a measure of the conservation status of river stretches, by virtue of
588 two metrics directly related to the conservation value of the fish community (loss of
589 native species and alien fish pressure) and two indirectly related additional metrics (age
590 structure and abundance of native fishes). This proposal is applicable to running waters
591 in Mediterranean areas and thus overcomes the two main shortfalls inherent in the RC
592 concept: the existence of pristine sites and the imposed lack of natural temporal
593 dynamics in river communities. In this case, the RC concept should be adapted to a
594 different ecological and environmental reality, but still be applicable to a collection of
595 sites with the highest ecological status/conservation value.

596

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603 **REFERENCES**

- 604 Abell, R. (2002). Conservation biology for the biodiversity crisis: a freshwater follow-
605 up. in *Conservation Biology*, 16(5), 1435–1437 [https://doi.org/10.1046/j.1523-](https://doi.org/10.1046/j.1523-1739.2002.01532.x)
606 [1739.2002.01532.x](https://doi.org/10.1046/j.1523-1739.2002.01532.x)
- 607 Abell, R., Allan, J.D. & Lehner, B. (2007). Unlocking the potential of protected areas
608 for freshwaters. *Biological Conservation*, 134(1), 48–63.
609 <https://doi.org/10.1016/j.biocon.2006.08.017>
- 610 Ales, R.F., Martin, A., Ortega, F. & Ales, E.E. (1992). Recent changes in landscape
611 structure and function in a mediterranean region of sw spain (1950-1984). *Landscape*
612 *Ecology*, 7(1), 3–18. <https://doi.org/10.1007/BF02573953>
- 613 Almodóvar, A., Nicola, G.G., Ayllón, D. & Elvira, B. (2012). Global warming threatens
614 the persistence of mediterranean brown trout. *Global Change Biology*, 18(5), 1549–
615 1560. <https://doi.org/10.1111/j.1365-2486.2011.02608.x>
- 616 Aparicio, E., Vargas, M.J., Olmo, J. M. & de Sostoa, A. (2000). Decline of native
617 freshwater fishes in a mediterranean watershed on the iberian peninsula: A quantitative
618 assessment. *Environmental Biology of Fishes*, 59, 11–19.
619 <https://doi.org/10.1023/A:1007618517557>
- 620 Aparicio, E., Carmona-Catot, G., Moyle, P.B. & García-Berthou, E. (2011).
621 Development and evaluation of a fish-based index to assess biological integrity of
622 mediterranean streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*,
623 21(4), 324–337. <https://doi.org/10.1002/aqc.1197>
- 624 Arias-García (2010). *ICTIO-TERM. Glosario alfabetico de nombre vernaculos*.
625 Available at: http://www.ictioterm.es/glosario_alfabetico_nombres_vernaculos.php
626 [Accessed June 2015]
- 627 Bailey, R.C., Kennedy, M.G., Dervish, M.Z. & Taylor, R.M. (1998). Biological
628 assessment of freshwater ecosystems using a reference condition approach: Comparing
629 predicted and actual benthic invertebrate communities in yukon streams. *Freshwater*
630 *Biology*, 39(4), 765–774. <https://doi.org/10.1046/j.1365-2427.1998.00317.x>
- 631 Blanchet, S., Leprieur, F., Beauchard, O., Staes, J., Oberdorff, T. & Brosse, S. (2009).
632 Broad-scale determinants of non-native fish species richness are context-dependent.

- 633 *Proceedings of the Royal Society B: Biological Sciences*, 276, 2385–2394.
634 <https://doi.org/10.1098/rspb.2009.0156>
- 635 Blanco-Garrido, F., Sánchez-Polaina, F. J. & Prenda, J. (2003). Summer diet of the
636 iberian chub (*Squalius pyrenaicus*) in a mediterranean stream in sierra morena (Yeguas
637 stream, Córdoba, Spain). *Limnetica*, 22(2), 99–105. <http://hdl.handle.net/10272/3211>
- 638 Boivin, N. L., Zeder, M.A., Fuller, D.Q., Crowther, A., Larson, G., Erlandson, J.M., et
639 al. (2016). Ecological consequences of human niche construction: Examining long-term
640 anthropogenic shaping of global species distributions. *Proceedings of the National
641 Academy of Sciences of the United States of America*, 113(23), 6388–6396.
642 <https://doi.org/10.1073/pnas.1525200113>
- 643 Boon, P.J., Lee, A. (2005). Falling through the cracks: are european directives and
644 international conventions the panacea for freshwater nature conservation? *Freshwater
645 Forum*, 24, 24–37.
- 646 Boon, P.J. (2012). Revisiting the case for river conservation. In: P.J. Boon & P.J. Raven
647 (Eds) *River Conservation and Management*. John Wiley, Chichester, 3–14.
- 648 Bouleau, G. & Pont, D. (2015). Did you say reference conditions? Ecological and socio-
649 economic perspectives on the european water framework directive. *Environmental
650 Science and Policy*, 47, 32–41. <https://doi.org/10.1016/j.envsci.2014.10.012>
- 651 Butzer, K.W. (2005). Environmental history in the mediterranean world: Cross-
652 disciplinary investigation of cause-and-effect for degradation and soil erosion. *Journal
653 of Archaeological Science*, 32 (12), 1773–1800.
654 <https://doi.org/10.1016/j.jas.2005.06.001>
- 655 Carpintero, G.M. (2015). Characterization of suspended sediments in the Guadalquivir
656 estuary at modeled scale. (Doctoral thesis, University of Córdoba, 151pp. Córdoba,
657 Spain. Retrieved from <http://hdl.handle.net/10396/12706>
- 658 Carrión, J.S., Sánchez-Gómez, P., Mota, J.F., Yll, R. & Chaín, C. (2003). Holocene
659 vegetation dynamics, fire and grazing in the sierra de Gádor, southern Spain. *Holocene*,
660 13(6), 839–849. <https://doi.org/10.1191/0959683603hl662rp>
- 661 Chakona, A., Gouws, G., Kadye, W.T., Jordaan, M.S. & Swartz, E.R. (2020).
662 Reconstruction of the historical distribution ranges of imperilled stream fishes from a
663 global endemic hotspot based on molecular data: Implications for conservation of
664 threatened taxa. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 30(1),
665 144–158. <https://doi.org/10.1002/aqc.3251>
- 666 Clavero, M., Blanco-Garrido, F. & Prenda, J. (2004). Fish fauna in iberian
667 mediterranean river basins: Biodiversity, introduced species and damming impacts.
668 *Aquatic Conservation: Marine and Freshwater Ecosystems*, 14(6), 575–585.
669 <https://doi.org/10.1002/aqc.636>

- 670 Clavero, M. & Hermoso, V. (2015). Historical data to plan the recovery of the european
671 eel. *Journal of Applied Ecology*, 52(4), 960-968. <https://doi.org/10.1111/1365->
672 2664.12446
- 673 Clavero, M., Ninyerola, M., Hermoso, V., Filipe, A.F., Pla, M., Villero, D., et al.
674 (2017). Historical citizen science to understand and predict climate-driven trout decline.
675 *Proceedings of the Royal Society B: Biological Sciences*, 284(1846).
676 <https://doi.org/10.1098/rspb.2016.1979>
- 677 Clavero, M. & Villero, D. (2014). Historical ecology and invasion biology: Long-term
678 distribution changes of introduced freshwater species. *BioScience*, 52(4), 960–968.
679 <https://doi.org/10.1093/biosci/bit014>
- 680 Confederación Hidrográfica del Guadalquivir (2015). Plan hidrológico de la
681 demarcación hidrográfica del Guadalquivir. (<http://www.chguadalquivir.es>. Accessed
682 20 December 2016).
- 683 Connor, S.E., Vannièrè, B., Colombaroli, D., Anderson, R.S., Carrión, J.S., Ejarque, A.
684 et al. (2019). Humans take control of fire-driven diversity changes in mediterranean
685 iberia’s vegetation during the mid–late holocene. *Holocene*, 29(5), 886–901.
686 <https://doi.org/10.1177/0959683619826652>
- 687 Council of the European Communities (2000). Directive 2000/60/EC of the european
688 parliament and of the council of 23 October 2000 establishing a framework for
689 community action in the field of water policy. Official Journal of the European
690 Communities, L327, 1–73. doi:10.1039/ap9842100196
- 691 Dallas, H.F. (2013). Ecological status assessment in mediterranean rivers: complexities
692 and challenges in developing tools for assessing ecological status and defining reference
693 conditions. *Hydrobiologia*, 719(1), 483–507. <http://dx.doi.org/10.1007/s10750-012->
694 [1305–8](http://dx.doi.org/10.1007/s10750-012-1305-8).
- 695 Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque
696 C., et al. (2006). Freshwater biodiversity: Importance, threats, status and conservation
697 challenges. *Biological Reviews of the Cambridge Philosophical Society*, 81(2), 163–82.
698 <https://doi.org/10.1017/S1464793105006950>
- 699 Dumont, A., Salmoral, G. & Llamas, M.R. (2013). The water footprint of a river basin
700 with a special focus on groundwater: The case of Guadalquivir basin (Spain). *Water*
701 *Resources and Industry*, 1-2, 70–76. <https://doi.org/10.1016/j.wri.2013.04.001>
- 702 Fairchild, G.W., Horwitz, R.J., Nieman, D.A., Boyer, M.R. & Knorr, D.F. (1998).
703 Spatial variation and historical change in fish communities of the Schuylkill river
704 drainage, southeast pennsylvania. *The American Midland Naturalist*, 139(2), 282–295.
705 [https://doi.org/10.1674/0003-0031\(1998\)139\[0282:svahci\]2.0.co;2](https://doi.org/10.1674/0003-0031(1998)139[0282:svahci]2.0.co;2)

