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1 **Stream restorations with meanders increase dragonfly and damselfly diversity and**
2 **abundance, including an endangered species**

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9

10

11 **HIGHLIGHTS**

- 12 • Many streams were artificially linearized for human usage, but with probable
13 negative effect on biodiversity.
- 14 • Two streams were restored with meanders and diggings allowing water to run again.
- 15 • On both restored streams, diversity and abundance of Odonata (dragonflies &
16 damselflies) significantly increased.
- 17 • The endangered southern damselfly (*Coenagrion mercurial*) colonized both streams
18 after restorations.

19

20 **SUMMARY**

21 This study presents examples of successful restoration projects for biodiversity conservation.
22 In West France, the Pinail National Nature Reserve is a protected wetland interspersed with
23 more than 6000 ponds. This wetland is inhabited by 50 species of Odonata and thus is a key
24 biodiversity area for damselflies and dragonflies conservation. In the past, when the
25 limestone was exploited, the streams of the plateau were artificially channeled rectilinearly,
26 running to the Vienne River. Eventually streams were blocked by biomass and sediments
27 resulting in water flowing mainly underground. In 2011, two restoration projects dug and
28 recreated lost habitats such as running streams and meanders by openly reconnecting
29 bodies of standing water (two sites: *Rivau* (20m) and *Hutte* (400m) streams). The Odonata
30 species diversity and abundance are annually monitored following transect inventories since
31 1995 and still ongoing. Diversity and abundance were compared before and after the
32 restoration. The abundance and species diversity increased at both sites due to the addition
33 of lotic habitats and consequently additional new species. The number of observed species
34 almost doubled on the Rivau (from 5.4 observed species to 9.9 spp). By extrapolation the
35 total species number on site increased from 15-18 spp to 29-37 spp. The abundance also
36 greatly increased with 770% more individuals on the Rivau. Similarly, on the 400m Hutte
37 stream, the extrapolated diversity increased from 31-38 spp to 35-43 spp; as well as the
38 abundance with 475% more individuals. These restoration projects created new habitats
39 leading to local biodiversity enrichment and conservation success. More specifically,
40 *Coenagrion mercuriale* (Odonata: Zygoptera), one of Europe's most threatened damselflies
41 and listed in the European Habitats directive, successfully recolonized the Rivau stream and
42 colonized the Hutte stream.

43 **Key Words:** Dragonflies, Damselflies, Freshwater, Wetland, Insect conservation, meander,
44 Zygoptera, Ephemeroptera, lotic water, *Coenagrion mercuriale*

45 **1. Introduction**

46 The Odonata order including damselflies (Zygoptera) and dragonflies (Epiprocta) did not
47 escape the global change and overall biodiversity crises. One tenth of the species are listed
48 as threatened of extinction (assigned as critically endangered, endangered, or vulnerable in
49 the IUCN Red List; (Clausnitzer *et al.*, 2009). The group varies from ubiquitous species
50 adapted to degraded water through to pristine water specialists sensitive to ecological
51 change. Most of them are affected by pollution, habitat destruction, and climate change
52 which impact their aquatic ecosystems (Clausnitzer and Jodicke, 2004). Hence this group is
53 considered to be a bioindicator for wetlands (De paiva Silva *et al.*, 2010; Kutcher and Bried,
54 2014). Human activities can degrade wetlands and lead to ecosystem simplification and
55 homogenization (Lougheed *et al.*, 2008) and remaining protected areas are rare and isolated
56 in an ocean of anthropogenic systems (Trombulak and Frissell, 2000; Cushman, 2006). That is
57 why wetland conservation involves habitat restoration projects. For Odonata, habitat
58 conservation/restoration projects can be categorized for two types of systems: lotic and
59 lentic.

60 There are Odonata in lotic (flowing water) and lentic (still water) ecosystems. Examples of a
61 lotic ecosystem include: creeks, streams, runs, rivers, springs, brooks and channels. Ponds,
62 basin marshes, ditches, reservoirs, seeps, lakes, and vernal/ephemeral pools are lentic
63 systems. Globally the lotic species are at greater risk than those in lentic waters (Clausnitzer
64 *et al.*, 2009) because lotic habitats are less predictable and stable in space and time. Lentic
65 species are more generalized and have a higher dispersal capacity, resulting in larger ranges
66 and wider ecological preferences (Korkeamäki and Suhonen, 2002; Corbet, 2004; Grewe *et*
67 *al.*, 2013).

68 In protected areas, stakeholders have to conserve habitat diversity in order to keep
69 ecological functioning and biological diversities. This mission implies sometimes, active
70 restoration projects of previous existing habitat such as lotic habitats; artificially degraded,
71 destroyed or plugged. In the West of France, the Pinail National Nature Reserve, is the only
72 National Reserve of the Vienne department. Among the Odonata order (Insecta: Pterygota),
73 50 from 138 described in Europe were assessed in the reserve (Sellier, unpub. Data).
74 Restoration projects aiming to recreate running water by meandering were undertaken
75 (during winter of 2010 and 2011) in order to increase ecological niches and thus biodiversity
76 (especially for lotic species). To our knowledge, there is no study to date illustrating the
77 positive ecological impact of stream meandering on Odonata diversity and abundance
78 comparing data before and after restoration. This study aims to illustrate this positive
79 ecological restoration effect.

80 The study focused on southern damselfly *Coenagrion mercuriale* (Charpentier, 1840)
81 (Odonata: Zygoptera) return or neocolonization in streams that were blocked and slowed-
82 down by biomass accumulation. *C. mercuriale* is one of Europe's most threatened
83 damselflies and is listed in the European Habitats directive. *C. mercuriale* has a south-west
84 European distribution and has declined in countries on the northern and eastern boundaries
85 of its distribution, including Britain. It is highly specialized, being restricted to fragmented
86 biotopes in Europe. These are small lowland heathland streams and ditches on old water
87 meadow ditch systems on chalk streams. Within these biotopes, a requirement for a
88 thermally advantageous microclimate (e.g. use of unshaded, permanently flowing, small
89 watercourses with perennial herbaceous aquatic vegetation) restricts the species further to
90 patches of early successional habitat with lotic water (Purse *et al.*, 2003). *C. mercuriale* was
91 occasionally observed within the reserve from 1988 in the Hutte and Rivau streams.

