

Are surface water characteristics efficient to locate hyporheic biodiversity hotspots?

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30 Abstract.

Location of river-groundwater exchange zones and biodiversity hotspot is essential for a river 31 32 management plan, especially for Mediterranean karstic rivers. This location is often difficult and timeconsuming when long river sectors are considered. We studied a 13 km-long sector of the Cèze River 33 34 (Southern France) located in a karstic canyon. We compared five indicators of river-groundwater exchanges: longitudinal profiles of temperature, electrical conductivity and ²²²Rn concentrations in the 35 36 surface water of the river, chemical characteristics of the hyporheic water and hyporheic biodiversity. 37 Upwelling zones occurred downstream of geomorphological heterogeneities (here at the tail of gravel bars). Surface water chemistry, especially electrical conductivity and ²²²Rn concentrations, clearly 38 39 traces large scale gaining sections, which were not associated to valley narrowing but with lateral 40 springs, suggesting a crucial role of the geological structuration of the karstic plateau of Méjanne-le-41 Clap. Hyporheic water chemistry fits with the large-scale hydrological pattern, but with a high 42 variability corresponding to local heterogeneities. The stygobite fauna (obligate groundwater 43 organisms) and benthic EPTC (Ephemeroptera, Plecoptera, Trichoptera and Coleoptera) occurred 44 preferentially in the gaining sections fed by groundwater, likely because of oligotrophic water and 45 cooler temperature. The spatial distribution of river-groundwater exchange zone and hyporheic biodiversity may be thus predicted using changes in surface water chemistry, especially for electrical 46 47 conductivity and ²²²Rn concentrations.

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49 Key words.

50 Macro-invertebrates, stygobite, groundwater, karstic aquifer, ²²²Rn

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53 1-Introduction

54 The relationship between a river and the surrounding groundwater may vary in space and time 55 (Winter, 1995; Sophocleous, 2002). None or very low vertical exchanges of water are observed in rivers 56 flowing over an impermeable substratum or when it is perched above an unsaturated zone (cases a, b, 57 e in Malard et al., 2002). In contrast, important exchanges occur when the river is in contact with a porous aquifer containing a well-developed groundwater system (case c in Malard et al., 2002). In this 58 59 situation, water exchange can occur from the river to the groundwater or reversely from the aquifer 60 to the surface system (Schaller & Fan, 2009). These exchanges vary in time, according to season or 61 more generally to the relative level of water in the river and the aquifer (Baxter et al., 2003). The 62 drainage of groundwater by a river generally occurs during low-water period when water levels in the 63 river are lower than in the aquifer (Rouch, 1992). The inflow of groundwater inside a river affects 64 surface-water characteristic (Ward et al., 1999; Hayashi et al., 2012), for example, modifying of the 65 thermic regime (Wawrzyniak et al., 2016) or solute concentrations (Ca, K... Grasby el al., 1999; Cook, 66 2013). Nutrient contents are also partly controlled by groundwater inputs: organic carbon (Ford & 67 Naiman, 1989), nitrate (Deutsch et al., 2006) or silica (Ward et al., 1999).

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69 The inputs of groundwater influence the benthic and hyporheic biological processes, because 70 groundwater nutrient loads stimulate biofilm biomass and activity (Claret & Fontvieille, 1997) and also 71 aquatic invertebrates. For example, Hunt et al. (2010) showed that abundances of benthic invertebrate 72 increased by 35% in groundwater-fed areas compared to surface water fed zones. In the same way, 73 Gray et al. (2010) found more diverse invertebrate communities in groundwater-fed springs than in 74 nearby stream (two times less) or in the main river (four times less). Similarly, hyporheic biodiversity 75 is mainly controlled by river-groundwater exchanges (Dole-Olivier & Marmonier, 1992). When 76 sediment interstices are fed by surface water well oxygenated and rich in labile organic matter (i.e. 77 downwelling zones) the benthic fraction of the hyporheic fauna is dominant, while in groundwater-fed areas with low dissolved oxygen, high oligotrophy and thermic stability (i.e. upwelling zones), the 78 79 stygobites are dominant in the assemblages (Dole-Olivier & Marmonier, 1992; Brunke & Gonser, 1999). 80 Thus, these downwelling/upwelling successions play a crucial role in the functioning of river sediments 81 and must be taken into account for the location of biodiversity hotspots (Dole-Olivier, 1998) and more 82 generally for river management (Marmonier et al., 2012).