706 Fernández-Pasquier, V. (1999). Acipenser sturio L. in the Guadalquivir river, Spain.
707 Water regulation and fishery as factors in stock decline from 1932 to 1967. *Journal of*
708 *Applied Ichthyology*, 15(4-5), 133-135. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0426.1999.tb00222.x)
709 0426.1999.tb00222.x

710 Filipe, A.F., Araújo, M.B., Doadrio, I., Angermeier, P.L. & Collares-Pereira, M.J.
711 (2009). Biogeography of iberian freshwater fishes revisited: The roles of historical
712 versus contemporary constraints. *Journal of Biogeography*, 36(11), 2096–
713 2110.<https://doi.org/10.1111/j.1365-2699.2009.02154.x>

714 Freyhof, J. & Brooks, E. (2011). European red list of freshwater fishes. In *Luxembourg:*
715 *Publications office of the European Union*, pp 61. <https://doi.org/10.2779/85903>

716 Gasith, A., & Resh, V. H. (1999). Streams in Mediterranean climate regions: abiotic
717 influences and biotic responses to predictable seasonal events. *Annual review of ecology*
718 *and systematics*, 30(1), 51-81. <https://doi.org/10.1146/annurev.ecolsys.30.1.51>

719 Geri, F., Amici, V. & Rocchini, D. (2010). Human activity impact on the heterogeneity
720 of a Mediterranean landscape. *Applied Geography*, 30(3), 370–379.
721 <https://doi.org/10.1016/j.apgeog.2009.10.006>

722 González-Ortegón, E., Cuesta, J.A., Pascual, E. & Drake, P. (2010). Assessment of the
723 interaction between the white shrimp, *Palaemon longirostris*, and the exotic oriental
724 shrimp, *Palaemon macrodactylus*, in a European estuary (SW Spain). *Biological*
725 *Invasions*, 12(6), 1731–1745. <https://doi.org/10.1007/s10530-009-9585-2>

726 Granado-Lorencio, C. (1991). The effect of man on the fish fauna of the river
727 Guadalquivir, Spain. *Fisheries Research*, 12(2), 91–100. [https://doi.org/10.1016/0165-](https://doi.org/10.1016/0165-7836(91)90034-D)
728 7836(91)90034-D

729 Grossman, G.D. & Freeman, M.C. (1987). Microhabitat use in a stream fish
730 assemblage. *Journal of Zoology*, 212(1), 151–176. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7998.1987.tb05121.x)
731 7998.1987.tb05121.x

732 Habit, E., Piedra, P., Ruzzante, D.E., Walde, S.J., Belk, M.C., Cussac, V.E., et al.
733 (2010). Changes in the distribution of native fishes in response to introduced species
734 and other anthropogenic effects. *Global Ecology and Biogeography*, 19(5), 697–710.
735 <https://doi.org/10.1111/j.1466-8238.2010.00541.x>

736 Haidvogel, G., Hoffmann, R., Pont, D., Jungwirth, M. & Winiwarter, V. (2015a).
737 Historical ecology of riverine fish in Europe. In *Aquatic Sciences*, 77, 315–324.
738 <https://doi.org/10.1007/s00027-015-0400-0>

739 Haidvogel, G., Pont, D., Dolak, H. & Hohensinner, S. (2015b). Long-term evolution of
740 fish communities in European mountainous rivers: past log driving effects, river

741 management and species introduction (Salzach River, Danube). *Aquatic Sciences*, 77,
742 395–410. <https://doi.org/10.1007/s00027-015-0398-3>

743 Hermosin, M.C., Calderon, M.J., Real, M. & Cornejo, J. (2013). Impact of herbicides
744 used in olive groves on waters of the Guadalquivir river basin (southern Spain).
745 *Agriculture, Ecosystems and Environment*, 164(1), 229–243.
746 <https://doi.org/10.1016/j.agee.2012.09.021>

747 Hermoso, V., Cattarino, L., Kennard, M.J., Watts, M. & Linke, S. (2015). Catchment
748 zoning for freshwater conservation: Refining plans to enhance action on the ground.
749 *Journal of Applied Ecology*, 52(4), 940–949. <https://doi.org/10.1111/1365-2664.12454>

750 Hermoso, V. & Clavero, M. (2013). Revisiting ecological integrity 30 years later: Non-
751 native species and the misdiagnosis of freshwater ecosystem health. *Fish and Fisheries*,
752 14(3), 416–423. <https://doi.org/10.1111/j.1467-2979.2012.00471.x>

753 Hobbs, R. J., Arico, S., Aronson, J., Baron, J.S., Bridgewater, P., Cramer, V.A., et al.
754 (2006). Novel ecosystems: theoretical and management aspects of the new ecological
755 world order. *Global ecology and biogeography*, 15(1), 1–7.
756 <https://doi.org/10.1111/j.1466-822X.2006.00212.x>

757 Hoffmann, R.C. (2005). A brief history of aquatic resource use in medieval Europe.
758 *Helgoland Marine Research*, 59(1), 22–30. <https://doi.org/10.1007/s10152-004-0203-5>

759 IUCN. (2015). The IUCN Red List of Threatened Species. Version 2015-1.
760 <https://www.iucnredlist.org>

761 Jalut, G., Dedoubat, J.J., Fontugne, M. & Otto, T. (2009). Holocene circum-
762 Mediterranean vegetation changes: Climate forcing and human impact. *Quaternary*
763 *International*, 200(1-2), 4–18. <https://doi.org/10.1016/j.quaint.2008.03.012>

764 Jungwirth, M., Muhar, S. & Schmutz, S. (2002). Re-establishing and assessing
765 ecological integrity in riverine landscapes. *Freshwater Biology*, 47(4), 867–887.
766 <https://doi.org/10.1046/j.1365-2427.2002.00914.x>

767 Karr, J.R. (1981). Assessment of biotic integrity using fish communities. *Fisheries*,
768 6(6), 21–27. [https://doi.org/10.1577/1548-8446\(1981\)006<0021:aobiuf>2.0.co;2](https://doi.org/10.1577/1548-8446(1981)006<0021:aobiuf>2.0.co;2)

769 Karr, J.R., Fausch, K.D., Angermeier, P.L., Yant, P.R. & Schlosser, I. J. (1986).
770 Assessing biological integrity in running waters: A method and its rationale. *Illinois*
771 *Natural History Survey Special Publication 5*, pp24.