92 However, occurrence decreased over time hence the stream restoration project's primary
93 objective is to restore local populations of *C. mercuriale*.
94 The goal of this paper is to assess whether Odonata (dragonflies and damselflies) community
95 abundance and species richness is influenced by restoration of lotic habitats. The study
96 focusses on the endangered species *C. mercuriale*.
97 We test the hypothesis that additional species (such as lotic species) would be recruited to
98 the study sites, specifically *Coenagrion mercuriale*.

99

100 **2. Materials and Methods**

101

102 *2.1 Study area*

103 The National Nature Reserve of the Pinail (N 46° 42' 2.698"- E 0° 31' 13.378"), (Réserve
104 Naturelle Nationale du Pinail, www.reserve-pinail.org) is a unique concentration of >6000
105 ponds with 3000 permanent water holes distributed over 142 ha (Préau *et al.*, 2017, Figure
106 1). It is surrounded by 4166 ha of forest mainly classified within Natura 2000 status (and also
107 ZSC: special zone of preservation and ZPS: zone of special protection), ZICO (zone of Europe
108 community interest for birds) (European Nature Protection area network) and ZNIEFF of 1
109 and 2 types: natural Zone of Ecological Interest Fauna and Flora (French natural inventory of
110 biodiversity) and a RAMSAR site in 2020. The reserve is covered with diversified *Erica* moors
111 on acid and oligotrophic ground (podzol). This site forms a very rich ecocomplex where 2 500
112 species (fauna, flora, fungi) are listed (Beaune *et al.*, 2017; Beaune *et al.*, 2018; Beaune *et al.*,
113 2019).

114 *2.2 Lentic to lotic restoration*

115 Historically, humans have extracted rock from the site for more than 1200 years. This
116 includes the extraction of grindstone (*Pierre meulière*) resulting in thousands of open ponds.
117 They also dug channels, probably to transport materials. The channels ran from the Pinail
118 plateau to the lower streams linked to the rivers Vienne and Clain. Human exploitation has
119 stopped since the 20th century and protection of the site officially started in 1980. With
120 phytomass accumulation plus limited water flow, the channels of 'La Hutte', 'Rivau' and 'Reu'
121 were blocked and eventually a succession of long ponds formed. Permanent water flows run
122 underground except during exceptionally dry events. Restoration consisted of the digging of
123 a succession of meanders increasing the length of the stream and the microhabitat
124 heterogeneity, and the reconnection of water bodies to restore water flow and current.
125 Restoration actions were achieved in the winter of 2011 for 'la Hutte' and 'Rivau', consisting
126 of unblocking channels and recreating meanders (increasing sinuosity) thereby improving in-
127 stream habitat heterogeneity with some islands and banks of sand or pebbles or rock. See
128 Figure 2 & 3.

129 Regarding the Hutte stream, work was performed over 9 days and 400 m. Restoration began
130 in September 2011 with vegetation clearing. Work using a 20-ton mechanical excavator
131 began on December 12, 2011 with the unblocking of three landings that blocked the stream
132 flow. This classic public works excavator has proven to be unsuitable for the finer works of
133 meandering. Work therefore resumed from October 1, 2012 with a 6-tonne excavator. A
134 final day of fine work (reconstruction of banks) was done by hand during the winter. The
135 cost of this intervention was € 11,500.

136 For the Rivau (20 m), the works took place on a single day in October 2010. Work was
137 completed by the Nature Reserve team with a 3.6-ton mini-excavator. In addition to the
138 remodeling of the stream, a pond has been created in parallel with the Rivau.

139 For the two restorations, no external material (gravel) was added. The sands, gravel and
140 stones came from the site avoiding changes in the water pH and other components.
141 Research and restoration protocols have been approved by the direction régionale de
142 l'Environnement, de l'Aménagement et du Logement Nouvelle-Aquitaine (DREAL N.A.) and
143 the National Reserve scientific committee.

144 *2.3 Odonata inventories*

145

146 Since 1995, Odonata (diversity and abundance) have been monitored on the site at least one
147 morning in June and July of each year following (Langlois and Gilg, 2007). The day was selected
148 according to favorable weather conditions: sunny without rain nor wind, which could bias the
149 census. For each transect inventory, one experienced biologist walked the same stream bank
150 at a constant speed of 2 km/h. Each imagines (adults) were recorded in a virtual corridor of
151 5m wide (5m high from the ground and 2.5m on each side of the biologist) and were
152 exhaustively identified at the species level by direct recognition or by capture with a
153 butterfly net for difficult species to identify (i.e. *Lestes* spp). The two streams: La Hutte and
154 Rivau were monitored between 10:30-13:00 with a total transect length of 400m and 90m
155 respectively. Censuses are still ongoing and are also performed at other sites of the reserve
156 following the same protocol.

157 The restoration's positive impacts on species richness, species emergence and species
158 abundance were tested for 1/La Hutte stream (over its entire length: 400 m) and 2/ the Rivau
159 stream (area limited to the restored portion: 20 m).

160

161 *2.4 Community analyses*

162

163 Species richness and abundance (individual number) were calculated for each stream;
164 Hutte and Rivau with one transect inventory in June and one in July (annual census that
165 failed these conditions were excluded, see Table 3 & 4 for the analyzed years). The actual
166 species richness was not attained with the monitorings. Using R (R Development Core Team,
167 2020), the extrapolated cumulative diversity richness was estimated by random resampling
168 using several methods: bootstrap, jackknife of degrees 1 and 2, and Chao (Palmer, 1990).
169 These four estimation methods consider differently the occurrences of rare species and the
170 importance of replication (Chao, 1987; Colwell and Coddington, 1994) and were calculated
171 with the SPECPOOL function of the Vegan-R-package (Oksanen *et al.*, 2019). Cumulative
172 diversity richness curves provide a good estimate of the representativeness of the sampling
173 effort and were also drawn to estimate the number of species by extrapolation with infinite
174 sampling and compare the species diversity of the streams BEFORE and AFTER the
175 restoration. The first year after restoration, the curves were obtained with the SPECACCUM
176 function Vegan-R package.

177 Classical species diversity indices were calculated for each available year of census at the
178 two streams: Shannon-Wiener Index (H ; H is low under strong dominance of one single
179 species and higher as the number of species increases in an assemblage, especially if they
180 are equally distributed), Simpson's Index (λ ; the index represents the probability that two

181 individuals randomly selected from a sample belong to different species, ranging from 0 to 1)
182 and Pielou evenness (J ; constrained between 0 and 1. The less variation in communities
183 between the species, the higher J is. In other words, an even community is 1).