772 Kopf, R.K., Finlayson, C.M., Humphries, P., Sims, N.C. & Hladysz, S. (2015).
773 Anthropocene Baselines: Assessing change and managing biodiversity in human-
774 dominated aquatic ecosystems. *BioScience*, 65(8), 798–811.
775 <https://doi.org/10.1093/biosci/biv092>

- 776 Kottelat, M. & Freyhof, J. (2007). Handbook of european freshwater fishes. In *Copeia*,
777 2008(3), 725-727. <https://doi.org/10.1643/OT-08-098a.1>
- 778 Labay, B.J., Hendrickson, D.A., Cohen, A.E., Bonner, T.H., King, R., Kleinsasser, L. J.,
779 et al. (2015). Can species distribution models aid bioassessment when reference sites are
780 lacking? Tests based on freshwater fishes. *Environmental Management*, 56(4):835–846.
781 <https://doi.org/10.1007/s00267-015-0567-0>
- 782 Leblanc, M., Morales, J.A., Borrego, J. & Elbaz-Poulichet, F. (2000). 4,500-year-old
783 mining pollution in southwestern Spain: Long-term implications for modern mining
784 pollution. *Economic Geology*, 95 (3): 655–662.
785 <https://doi.org/10.2113/gsecongeo.95.3.655>
- 786 Leunda, P.M., Elvira, B., Ribeiro, F., Miranda, R., Oscoz, J., Alves, M., et al. (2009).
787 International standardization of common names for Iberian endemic freshwater fishes.
788 *Limnetica*, 28(2), 189–202.
- 789 Leyton, J.M., Puerto Marchena, A. & Martín Vicente, Á. (2017). Land use and land
790 cover dynamics in the dehesa of Sierra Morena Biosphere Reserve (Sierra Norte de
791 Sevilla Natural Park, Spain), 1956-2007. *Madera y Bosques*, 23(2), 133–143.
792 <https://doi.org/10.21829/myb.2017.232552>
- 793 Lopes-Cunha, M., Aboim, M.A., Mesquita, N., Alves, M.J., Doadrio, I., & Coelho,
794 M.M. (2012). Population genetic structure in the Iberian cyprinid fish
795 *Iberochondrostoma lemmingii* (Steindachner, 1866): Disentangling species
796 fragmentation and colonization processes. *Biological Journal of the Linnean Society*,
797 105(3), 559–572. <https://doi.org/10.1111/j.1095-8312.2011.01827.x>
- 798 López-Doval, J.C., Ginebreda, A., Caquet, T., Dahm, C.N., Petrovic, M., Barceló, D., et
799 al. (2013). Pollution in Mediterranean-climate rivers. *Hydrobiologia*, 719(1), 427–45.
800 <http://dx.doi.org/10.1007/s10750-012-1369-5>.
- 801 Lotze, H.K. & Worm, B. (2009). Historical baselines for large marine animals. In
802 *Trends in Ecology and Evolution*, 24(5), 254–262.
803 <https://doi.org/10.1016/j.tree.2008.12.004>
- 804 Madoz, P, ed. (1846-1850). Diccionario geográfico, estadístico e histórico de España, y
805 sus posesiones de ultramar. 16 vols. Madoz and L. Sagasti, Madrid, Spain. Available at
806 <http://www.bibliotecavirtualdeandalucia.es>
- 807 Magny, M., Bégeot, C., Guiot, J. & Peyron, O. (2003). Contrasting patterns of
808 hydrological changes in europe in response to holocene climate cooling phases.
809 *Quaternary Science Reviews*, 22(15–17), 1589-1596. [https://doi.org/10.1016/S0277-](https://doi.org/10.1016/S0277-3791(03)00131-8)
810 [3791\(03\)00131-8](https://doi.org/10.1016/S0277-3791(03)00131-8)

- 811 Maidment, D.R. (2002). *Arc Hydro: GIS for water resources*. ESRI, Inc. 203pp. In
812 *Agriculture*. ISBN: 1589480341, 9781589480346.
- 813 Manzano, S., Carrión, J.S., García-Murillo, P. & López-Merino, L. (2019). When
814 dynamism is the baseline: long-term ecology of a Mediterranean seasonal wetland in the
815 Doñana National Park (Southwestern Europe). *Biodiversity and Conservation*, 28, 501–
816 522. <https://doi.org/10.1007/s10531-018-1674-z>
- 817 Martín-Ortega, J., Gutiérrez Martín, C. & Berbel, J. (2008). Caracterización de los usos
818 del agua en la demarcación del Guadalquivir en aplicación de la Directiva Marco de
819 Aguas. *Revista de Estudios Regionales*. 81, 45-76.
- 820 Mateus, C.S., Rodríguez-Muñoz, R., Quintella, B.R., Alves, M.J. & Almeida, P.R.
821 (2012). Lampreys of the Iberian Peninsula: Distribution, population status and
822 conservation. In *Endangered Species Research*, 16, 183–198.
823 <https://doi.org/10.3354/esr00405>
- 824 Moore, M.M., Covington, W.W. & Fulé, P.Z. (1999). Reference conditions and
825 ecological restoration: A southwestern ponderosa pine perspective. In *Ecological*
826 *Applications*, 9(4), 1266–1277. [https://doi.org/10.1890/1051-](https://doi.org/10.1890/1051-0761(1999)009[1266:RCAERA]2.0.CO;2)
827 [0761\(1999\)009\[1266:RCAERA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1999)009[1266:RCAERA]2.0.CO;2)
- 828 Moss, B. (2008). The Water Framework Directive: total environment or political
829 compromise? *Science of the total environment*, 400(1-3), 32–41.
830 <https://doi.org/10.1016/j.scitotenv.2008.04.029>
- 831 Muñoz, M. & Casadevall, M. (1997). Fish remains from Arbreda Cave (Serinyà,
832 Girona), northeast Spain, and their palaeoecological significance. *Journal of Quaternary*
833 *Science*, 12(2), 111–115 [https://doi.org/10.1002/\(SICI\)1099-](https://doi.org/10.1002/(SICI)1099-1417(199703/04)12:2<111::AID-JQS294>3.0.CO;2-P)
834 [1417\(199703/04\)12:2<111::AID-JQS294>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1099-1417(199703/04)12:2<111::AID-JQS294>3.0.CO;2-P)
- 835 Nijboer, R.C., Johnson, R.K., Verdonschot, P.F.M., Sommerhäuser, M. & Buffagni, A.
836 (2004). Establishing reference conditions for European streams. *Hydrobiologia*, 516,
837 91–105. <https://doi.org/10.1023/B:HYDR.0000025260.30930.f4>
- 838 Pont, D., Logez, M., Carrel, G., Rogers, C. & Haidvogel, G. (2015). Historical change in
839 fish species distribution: shifting reference conditions and global warming effects.
840 *Aquatic Sciences*, 77, 441–453. <https://doi.org/10.1007/s00027-014-0386-z>
- 841 Prenda, J., Armitage, P.D. & Grayston, A. (1997). Habitat use by the fish assemblages
842 of two chalk streams. *Journal of Fish Biology*, 51 (1), 64–79.
843 <https://doi.org/10.1006/jfbi.1997.0410>
- 844 Prenda, J., Clavero, M., Blanco-Garrido, F., Menor, A. & Hermoso, V. (2006). Threats
845 to the conservation of biotic integrity in Iberian fluvial ecosystems. *Limnetica*, 25(1–2),
846 377-388.

847 R Core Team (2016). R: A language and environment for statistical computing. R
848 Foundation for Statistical Computing, Vienna, Austria. Available online at
849 <https://www.R-project.org/>.

850 Ramos-Merchante, A. & Prenda, J. (2017). Macroinvertebrate taxa richness uncertainty
851 and kick sampling in the establishment of Mediterranean rivers ecological status.
852 *Ecological Indicators*, 72, 1–12. <https://doi.org/10.1016/j.ecolind.2016.07.047>

853 Ramos-Merchante, A., & Prenda, J. (2018). The ecological and conservation status of
854 the Guadalquivir river basin (s Spain) through the application of a fish-based
855 multimetric index. *Ecological Indicators*, 84, 45–59.
856 <https://doi.org/10.1016/j.ecolind.2017.08.034>

857 Riis, T. & Sand-Jensen, K. (2001). Historical changes in species composition and
858 richness accompanying perturbation and eutrophication of Danish lowland streams over
859 100 years. *Freshwater Biology*, 46(2), 269–280. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-2427.2001.00656.x)
860 [2427.2001.00656.x](https://doi.org/10.1046/j.1365-2427.2001.00656.x)

861 Robles, S., Toro, M., Nuño, C., Avilés, J., Alba-Tercedor, J., Álvarez, M., et al. (2002).
862 Descripción de las cuencas mediterráneas seleccionadas en el proyecto GUADALMED.
863 *Limnetica*, 21(2), 35–61.

864 Rodríguez Díaz, J.A., Weatherhead, E.K., Knox, J.W. & Camacho, E. (2007). Climate
865 change impacts on irrigation water requirements in the Guadalquivir river basin in
866 Spain. *Regional Environmental Change*, 7, 149–159. [https://doi.org/10.1007/s10113-](https://doi.org/10.1007/s10113-007-0035-3)
867 [007-0035-3](https://doi.org/10.1007/s10113-007-0035-3)

868 Sánchez-Polaina, F.J. & Fernández-Delgado, C. (1997). Historia de las introducciones
869 de peces en la Reserva Natural de la laguna de Zóñar (Córdoba, España). *Oxyura*, 9(1),
870 135–140.

871 Schletterer, M., Füreder, L., Kuzovlev, V.V., Zhenikov, Y.N. & Grigorieva, I.L. (2014).
872 Lowland river reference condition: spatial and temporal patterns of the zoobenthos
873 community in the Volga headwaters (2006–2010). *Hydrobiologia*, 729, 175–189.
874 <https://doi.org/10.1007/s10750-013-1466-0>

875 Schletterer, M., Kuzovlev, V.V., Zhenikov, Y.N., Tuhtan, J.A., Haidvogel, G., Friedrich,
876 T., et al. (2018). Fish fauna and fisheries of large European rivers: examples from the
877 Volga and the Danube. In *Hydrobiologia*, 814, 45–60. [https://doi.org/10.1007/s10750-](https://doi.org/10.1007/s10750-017-3370-5)
878 [017-3370-5](https://doi.org/10.1007/s10750-017-3370-5)

879 Simon, T.P. & Lyons, J. (1995). Application of the index of biotic integrity to evaluate
880 water resource integrity in freshwater ecosystems. In *Biological Assessment and*
881 *Criteria: Tools for water resource planning and decision making*. Davis WS, Simon TP
882 (eds). Lewis Publishers: Boca Raton, FL; 245–262.

883 Soranno, P.A., Wagner, T., Martin, S.L., McLean, C., Novitski, L.N., Provence, C.D., et
884 al. (2011). Quantifying regional reference conditions for freshwater ecosystem
885 management: A comparison of approaches and future research needs. *Lake and*
886 *Reservoir Management*, 7(2), 138–148. <https://doi.org/10.1080/07438141.2011.573614>

887 Steffen, W., Crutzen, P.J. & McNeill, J.R. (2007). The anthropocene: Are humans now
888 overwhelming the great forces of nature? *Ambio*, 36, 614–621.
889 [https://doi.org/10.1579/0044-7447\(2007\)36\[614:TAAHNO\]2.0.CO;2](https://doi.org/10.1579/0044-7447(2007)36[614:TAAHNO]2.0.CO;2)

890 Stoddard, J.L., Larsen, D.P., Hawkins, C.P., Johnson, R.K. & Norris, R.H. (2006).
891 Setting expectations for the ecological condition of streams: The concept of reference
892 condition. In *Ecological Applications*, 16(4):1267–76. [https://doi.org/10.1890/1051-0761\(2006\)016\[1267:SEFTEC\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[1267:SEFTEC]2.0.CO;2)

894 Swetnam, T.W., Allen, C.D. & Betancourt, J.L. (1999). Applied historical ecology:
895 Using the past to manage for the future. *Ecological Applications*, 9(4), 1189–1206.
896 [https://doi.org/10.1890/1051-0761\(1999\)009\[1189:AHEUTP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1999)009[1189:AHEUTP]2.0.CO;2)

897 Tingley, M.W. & Beissinger, S.R. (2009). Detecting range shifts from historical species
898 occurrences: new perspectives on old data. *Trends in Ecology and Evolution*, 24(11),
899 625–633. <https://doi.org/10.1016/j.tree.2009.05.009>

900 UNE-EN 14011:2003. (2003). Water quality - Sampling of fish with electricity.
901 <https://www.une.org/encuentra-tu-norma/busca-tu-norma/norma/?c=N0029997>.

902 Urbietta, I.R., Zavala, M.A. & Marañón, T. (2008). Human and non-human determinants
903 of forest composition in southern Spain: Evidence of shifts towards cork oak dominance
904 as a result of management over the past century. *Journal of Biogeography*, 35(9): 1688–
905 1670. <https://doi.org/10.1111/j.1365-2699.2008.01914.x>

906 Voulvoulis, N., Arpon, K.D. & Giakoumis, T. (2017). The EU Water Framework
907 Directive: From great expectations to problems with implementation. *Science of the*
908 *Total Environment*, 575(1), 358–366. <https://doi.org/10.1016/j.scitotenv.2016.09.228>

909 Weaver, L.A. & Garman, G.C. (1994). Urbanization of a watershed and historical
910 changes in a stream fish assemblage. *Transactions of the American Fisheries Society*,
911 123(2), 162–172. [https://doi.org/10.1577/1548-8659\(1994\)123<0162:uoawah>2.3.co;2](https://doi.org/10.1577/1548-8659(1994)123<0162:uoawah>2.3.co;2)

912

913 **TABLE RELATIONSHIP**

914 **Table 1.** List of freshwater fish species sampled in 2008 in the Guadalquivir basin,
 915 showing standard international name (Leunda *et al.* 2009) and frequency of occurrence
 916 (%). In parenthesis the name used in this article.

917

Family	Species	Common name	% occurrence
Anguillidae			
	<i>Anguilla anguilla</i> †	European eel (eel)	1.0
Atherinidae			
	<i>Atherina boyeri</i>	Big-scale sand smelt	1.5
Centrarchidae			
	<i>Lepomis gibbosus</i> ‡	Pumpkinseed	20.0
	<i>Micropterus salmoides</i> ‡	Largemouth black bass	22.1
Cobitidae			
	<i>Cobitis paludica</i>	Southern Iberian spined-loach (loach)	22.6
Cyprinidae			
	<i>Alburnus alburnus</i> ‡	Bleak	9.7
	<i>Carassius auratus</i> ‡	Goldfish	4.1
	<i>Cyprinus carpio</i> ‡	Common carp	20.5
	<i>Gobio lozanoi</i> ‡	Iberian gudgeon	<1
	<i>Iberochondrostoma lemmingii</i>	Iberian arched-mouth nase	3.1
	<i>Squalius pyrenaicus</i> †	Southern Iberian chub (chub)	11.8
	<i>Luciobarbus sclateri</i> †	Southern Iberian barbell (barbell)	71.3
	<i>Phoxinus phoxinus</i> ‡	Adour minnow	<1
	<i>Pseudochondrostoma willkommii</i> †	Southern straight-mouth nase (nase)	24.1
	<i>Squalius alburnoides</i>	Iberian-roach –calandino (calandino)	23.1
Mugilidae			
	<i>Liza ramada</i>	Grey mullet	<1
Poeciliidae			
	<i>Gambusia holbrooki</i> ‡	Eastern mosquitofish	17.4
Salmonidae			
	<i>Oncorhynchus mykiss</i> ‡	Rainbow trout	2.1
	<i>Salmo trutta</i> †	Brown trout (trout)	10.8

918 † native species used in historical distribution analyses.

919 ‡ non-native species

920

921 **Table 2.** Geographic extent of the fish community integrity in the Guadalquivir basin (S
 922 Spain) according to the local extirpation of native fish species and gain of non-native
 923 species between the 19th and 21st centuries. Lost = species that were cited in the 19th
 924 century work by Madoz (1846-1850), but were not captured in the samplings carried out
 925 in the 21st century, being thus supposedly extirpated; gained = non-native species that
 926 were captured during the 21st century field sampling and were not cited in Madoz work
 927 (1846-1850); FCI = a Fish Community Integrity index computed as the sum of number
 928 of native species lost plus non-native species gained among historical and current data
 929 (see Methods). Number of sub-basins analysed=137.