184 The compositional dissimilarity among the annual census BEFORE and AFTER restoration
185 was tested using the Bray-Curtis index (Bray and Curtis, 1957) and Horn-Morisita overlap
186 index, still using the Vegan-R-package (Oksanen *et al.*, 2019). The Bray–Curtis dissimilarity is
187 a statistic used to quantify the compositional dissimilarity between different sites (here the
188 same site but BEFORE and AFTER restoration), based on counts at each site. The Horn-
189 Morisita overlap index is less sensitive to hyper-abundant species. Ordinations of the annual
190 census are graphically displayed by non-metric multidimensional scaling (NMDS), in two
191 dimensions; using Bray–Curtis and Horn-Morisita distances separately. NMDS is an indirect
192 gradient analysis approach which produces an ordination based on a distance or dissimilarity
193 matrix. NMDS attempts to represent, as closely as possible, the pairwise dissimilarity
194 between objects (yearly census) in a low-dimensional space (with species as vectors).
195 Furthermore, the analysis of multivariate homogeneity of group (BEFORE and AFTER
196 restoration) dispersions (variances) is performed using BETADISPERSER function. Non-
197 Euclidean distances between objects and group centroids are handled by reducing the
198 original distances to principal coordinates (PCo); the distances of the group are eventually
199 tested with PERMUTEST for Permutation test of multivariate homogeneity of groups
200 dispersions (variances).

201 Differences in the species richness, abundance of Odonata, diversity indexes (H, λ , J) BEFORE
202 and AFTER restoration were tested with Wilcoxon rank sum test (non-parametric data) for
203 each stream: Hutte and Rivau. The first year after restoration was considered non-
204 representative as it was highly perturbed and was excluded from analyses (2011 for Rivau
205 and 2012 for Hutte). All statistical tests were considered significant at the 0.05 level.

206

207 *2.5 The southern damselfly*

208

209 Among species inventoried, the endangered *Coenagrion mercuriale* (Charpentier, 1840) was
210 a focus for annual abundance. This species was the main target for the restoration projects
211 due to its conservation and patrimonial status. Consequently, the total number of individuals
212 inventoried on June-July annual transects described below were compared BEFORE and
213 AFTER the restoration on both streams.

214

215 **3. Results**

216

217 *3.1 Odonata inventories*

218

219 The number of insects (abundance) and species (diversity) significantly increased in both
220 streams after restoration (Table 1, Figure 4, 5, 6).

221 Rivau : Before the restoration, an average of $6.8 \pm \text{SD } 2.6$ individual Odonata were counted
222 during the June -July annual census (N= 5 years) with transects, increasing significantly to
223 52.5 ± 40.0 Odonata after the restoration (N= 8) see Table 1, with W test); from $5.4 \pm \text{SD } 1.8$
224 species before to a significant increase of 9.9 ± 3.6 species. A total of 13 different species

225 were observed during the annual June-July census (N = 5) before restoration and 25 species
226 after restoration (8). The extrapolated calculated diversities of the Rivau range from 15 to 18
227 species before restoration and from 29 to 37 species after restoration (Table 2, Figure 4, 5).

228 Hutte : Before the restoration, an average of $64.1 \pm \text{SD } 21.9$ individual Odonata were counted
229 during June and July annual census (N= 10 years), increasing significantly to 303 ± 120
230 Odonata after the restoration (N= 8; Table 1, with W test) ; from 12.9 ± 3 species before to a
231 significant increase of 15.7 ± 3.4 species. A total of 28 different species were observed
232 during the annual June-July census (N=10) before restoration and 31 species after
233 restoration (7). The extrapolated calculated diversities of the Hutte range from 31 to 38
234 species before restoration and from 35 to 43 species after restoration (Table 2, Figure 4, 6).

235 Occurrence of some species happened after the restoration. It is not possible to confirm that
236 those species were not present in the streams before the restoration. However ten species
237 were observed in the Hutte stream only after the restoration namely : *Calopteryx virgo*
238 (Linnaeus, 1758), *Chalcolestes viridis* (Vander Linden, 1825), *Coenagrion scitulum* (Rambur,
239 1842), *Crocothemis erythraea* (Brullé, 1832), *Gomphus graslinii* Rambur, 1842, *Leucorrhinia*
240 *pectoralis* (Charpentier, 1825), *Onychogomphus forcipatus* (Linnaeus, 1758), *Orthetrum*
241 *cancellatum* (Linnaeus, 1758), *Somatochlora metallica* (Vander Linden, 1825), *Sympetrum*
242 *meridionale* (Selys, 1841). Some of these species inhabit running water systems (lotic; see
243 Table 3). Inversely some species were not observed anymore after 2011 including *Aeshna*
244 *affinis* Vander Linden, 1820, *Aeshna cyanea* (O. F. Müller, 1764), *Aeshna isoceles* (O. F.
245 Müller, 1767), *Aeshna mixta* Latreille, 1805, *Cordulia aenea* (Linnaeus, 1758), *Gomphus*
246 *simillimus* Selys, 1840. Some of these species inhabit lentic water systems (see Table 3). All
247 the species absent in the stream after restoration were observed within the reserve ponds.

248 Similarly on the Rivau : 13 species were observed after restoration, namely : *Calopteryx virgo*
249 (Linnaeus, 1758), *Coenagrion mercuriale* (Charpentier, 1840), *Coenagrion puella* (Linnaeus,
250 1758), *Coenagrion pulchellum* (Vander Linden, 1825), *Coenagrion tenellum* (Villers, 1789),
251 *Enallagma cyathigerum* (Charpentier, 1840), *Ischnura elegans* (Vander Linden, 1820),
252 *Ischnura pumilio* (Charpentier, 1825), *Lestes dryas* Kirby, 1890, *Libellula depressa* Linnaeus,
253 1758, *Libellula quadrimaculata* Linnaeus, 1758, *Platycnemis pennipes* (Pallas, 1771),
254 *Sympetrum meridionale* (Selys, 1841). Some of these species inhabit running water systems
255 (lotic; see Table 4). One lentic water species was not observed after the restoration namely
256 *Lestes virens vestalis* Rambur, 1842 (see Table 4). All the species absent in the stream after
257 restoration were observed within the reserve ponds.