930

Number of native species lost			Number of non-native species gained			FCI index		
	n sub-basins	%		n sub-basins	%		n sub-basins	%
0	11	8.0	0	92	67.2	0	7	5.1
1	43	31.4	1	24	17.5	1	30	21.9
2	53	38.7	2	13	9.5	2	44	32.1
3	18	13.1	3	4	2.9	3	26	19.0
4	10	7.3	4	2	1.5	4	21	15.3
5	2	1.5	5	2	1.5	5	4	2.9
						6	2	1.5
						7	3	2.2
						>7	0	0.0

931

932

933

934 **Table 3.** Historical and current distribution of fish species in the Guadalquivir basin,
 935 according to sub-basin area (km²). TOTAL: a) historical data *t* is the total area of sub-
 936 basins (km²) recording a given species in 19th century in Madoz (1846-1850); b) current
 937 data *t* is the total area of sub-basins (km²) recording a given species in 21st century.
 938 OVERLAPPED refers only to sub-basins registering fish distribution data in both
 939 historical and current periods. Historical data ϕ and current data ϕ are also shown for
 940 total sub-basin area per period and species. % change is the area lost (-) or gained (+)
 941 from historical to current data, taking historical data ϕ as 100%. Sector χ^2 shows the
 942 results of a chi-squared test in which the null hypothesis was “there is no difference
 943 between the two periods in the distribution of a given species” (see material and
 944 methods for details).

945

Species	TOTAL		OVERLAPPED			
	historical data <i>t</i> (km ²) (%)	current data <i>t</i> (km ²) (%)	historical data ϕ (km ²) (%)	current data ϕ (km ²) (%)	% change	sectors χ^2
<i>Anguilla anguilla</i>	43,026 (86.7)	1,322 (3.4)	30,076 (85.8)	1,003 (2.9)	- 96.7	***
<i>Salmo trutta</i>	21,450 (43.2)	2,538 (6.5)	15,393 (43.9)	2,538 (7.2)	- 83.5	***
<i>Luciobarbus sclateri</i>	37,515 (75.6)	25,895 (66.8)	27,618 (78.8)	23,212 (66.2)	- 15.9	n.s
<i>Pseudochondrostoma willkommii</i>	30,553 (61.6)	9,401 (24.2)	22,344 (63.7)	9,015 (25.7)	- 59.7	n.s
<i>Squalius pyrenaicus</i>	5,865 (11.8)	5,644 (14.6)	3,725 (10.6)	5,171 (14.7)	+ 38.8	n.s
Total surveyed area	49,618 (100.0)	38,780 (100.0)	35,061 (100.0)	35,061 (100.0)		

χ^2 test: *p<0.05; **p<0.01; ***p< 0.001; n.s: non-significant

946

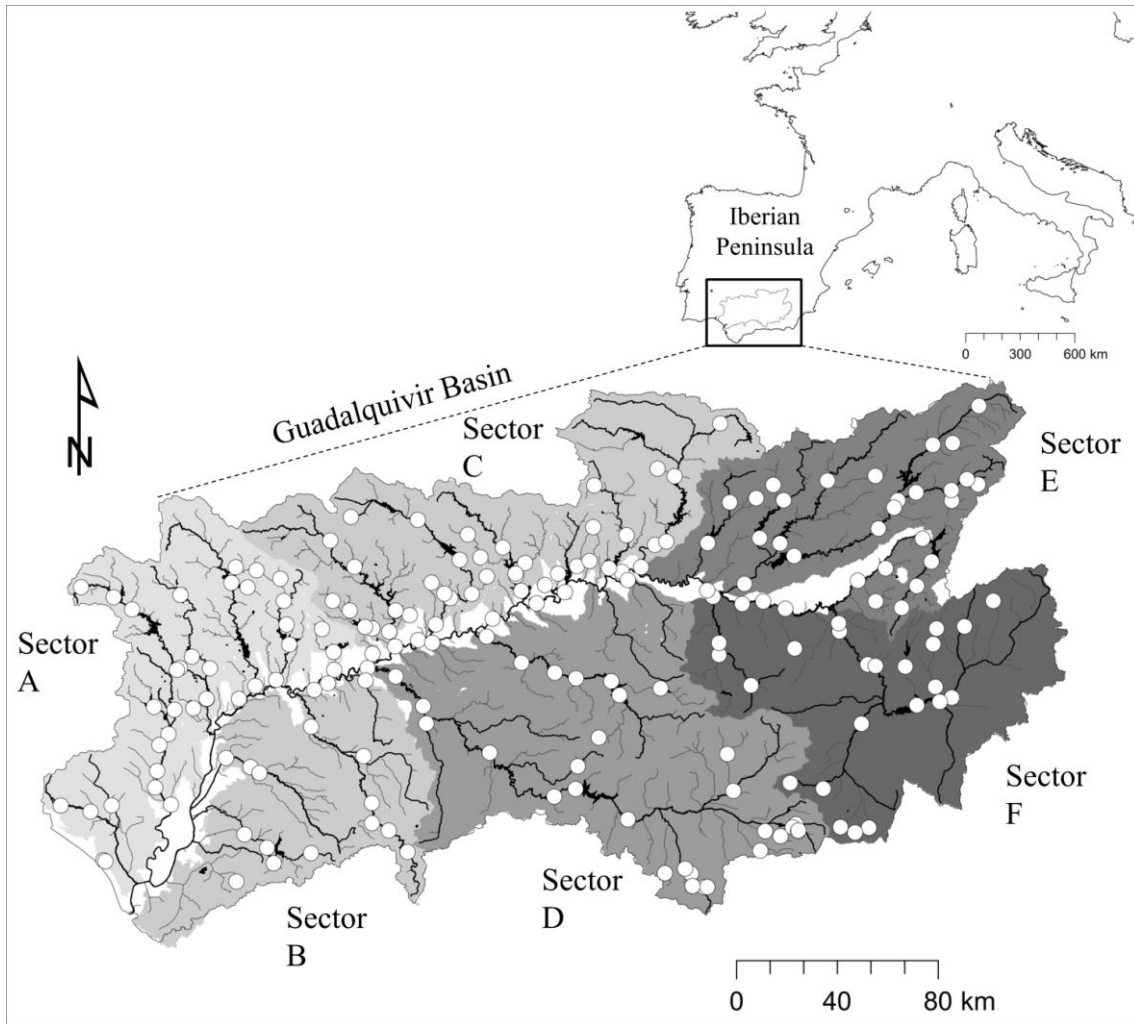
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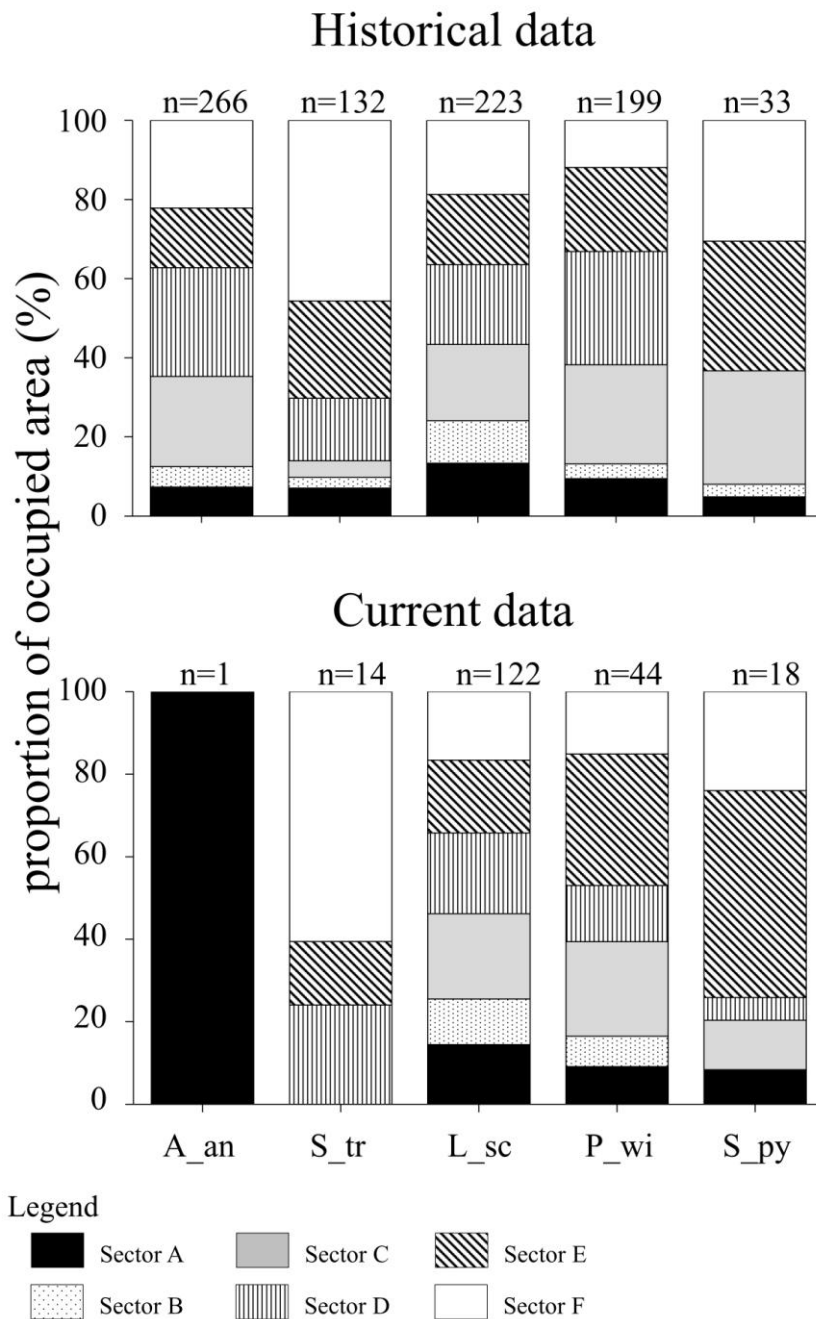
950 **FIGURE CAPTIONS**

951 Figure 1. Map of the Guadalquivir River basin (S Iberian Peninsula). The 195 sites
952 sampled in 2008 for fish and habitat variables are shown with white dots. The basin has
953 been divided into six sectors highlighting the lower (A, B), medium (C, D) and upper
954 (E, F) basin. Sectors are shown in different shades of grey.
955



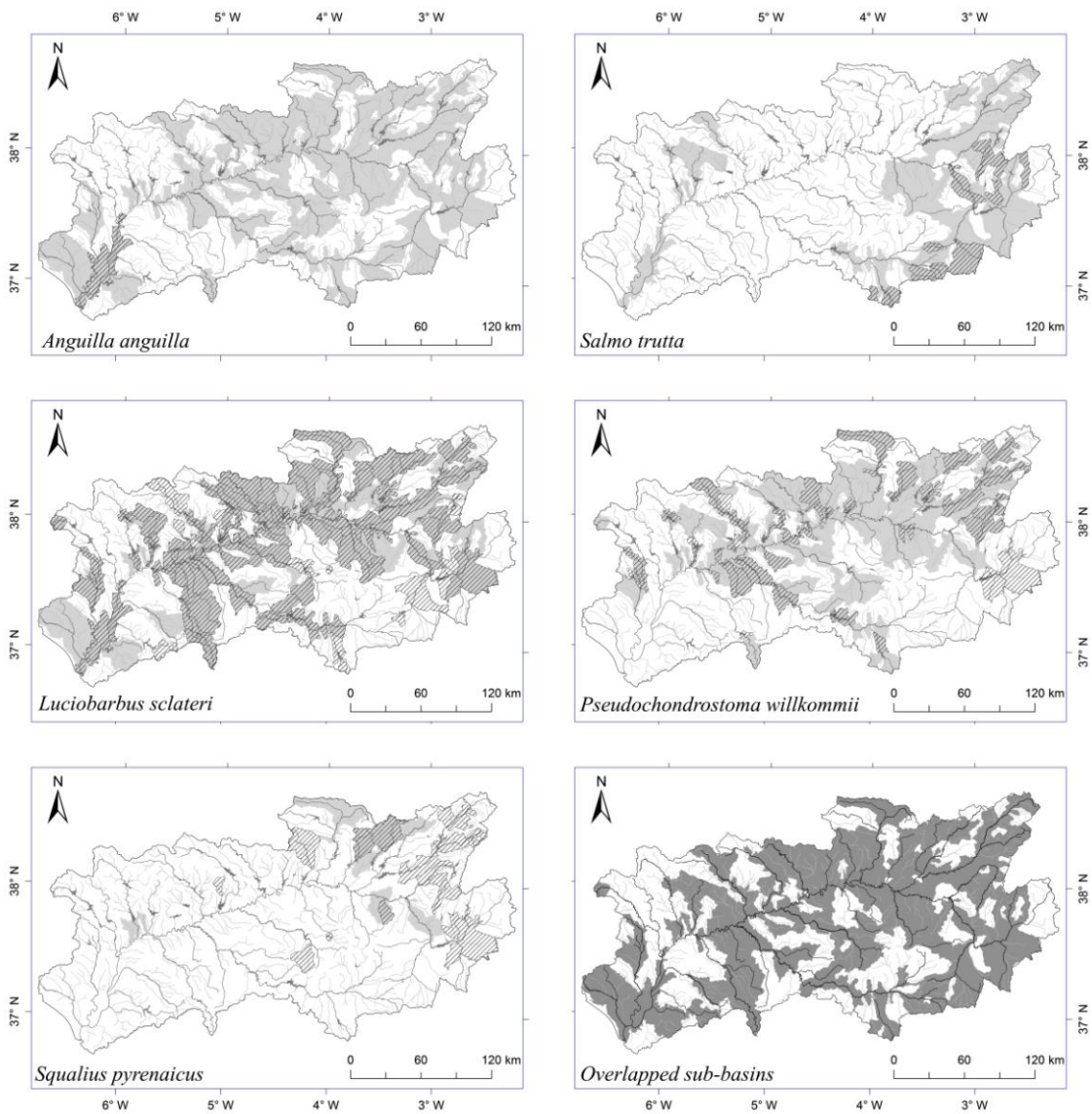
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
958 Figure 2. Relative presence (%) of five native fish species in the different sectors of
 959 Guadalquivir basin from historical and current periods using all data available in each
 960 period (n=338 and n=212, respectively). The historical and current use of basin sectors
 961 differed statistically for European eel and brown trout (Chi-squared test, p<0.001), but
 962 not for the rest of the species (see Table 2). Species codes A_an: *Anguilla anguilla*;
 963 S_tr: *Salmo trutta*; L_sc: *Luciobarbus sclateri*; P_wi: *Pseudochondrostoma willkommii*;
 964 S_py: *Squalius pyrenaicus*. n, total amount of observations for each species and each
 965 period.
 966