258 Some species including *Ceriagrion tenellum*, *Orthetrum coerulescens* became hyperabundant
259 (Tables 3 & 4), affecting the diversity indexes and evenness of the community (see Table 1).

260

261 3.2 Community analyses

262

263 Odonata communities BEFORE and AFTER the restoration seem dissimilar for both streams
264 Hutte and Rivau due to new species observed in both streams (see Table 3 & 4, and below
265 for details about the species, and the yearly transects analyzed). Graphically, the Odonata
266 communities ordinations by non-metric multidimensional scalings (NMDS, in two dimensions
267 display; using Bray–Curtis and Horn-Morisita distances) show no overlap for the small
268 community of the Rivau (20m) but slight overlapping for the larger Hutte 400m's community

269 (Figure 7). For the Rivau: the two centroids or spatial medians drawn draw by ‘spider
270 diagram’ where each point (annual census) is connected to the group centroid BEFORE and
271 AFTER restoration are distant; accordingly, the ellipsoid hulls, standard error ellipses of both
272 group coordinates are not overlapped (Figure 7a). For the Hutte: a slight overlap is visible
273 (Figure 7b). However, the analyses of multivariate homogeneity of groups BEFORE and
274 AFTER restoration using Permutation tests for homogeneity of multivariate dispersions did
275 not demonstrate significant dissimilarities. Using Bray-Curtis distance: Rivau: $F=0.0204$; p-
276 value = 0.9 and Hutte : $F=2.6992$; p-value = 0.12 (99 permutations). Using Horn-Morisita
277 distance= Rivau: $F=0.9705$; p-value = 0.28 and Hutte: $F= 2.7004$; p-value = 0.12 (99
278 permutations).

279 Indeed, several species are still present before and after restoration with 57% common
280 species for Hutte (21/37spp) and 46% for Rivau (12/26 spp). Thus, communities are not
281 significantly distant (i.e. not significantly different) accordingly to this test.

282

283 3.3 *The southern damselfly*

284

285 The restoration projects targeted the establishment or increase in the *Coenagrion mercurial*
286 populations. This damselfly was never observed on the Hutte stream and rarely on the Rivau
287 before restoration (see Table 2). *Coenagrion mercurial* was observed on both Hutte and
288 Rivau the first year after restoration (Figure 8). After the restoration the number of observed
289 insects was significantly higher in the Rivau ($W = 39$, p-value = 0.0289) and Hutte ($W = 71.5$,
290 p-value = 0.004501) than before (with great annual variations): the number is from 0 to 4.3
291 \pm SD 4.8 on the Rivau and from $1 \pm$ SD 2.6 to 9.9 ± 7.8 on the Hutte. See (Table 1,2 & Figure 9).

292

293 4. Discussion

294 Restoration of rectilinear streams aims to broaden the spectrum of relevant habitats to
295 increase stream biodiversity. In particular meanders create micro-current conditions with a
296 variety of current speeds and water acceleration, thus creating various substratum deposits,
297 from sandy banks to gravels. This restoration also alters the riparian flora such as into the
298 Rivau and Hutte. Firstly, *Characeae* appeared (*Chara virgata* Kütz., 1834, *Nitella translucens*
299 (Pers.) C.Agardh, 1824, *Nitella tenuissima* (Desv.) Coss. & Germ.), and various species of
300 *Juncus* became abundant (*Juncus articulatus* L., *Juncus acutiflorus* Ehrh. ex Hoffm., *Juncus*
301 *bulbosus* L.). Secondly, *Schoenus nigricans* L. and *Cladium mariscus* (L.) Pohl disappeared,
302 and *Carex viridula* Michx. subsp. *oedocarpa* (Andersson) B.Schmid, *Baldellia ranunculoides*
303 (L.) Parl., *Carex elata* subsp. *elata* All., 1785 and *Eleocharis multicaulis* (Sm.) Desv colonized
304 the area. Odonata respond to these riparian changes and actively select new habitats
305 (Carchini *et al.*, 2003; Remsburg and Turner, 2009). New habitats lead to additional species
306 into the system. Our study demonstrates the rapid increase in both Odonata species
307 diversity and abundance in two restored streams. The number of observed species almost
308 doubled on the Rivau (from 5.4 observed species to 9.9 spp). By extrapolation the total
309 species number on site increased from 15-18 spp to 29-37 spp. The abundance also greatly
310 increased with 770% more individuals on the Rivau. Similarly, on the 400m Hutte stream, the
311 extrapolated diversity increased from 31-38 spp to 35-43 spp; as well as the abundance with

312 475% more individuals. These restoration projects created new habitats leading to local
313 biodiversity enrichment and conservation success.

314 This was also described with *Coenagrion mercurial* selecting tussock-forming monocots,
315 *Juncus inflexus* L., 1753 and *Deschampsia cespitosa* (L.) P.Beauv., 1812 (Rouquette and
316 Thompson, 2007). These are used for oviposition, as perching sites and the roots provide the
317 main larval habitat (Rouquette and Thompson 2005). All these microhabitats provide for
318 several distinct and required activities such as foraging, mate-seeking, pairing, oviposition
319 and nocturnal roosting suiting more selective species (Corbet, 2004). Changes in flora can
320 affect the Odonata communities (Rouquette and Thompson, 2007). In our study the
321 Odonata quickly responded to new habitat availability and heterogeneity. Species such as
322 *Coenagrion mercurial* which favor running waters, gained new lotic habitats in both streams
323 after restoration (similarly to *Calopteryx virgo*, *Gomphus graslinii*, *Onychogomphus*
324 *forcipatus* who arrived after restoration). Additional populations of this endangered species
325 is promising, especially for a rather sedentary species and poor disperser (Watts *et al.*, 2004;
326 Purse *et al.*, 2003). Movements are more likely within than between patches of suitable
327 habitat over short to medium distances (50–300 m) and the likelihood of *C. mercuriale*
328 recolonizing sites naturally (with only 1-11% of emigration rate) is within 1–3 km of other
329 populations (particularly within large clusters) (Purse *et al.*, 2003). For this species it is vital
330 to add or increase existing populations within suitable sites to facilitate stepping-stone
331 dispersal movements.