967
 968

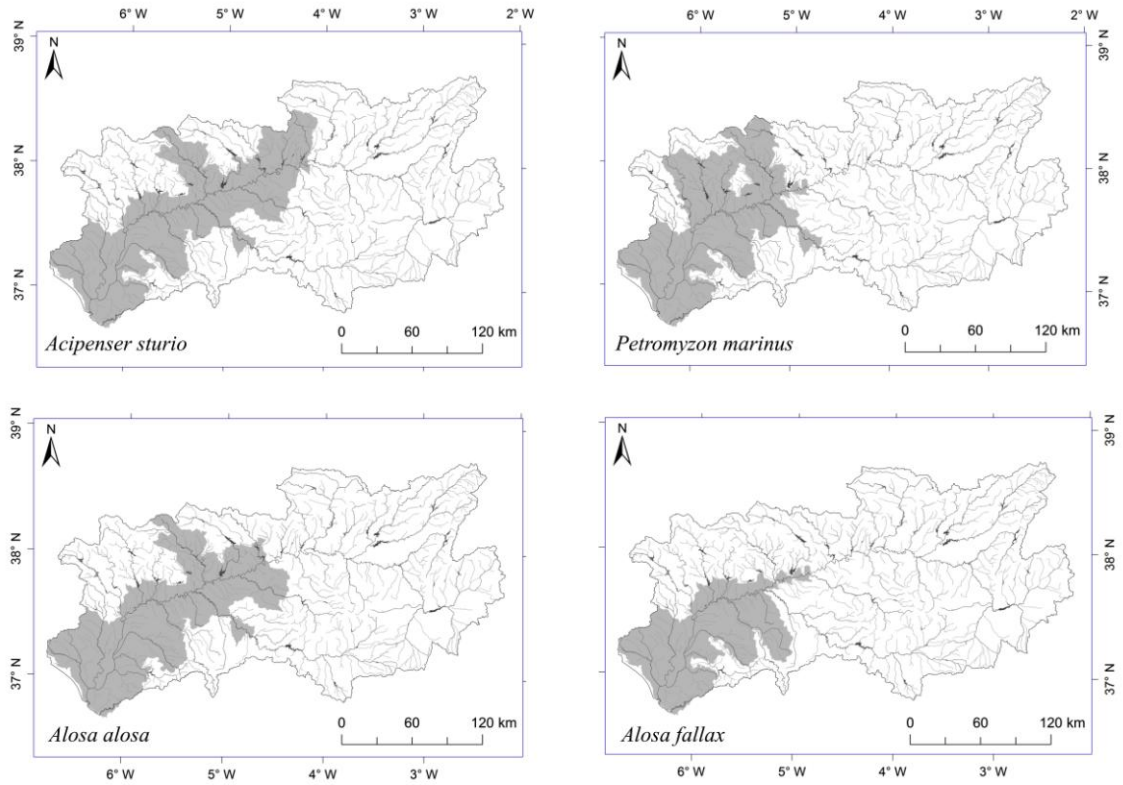
969 Figure 3. Distribution maps of the European eel (*Anguilla anguilla*), brown trout (*Salmo*
 970 *trutta*), southern Iberian barbel (*Luciobarbus sclateri*), southern straight-mouth nase
 971 (*Pseudochondrostoma willkommii*) and southern Iberian chub (*Squalius pyrenaicus*) in
 972 the Guadalquivir basin in historical and current data. The grey area highlights the
 973 species distribution in historical data and the striped areas represent the current data
 974 species distribution. Total of sub-basins with sampling data in both periods (n=187)
 975 where used to represent the comparison of specie distribution among historical and
 976 current data (see map 'overlapped sub-basins' at the botton of figure).
 977




Legend:  Historical sub-basins  Current sub-basins

978
 979

980 Figure 4. Distribution maps of extinct diadromous freshwater fish species (Atlantic
981 sturgeon, *Acipenser sturio*; sea lamprey, *Petromyzon marinus*; allis shad, *Alosa alosa*
982 and twaite shad, *Alosa fallax*) detected previously to 21st century in Guadalquivir River
983 basin. The grey area highlights the species distribution.
984



985 Legend:  Historical sub-basins
986

987 **Supplementary Data. Table A.** List of extinct diadromous freshwater fish species and
 988 sites where were detected previously to 21st century in the Guadalquivir basin.
 989

Species	Date	Locality	Reference
<i>Acipenser sturio</i>	1758	Cádiz (NW, coast) and Guadalquivir River (up to Córdoba)	Machado, 1857
	1826-29	Guadalquivir River	Miñano, 1829
	1846-1950	Córdoba	Madoz, 1850
	1846-1950	Cantillana	Madoz, 1850
	1846-1950	Lora del Río	Madoz, 1850
	1846-1950	Peñaflor	Madoz, 1850
	1846-1950	Guadalquivir River	Madoz, 1850
	1865	Sevilla	Steindachner, 1866
	1866	Córdoba	Steindachner, 1866
	1931	Cantillana	Classen, 1944
	1931	Villanueva del Río & Alcolea del Río	Classen, 1944
	1931	Lora del Río	Classen, 1944
	1932	Isla Mayor	Classen, 1944
	1932-34	Trebujena	Classen, 1944
	1932-35	Coria del Río	Classen, 1944
	1932-41	La Algaba	Classen, 1944
	1932-43	Alcalá del Río	Classen, 1944
	1933-34	Sanlúcar de Barrameda	Classen, 1944
	1933-42	Isla Menor & Isla Mínima	Classen, 1944
	1933-43	El Puntal & Caño de la Nueva	Classen, 1944
	1933-43	La mata	Classen, 1944
	1934-43	Figuerola	Classen, 1944
	1934	Tarfia	Classen, 1944
	1934	Sevilla	Classen, 1936
	1974	Alcalá del Río	Hernando, 1975
	1975	Alcalá del Río	Hernando, 1975
	1992	El Inglesillo (beach)	Elvira & Almodóvar, 1993
	no date	Guadalquivir River	Matallanas <i>et al.</i> , 1981

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991 **Supplementary Data.** Continued.

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Species	Date	Locality	Reference
<i>Alosa alosa</i>	1758	Cádiz (NW, coast) and Guadalquivir River	Machado, 1857
	1826-29	Andalucía	Miñano, 1829
	1826-29	Guadalquivir River	Miñano, 1829
	1846-1950	Córdoba	Madoz, 1850
	1846-1950	Palma del Río	Madoz, 1850
	1846-1950	Córdoba	Madoz, 1850
	1846-1950	Benacazón	Madoz, 1850
	1846-1950	Cantillana	Madoz, 1850
	1846-1950	Coria del Río	Madoz, 1850
	1846-1950	Gelves	Madoz, 1850
	1846-1950	Lora del Río	Madoz, 1850
	1846-1950	Peñaflor	Madoz, 1850
	1846-1950	Puebla junto a Coria	Madoz, 1850
	1846-1950	San Juan de Aznalfarache	Madoz, 1850
	1846-1950	Sanlúcar La Mayor	Madoz, 1850
	1846-1950	Lora del Río P.J.	Madoz, 1850
1846-1950	Guadalquivir River	Madoz, 1850	
<i>Alosa fallax</i>	1803	Cádiz (NW, coast)	Machado, 1857
	1846-1950	Peñaflor	Madoz, 1850
	1846-1950	Guadalquivir River	Madoz, 1850
<i>Petromyzon marinus</i>	1784	Guadalquivir River	Machado, 1857
	1846-1950	Fuente Obejuna	Madoz, 1850
	1846-1950	Viar River	Madoz, 1850
	1846-1950	Guadalquivir River	Madoz, 1850
	1973	Canal de Parladé	Hernando, 1974
	1973	Canal de los Portugueses	Hernando, 1974
	1973	Canal de los Portugueses	Hernando, 1974

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995 **Supplementary data references (with data)**