332 Another management action to conserve this species within perennial herbaceous aquatic
333 vegetation in small watercourses, is to maintain early successional habitat by rotational
334 cutting or grazing. Long-term regional persistence is likely to depend on metapopulation
335 dynamics and insects occupying a shifting habitat mosaic (Thomas & Hanski 1997). In our
336 study, annual change seems to be present. Is it due to weather factors, drying up of the
337 stream (drought), and possibly the current change in plants? Indeed, severe 2017 and 2019
338 droughts with negative effects on Odonata abundance are visible in Table 3 and 4 and in the
339 *C. mercuriale* occurrences (Figure 8) and should be investigated. The lowest occurrences
340 post restoration are in 2017 and 2019. Climate change may reduce the water amount input
341 to the reserve in the near future as suggested by recent models (Préau *et al.*, 2020; Préau *et*
342 *al.*, 2019) and this may counteract the positive effect of the restoration. Change in species
343 occurrence could be affected by the change in the riparian flora succession and deserve
344 further investigations. Other species may appear and disappear over time. More generally
345 our study demonstrates that meandering stream increases the diversity and abundance of
346 Odonata and is a good restoration strategy for artificially rectilinearized streams. This was
347 demonstrated on two scales: fine scale with 20m streams and longer with 400m. On the
348 finest scale, changes in the community are more detectable with more dissimilar species
349 occupying the modified habitat. This kind of conservation action are beneficial for the
350 biodiversity of the river basin and thus support a good ecological status by improving its
351 biological quality, in agreement with one of the objectives of the EU Water Framework
352 Directive 2000/60/EC (EU Council 2000).

353

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361

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438 ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion
439 mercuriale*', *Molecular Ecology*, 13(10), pp. 2931-2945.

440

441 Table list:

442 Table 1 Mean Odonata species richness, abundance, diversity (H, λ , J indexes) (mean \pm SD) in
 443 June-July of two streams: Hutte 400m and Rivau 20m after annual census (June-July), before
 444 and after restoration. Pinail National Nature Reserve, Vienne. France. Statistical
 445 significances: *: p-value \geq 0.05; **: p-value \geq 0.01; ***: p-value \geq 0.001.

Stream	Rivau	Rivau	Rivau	Hutte	Hutte	Hutte
Restoration	Before (5)	After (8)	Wilcoxon rank sum test	Before (10)	After (7)	Wilcoxon rank sum test
Species diversity	5.4 \pm 1.8	9.9 \pm 3.6	* W = 34. p-value = 0.02315	12.9 \pm 3.0	15.7 \pm 3.4	* W = 55.5, p-value = 0.02365
Abundance	6.8 \pm 2.6	52.5 \pm 40	** W = 40. p-value = 0.002129	64.1 \pm 21.9	303 \pm 120.5	*** W = 70, p-value = 0.0003773
Shannon-Wiener Index (H)	1.59 \pm 0.33	1.73 \pm 0.38	NS W = 25. p-value = 0.2618	2.01 \pm 0.32	1.77 \pm 0.21	NS W = 19, p-value = 0.0615
Simpson's Index (λ)	0.65 \pm 0.32	0.77 \pm 0.07	NS W = 16. p-value = 0.7382	0.80 \pm 0.07	0.72 \pm 0.06	* W = 12, p-value = 0.01250
Pielou evenness (J)	0.49 \pm 0.07	0.34 \pm 0.07	** W = 2. p-value = 0.003108	0.31 \pm 0.03	0.26 \pm 0.03	*** W = 4, p-value = 0.000617
N <i>Coenagrion mercuriale</i>	0 \pm 0	3.8 \pm 4.7	* W = 39. p-value = 0.0289	1 \pm 1.5	9.9 \pm 7.7	** W = 71.5, p-value = 0.002250

446

447 Table 2 Observed and estimated (4 extrapolation methods: Chao, Jackknife degree 1 and 2,
 448 Bootstrap) Odonata species richness (mean \pm SD) of two streams: Hutte 400m and Rivau
 449 20m after annual census (June-July), before and after restoration. Pinail National Nature
 450 Reserve, Vienne. France.

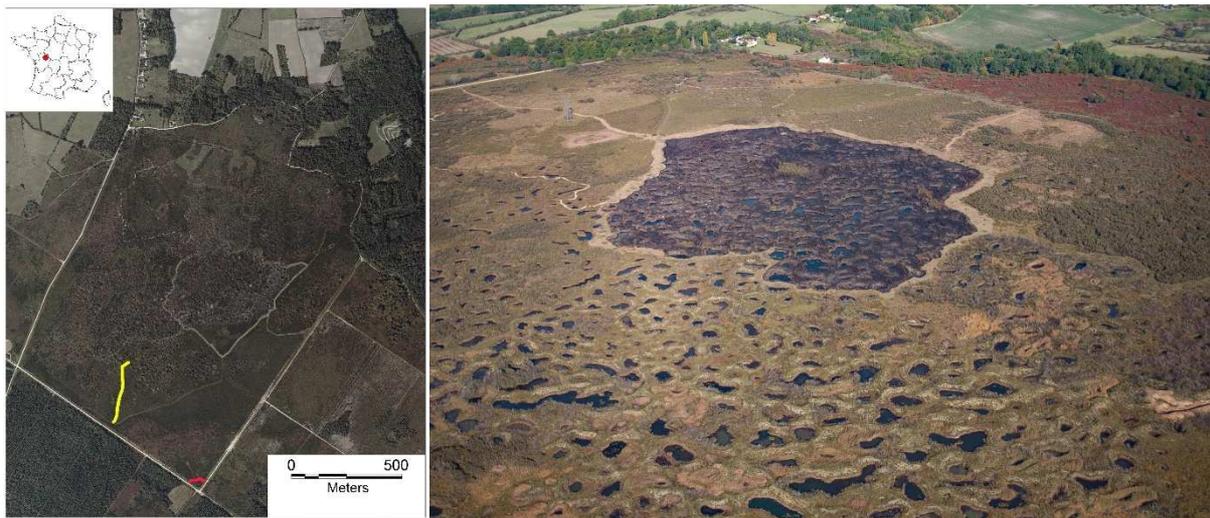
	Species observed	Chao	Jackknife1	Jackknife2	Bootstrap	Nb of annual census
Hutte (Before)	28	35.4 \pm 7.5	34.3 \pm 2.7	37.8	30.9 \pm 1.5	10
Hutte (After)	31	39.8 \pm 7.9	39.9 \pm 3.9	43.0	34.6 \pm 2.1	7
Rivau (Before)	13	15.5 \pm 3	17 \pm 1.8	18.2	15 \pm 1.2	5
Rivau (After)	25	33.9 \pm 8	32.9 \pm 4.2	37.1	28.6 \pm 2.2	8

458 Table 4 Rivau stream 20m monitoring of the Odonata, before and after restoration in winter
 459 2010. Numbers are individual observations per species for one day in June and one in July,
 460 with similar weather conditions (without rain and win). Blue bars are relative abundance
 461 Species are sorted in 4 habitat types (following Poitou-Charentes Nature, 2019). Pinail National
 462 Nature Reserve, Vienne. France.