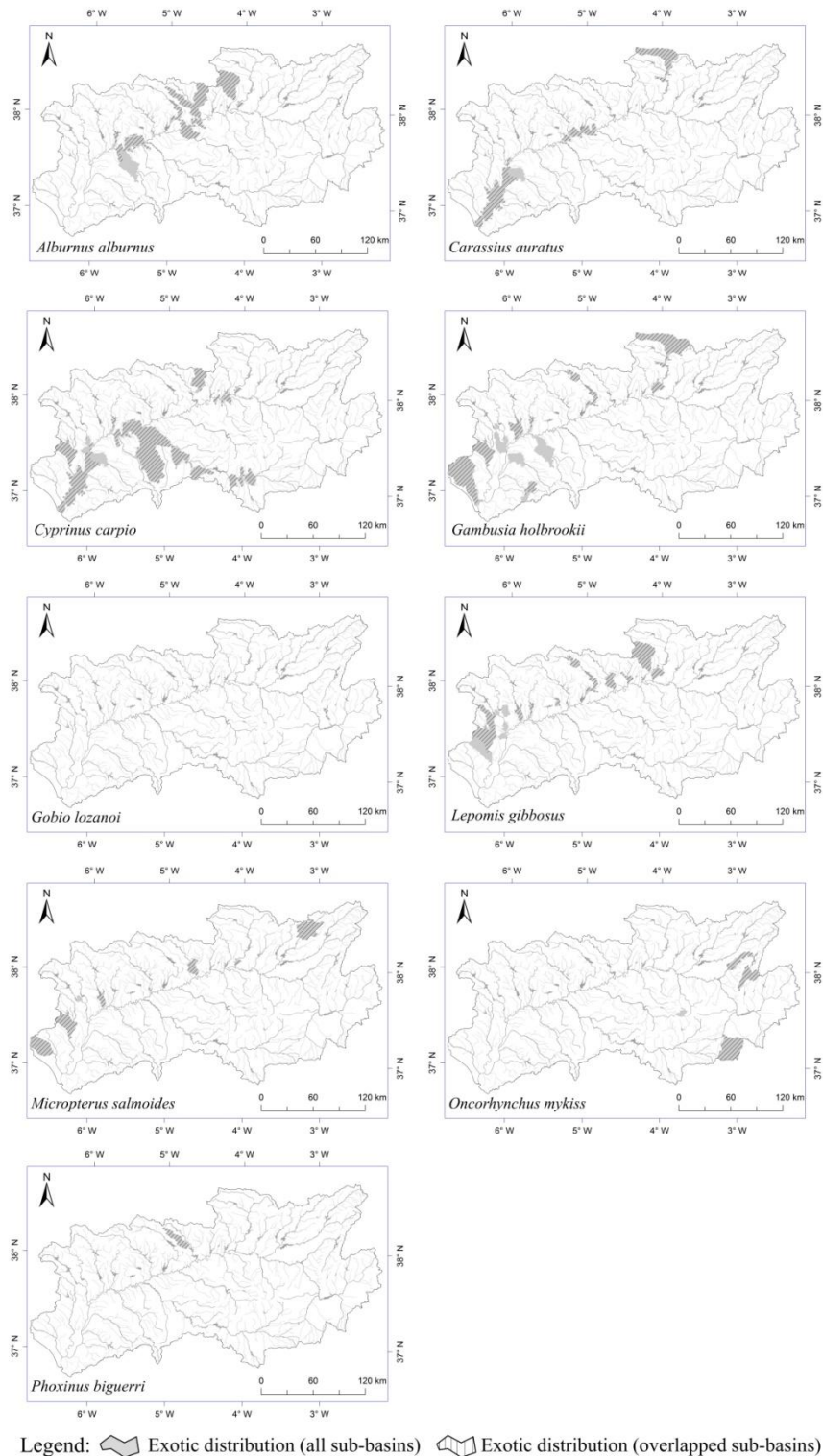
- 996 Classen, T.E.A. (1936). *Notas preliminares sobre la biología y el aprovechamiento del*
997 *esturión del Guadalquivir*. Publicaciones de la Sección de Pesca, Ministerio de
998 Agricultura, Industria y Comercio, ser. 1, 2: 15–41.
- 999 Classen, T.E.A. (1944). *Estudio bio-estadístico del esturión o sollo del Guadalquivir*
1000 *(Acipenser sturio L.)*. Ministerio de Marina. Instituto Español de Oceanografía.
1001 Trabajos, 19. 112 pp.
- 1002 Elvira, B. & Almodóvar, A. (1993). Notice about the survival of sturgeon (*Acipenser*
1003 *sturio* L., 1758) in the Guadalquivir estuary (SW Spain). *Archiv fur Hydrobiologie*,
1004 129(2), 253.
- 1005 Hernando Casal, J.A. (1974). Captura de *Petromyzon marinus* L. en las marismas del
1006 Guadalquivir. Doñana. *Acta vertebrata*, 1(2), 1–4.
- 1007 Hernando Casal, J.A. (1975). Notas sobre la distribución de los peces fluviales en el
1008 Suroeste de España. *Doñana, Acta vertebrata*, 2(2), 263–264.
- 1009 Madoz, P. (1845-1850). *Diccionario geográfico-estadístico-histórico de España y sus*
1010 *posesiones de ultramar*. 16 Vols. P. Madoz and L. Sagasti, Madrid, Spain. Available at
1011 <http://www.bibliotecavirtualdeandalucia.es>
- 1012 Machado, A. (1857). *Catálogo de los peces que habitan o frecuentan las costas de*
1013 *Cádiz y Huelva, con inclusión de los del Río Guadalquivir*. Librería española y
1014 extranjera, Sevilla. [http://fondosdigitales.us.es/fondos/libros/3515/14/catalogo-de-los-](http://fondosdigitales.us.es/fondos/libros/3515/14/catalogo-de-los-peces-que-habitan-o-frecuentan-las-costas-de-cadiz-y-huelva-con-inclusion-de-los-del-rio-guadalquivir-por-antonio-machado/)
1015 [peces-que-habitan-o-frecuentan-las-costas-de-cadiz-y-huelva-con-inclusion-de-los-del-](http://fondosdigitales.us.es/fondos/libros/3515/14/catalogo-de-los-peces-que-habitan-o-frecuentan-las-costas-de-cadiz-y-huelva-con-inclusion-de-los-del-rio-guadalquivir-por-antonio-machado/)
1016 [rio-guadalquivir-por-antonio-machado/](http://fondosdigitales.us.es/fondos/libros/3515/14/catalogo-de-los-peces-que-habitan-o-frecuentan-las-costas-de-cadiz-y-huelva-con-inclusion-de-los-del-rio-guadalquivir-por-antonio-machado/)
- 1017 Matallanas, J., Ibáñez, M., San Millán, M.D. & Riba, G. (1981). *Catálogo de los Peces*
1018 *Marinos de la colección del Museo Nacional de Ciencias Naturales de Madrid*. Ed.
1019 Universidad Autónoma de Barcelona, Bellatema, 1, 138 pp.
- 1020 Miñano, S. (1826-1829). *Diccionario geográfico-estadístico de España y Portugal*. 11
1021 Vols. Imprenta Pierart-Peralta, Madrid, Spain. Available at
1022 <http://www.bibliotecavirtualdeandalucia.es>
- 1023 Steindachner, F. (1866). Ichthyologischer bericht iiber eine nach Spanien und Portugal
1024 unternommene Reise. Zur Flussfishfaune des siidlichentheiles von Spanien und
1025 Portugal. *Sitzungsber. Kais. Akad. Wiss.*, 54:261-272.
- 1026 **Supplementary data References (with no data)**
- 1027 Muñoz y Romero, T. (1858). *Diccionario bibliográfico-histórico de los antiguos reinos,*
1028 *provincias de España*. Imprenta y estereotipia de M. Rivadeneiva: Madrid. 329 p.

- 1029 Sañez Reguart, A. (1791). *Diccionario histórico de las artes de pesca nacional*. Edit.
1030 Maxtor: Madrid. ISSN: 8497616006
- 1031 Cazurro, M. (1871-1900). *Actas de la Sociedad Española de Historia Natural* 23: 188-
1032 226. Available at <http://bibdigital.rjb.csic.es/spa/Volumenes.php?Libro=1110>
- 1033 Graells, MP. (1897). *Memorias de la Real Academia de Ciencias Exactas Físicas y*
1034 *Naturales* 17: 1-806. Available at
1035 [http://www.archive.org/stream/memoriasdelareal1797real/memoriasdelareal1797real_dj](http://www.archive.org/stream/memoriasdelareal1797real/memoriasdelareal1797real_djvu.txt)
1036 [vu.txt](http://www.archive.org/stream/memoriasdelareal1797real/memoriasdelareal1797real_djvu.txt)
- 1037 Martínez-Reguera, L. (1881). *La Fauna de Sierra Morena: catálogo descriptivo de los*
1038 *mamíferos del término de Montoro*. Imp. de M. Romero, Madrid, Spain. Note: available
1039 at the virtual library of Andalucía
1040 ([http://www.bibliotecavirtualdeandalucia.es/catalogo/catalogo_imagenes/grupo.cmd?pat](http://www.bibliotecavirtualdeandalucia.es/catalogo/catalogo_imagenes/grupo.cmd?pat_h=1001741)
1041 [h=1001741](http://www.bibliotecavirtualdeandalucia.es/catalogo/catalogo_imagenes/grupo.cmd?pat_h=1001741))
- 1042 Navarro, A.J. (1798). La ciudad y territorio de Baza. Published in 1917. In: *Boletín de*
1043 *la Real Academia de Historia* 70: 259-286. Available at Biblioteca Virtual Miguel de
1044 Cervantes, Alicante, Spain ([http://www.cervantesvirtual.com/obra/la-ciudad-y-](http://www.cervantesvirtual.com/obra/la-ciudad-y-territorio-de-baza-0/)
1045 [territorio-de-baza-0/](http://www.cervantesvirtual.com/obra/la-ciudad-y-territorio-de-baza-0/))
- 1046

1047 **Supplementary Data.**

1048 **Figure A.** Distribution maps of non-native species in the Guadalquivir basin at twenty-
1049 first century.

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