	Ubiquitous					Lentic					Lentic to slow running water					Running water											
Year	Coenagrion pulchellum (Vander Linden, 1825)	Ischnura elegans (Vander Linden, 1820)	Orthetrum brunneum (Boyer de Fonscolombe, 1837)	Platynemis pennipes (Pallas, 1771)	Pyrrhosoma nymphula (Sulzer, 1776)	Sympetrum sanguineum (O. F. Müller, 1764)	Lestes dryas Kirby, 1890	Lestes sponsa (Hansemann, 1823)	Lestes virens vestalis Rambur, 1842	Somatochlora flavomaculata (Vander Linden, 1825)	Anax imperator Leach, 1815	Ceragrion tenellum (Villers, 1789)	Chalcolestes viridis (Vander Linden, 1825)	Coenagrion puella (Linnaeus, 1758)	Coenagrion tenellum (Villers, 1789)	Enallagma cyathigerum (Charpentier, 1840)	Ischnura pumilio (Charpentier, 1825)	Libellula depressa Linnaeus, 1758	Libellula quadrimaculata Linnaeus, 1758	Somatochlora metallica (Vander Linden, 1825)	Sympetrum meridionale (Selys, 1841)	Calopteryx splendens (Harris, 1780)	Calopteryx virgo (Linnaeus, 1758)	Coenagrion mercuriale (Charpentier, 1840)	Cordulegaster boltonii (Donovan, 1807)	Orthetrum coerulescens (Fabricius, 1798)	Total
1997	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	9
1998	0	0	0	0	0	2	0	0	1	1	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	9
2002	0	0	1	0	0	1	0	1	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	9
2009	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	5
2010	0	0	0	0	0	3	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	8
2011	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2012	0	2	1	0	0	2	0	4	0	0	2	1	0	8	3	0	0	1	0	1	2	0	0	0	0	2	29
2013	0	0	0	0	0	2	0	1	0	0	2	5	2	11	0	0	0	0	0	0	0	0	0	0	0	7	30
2014	0	3	0	1	27	3	0	7	0	1	3	26	0	12	0	2	0	1	2	0	0	0	3	5	0	12	108
2015	2	6	0	0	0	8	1	5	0	0	1	77	0	6	0	0	0	0	0	0	0	0	1	4	1	5	112
2016	0	2	0	0	2	3	5	3	0	1	0	30	0	6	0	0	0	0	0	0	0	0	1	2	1	4	60
2017	0	0	0	0	0	0	3	1	0	0	0	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	11
2018	0	2	0	0	0	3	1	15	0	0	0	11	0	0	0	0	0	0	0	0	0	1	1	4	1	6	45
2019	0	1	0	0	3	0	0	0	0	0	1	8	0	5	0	1	0	0	1	0	0	0	0	0	0	0	20

463

464 Figures:



465

466 Figure 1 Map of the study site, Pinail Nature Reserve, Vienne, France with more than 3000
467 permanent in water ponds and location of Rivau stream (red line), Hutte stream (yellow
468 line). With view from sky (right). Dark area in the middle is burnt patch due to annual fire
469 control artificially maintaining mosaic of flora succession ©Y. Sellier.

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472

473 Figure 2 The Hutte stream (400m) before 12/12/2011 (left) and after 01/01/2012 restoration
474 (meandering). Pinail Nature Reserve, Vienne. France. ©Y. Sellier

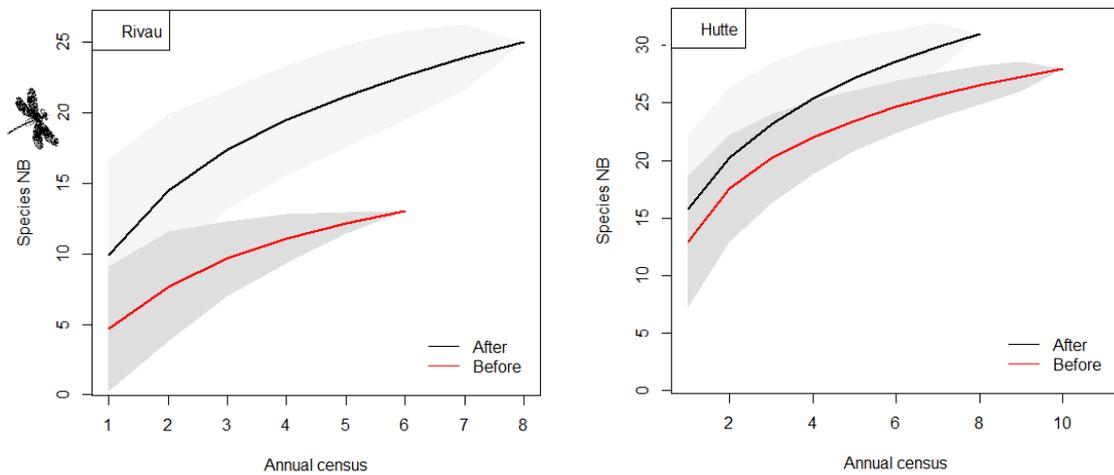


475

476 Figure 3 The Rivau stream (20m) before 23/03/2009 (left) and after 05/10/2010 restoration
 477 (meandering). Pinail Nature Reserve, Vienne. France. ©Y. Sellier

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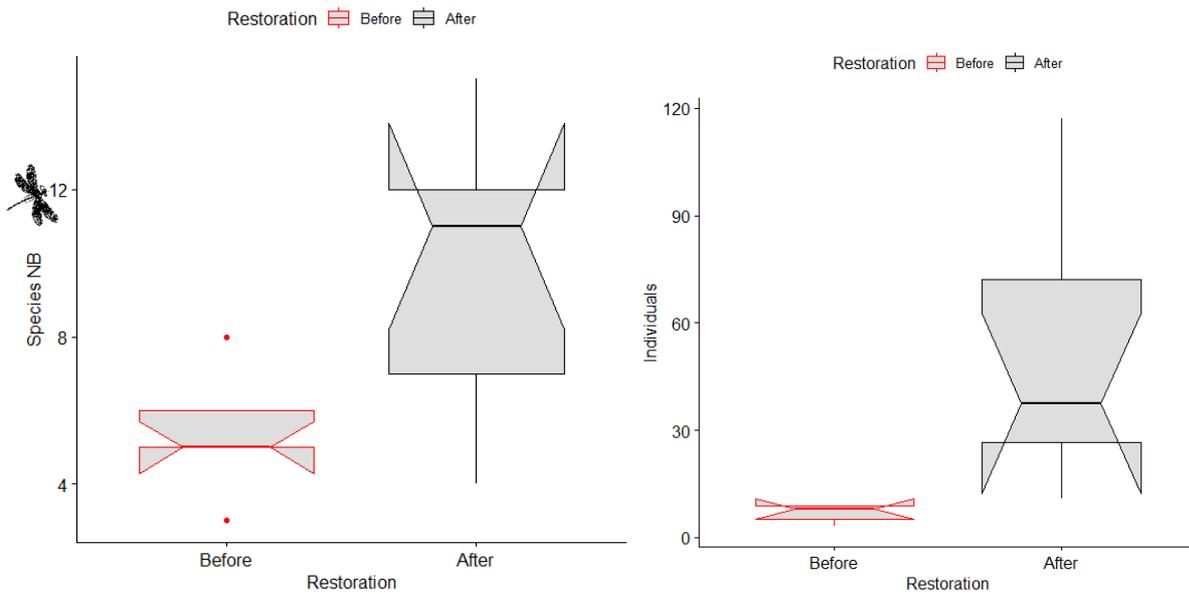


480

481 Figure 4 Species accumulation curves (with IC₉₅ in grey) of the annual census (June and July)
 482 of the Rivau 20m (left graph) and Hutte, 400m streams (right), before and after the
 483 restoration. Pinail National Nature Reserve, Vienne. France.

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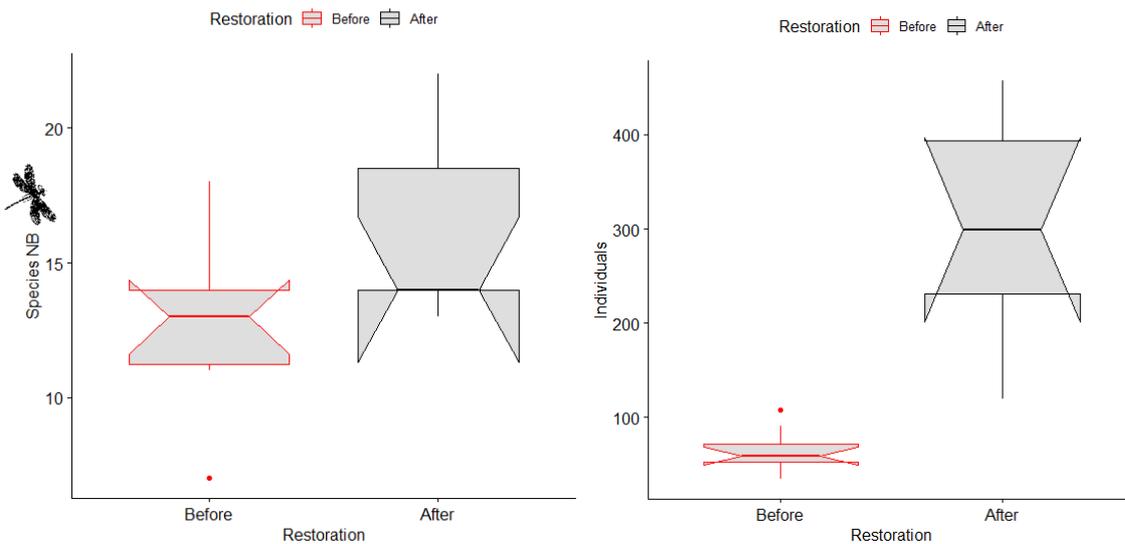
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488 Figure 5 Mean species number and abundance of Odonata on the Rivau (20m, June-July),
489 before (5 census) and after (8) restoration of the stream (boxplots displaying median, IC_{95%}
490 =notch, IQR). Pinail National Nature Reserve, Vienne. France.

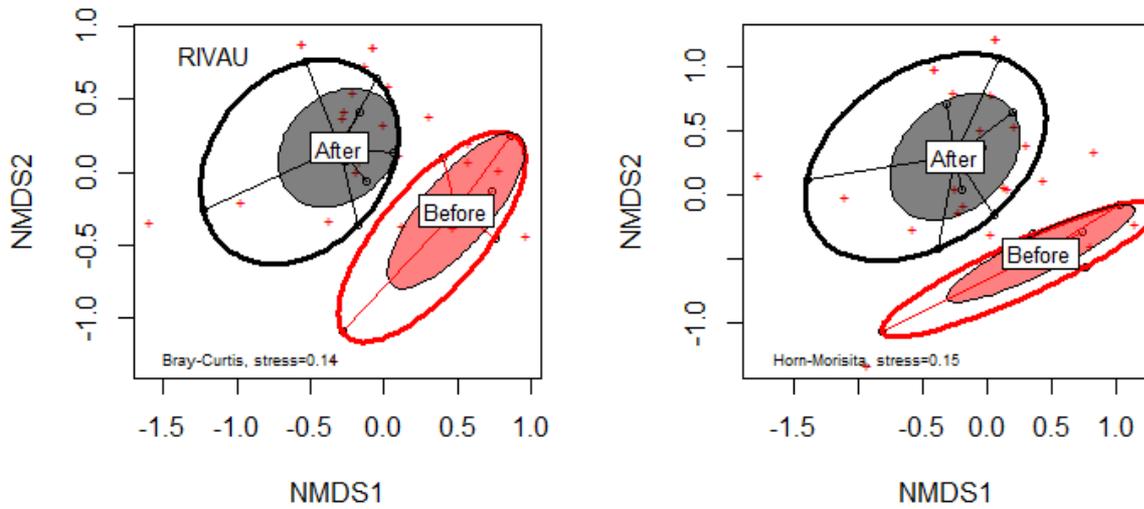


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492

493 Figure 6 Mean species number and abundance of Odonata on the Hutte (400m, June-July),
494 before (10 census) and after (7) restoration of the stream (boxplots displaying median, IC_{95%}
495 =notch, IQR). Pinail National Nature Reserve, Vienne. France.

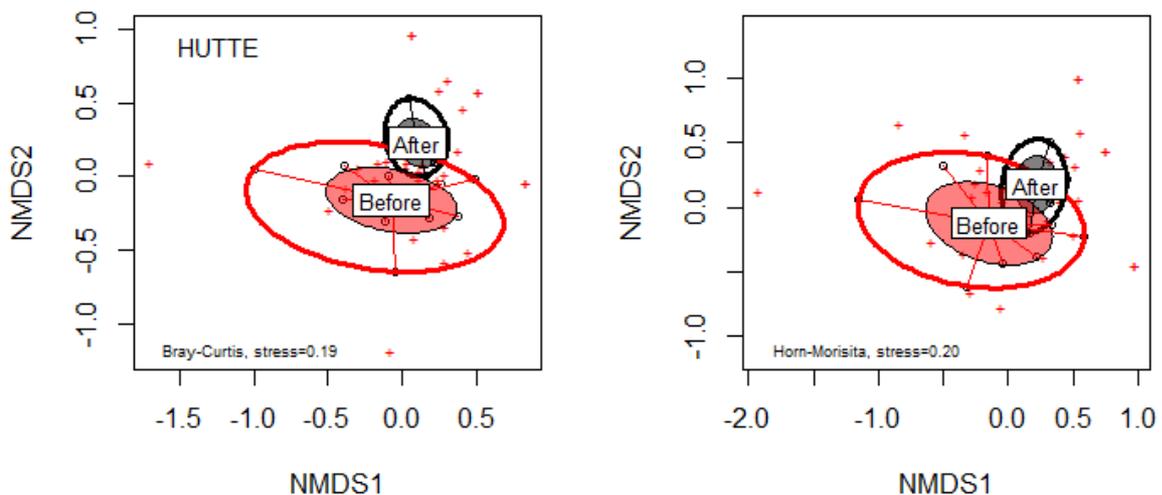
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497

498 Figure 7a Odonata community ordinations by non-metric multidimensional scaling (NMDS, in
 499 two dimensions display; using Bray-Curtis (left) and Horn-Morisita distances (right), with
 500 ellipsoid hull gathering all the group coordinates, 'spider web diagram' connecting all the
 501 annual census coordinates to the group centroids with standard error full ellipse displays) of
 502 the Rivau 20m stream (no overlap); showing differences in Odonata communities before and
 503 after the restoration. (each dot represents an annual census; each red cross represents a
 504 factor species). Pinail National Nature Reserve, Vienne. France.

505



506

507 Figure 7b Odonata community ordinations by non-metric multidimensional scaling (NMDS,
 508 in two dimensions display; using Bray-Curtis (left) and Horn-Morisita distances (right), with
 509 ellipsoid hull gathering all the group coordinates, 'spider web diagram' connecting all the
 510 annual census coordinates to the group centroids with standard error full ellipse displays) of

511 the Hutte 400m stream (overlap); showing differences in Odonata communities before and
512 after the restoration. (each dot represents an annual census; each red cross represents a
513 factor species). Pinail National Nature Reserve, Vienne. France.

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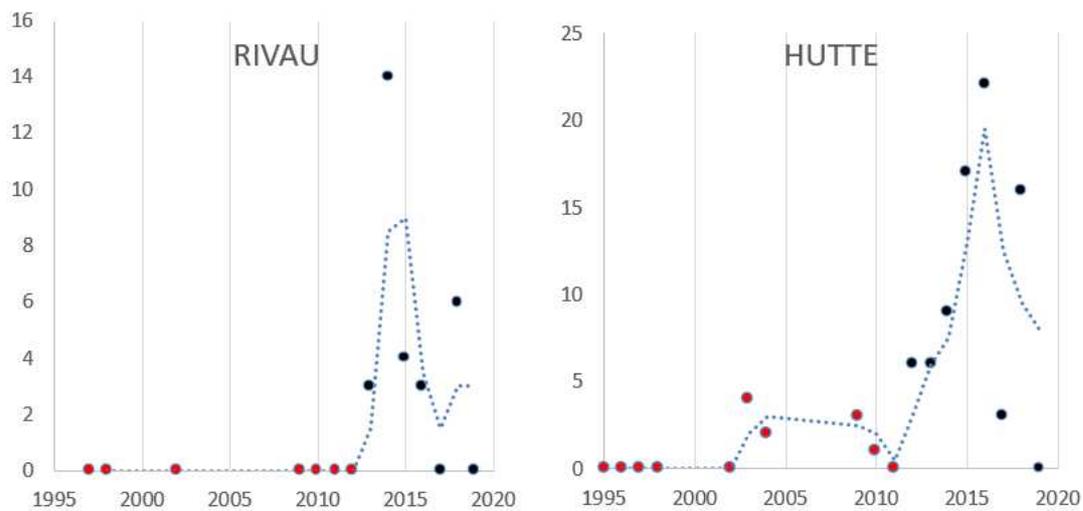
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518 Figure 8 Southern damselfly *Coenagrion mercuriale* (Charpentier, 1840) (Odonata:
519 Zygoptera) in 2016 at the Hutte 400m stream, present after restoration. Pinail Nature
520 Reserve, Vienne. France. ©Y. Sellier



521
 522 Figure 9 Occurrence of *Coenagrion mercuriale* on the Rivau 20m and Hutte 400m streams
 523 (June-July census), before (red dots) and after (black) restoration. Pinail National Nature
 524 Reserve, Vienne, France.

525

526



527
 528 Figure 10 The Rivau 20m (left) and Hutte 400m streams after restoration. Pinail Nature
 529 Reserve, Vienne, France. ©Y. Sellier