83

The integration of river-groundwater exchanges in a river management plan is limited by the difficulty to localise these interaction zones at a pluri-kilometer scale of 10 or 20 km (Graillot et al., 2014; Dole-Olivier et al., 2019). On one hand, surface water chemistry is not adequate to detect exchanges in large-scale losing sections making them hard to localise (Dole-Olivier et al., 2019). On the other hand,

88 in large-scale gaining sections, the chemical changes may be hidden by local conditions (e.g. inputs of 89 solutes linked to tributaries) or temporal cycles (e.g. effect of day-night alternation for thermic regime, 90 Tonolla et al., 2010; Wawrzyniak et al., 2012, 2013). Despite these difficulties, general models of the 91 spatial distribution of surface water/groundwater exchanges along a river were elaborated for alluvial 92 valleys (e.g. Toth, 1963; Stanford & Word, 1993; Capderrey et al., 2013) combining two scales for 93 geomorphological and geological characteristics: (1) at the scale of the geomorphological units, (e.g. gravel bars, riffles), local downwellings generally occur upstream of these morphological 94 95 discontinuities, while local upwellings occur downstream (Hendricks & White, 1988; Dole-Olivier & 96 Marmonier, 1992). (2) At the pluri-kilometer scale of the river section, the geological context becomes 97 essential to locate losing and gaining sections. For example, an increase in the thickness of the alluvium 98 linked to a depression in the impermeable substratum induces a massive surface water loss to the 99 aquifer (losing sections, Toth, 1963, Malard et al., 2006; Capderrey et al., 2013). On the contrary, a 100 reduction of the alluvial valley width related to a bedrock outcrop generally induces massive inputs of 101 groundwater to the river (i.e. gaining sections, Doering et al., 2007; Marmonier et al., 2019). The 102 interaction between these two scales makes the location of gaining (groundwater inputs inside the 103 river) and losing areas (surface water infiltration toward the aquifer) difficult to predict: in a gaining 104 section, the local upwellings are mostly fed by deep groundwater, while in a losing section upwellings, 105 if present, are fed by hyporheic water that originates from the surface and follows a short hyporheic 106 flowpath before returning to the river (Dole-Olivier et al., 2019, see supplementary material Fig. S1). 107 Indicators of such patterns can be found in upstream-downstream profiles of surface water chemistry, for temperature or solute contents (Ward et al., 1999; Wawrzyniak et al., 2016). The ²²²Rn is a 108 109 radioactive tracer used to estimate the inflow of surface water to the hyporheic zone (Bourke et al., 110 2014) or groundwater contribution to rivers (Ellins et al., 1990; Cook et al., 2006; Guida et al., 2013) when surrounding rocks or bottom sediment are a source of ²²²Rn (e.g. like granite in the Cockburn 111 River, Cook et al., 2006). The potential link between ²²²Rn and hyporheic biodiversity was never 112 113 brought to attention before.

114

115 The objectives of the present study were to localise river-groundwater exchange zones at a pluri-116 kilometric scale (i.e. gaining and losing sections) to predict the location of hyporheic biodiversity hotspots along a 13 km long canyon of a karstic river (the Cèze River, Southern France). We combined 117 118 five indicators of river-groundwater exchanges: the longitudinal profiles of surface water temperature, electrical conductivity (using upstream-downstream continuous measure), ²²²Rn concentrations (using 119 high-resolution field measurements), hyporheic water chemistry and hyporheic biodiversity. 120 121 Downwelling zones are all fed by surface water and generally harbour a hyporheic fauna dominated 122 by organisms living in surface water, whatever their location in a losing or a gaining section (Creuze des Châtelliers & Reygrobellet, 1990). To identify hydrologic patterns (i.e. losing versus gaining sections), we thus focused on upwelling zones (i.e. downstream end of gravel bars) where the hyporheic water and fauna may be influenced directly by groundwater (in gaining sections) or by surface water that upwells after a short flow path inside sediment (in losing sections, see Fig. S1). Four hypotheses were considered:

128 - In a river with a heterogeneous watershed, such as the Cèze River (metamorphic upstream sector 129 followed by a calcareous canyon), the chemical characteristics of the surface water differ from the 130 surrounding karstic groundwater. In the same way, the temperature of a Mediterranean river and the 131 surrounding groundwater varies, especially during the warm season. We firstly hypothesised that 132 surface water chemistry is modified by groundwater inflow in gaining sections compared to losing 133 sections or no-exchange zones (H1, Cook, 2013). For this purpose, we used surface water temperature, electrical conductivity profiles and changes in ²²²Rn concentrations as indicators of exchanges. The 134 135 ²²²Rn content was chosen because of the metamorphic origin of the river sediment inside the canyon. 136 - Assuming that groundwater-surface water exchange patterns described in alluvial rivers (Capderrey 137 et al., 2013) are similar in all types of rivers, we can hypothesise locations of exchange zones at two 138 scales. Firstly, at the scale of a gravel bar, as we sampled only at the downstream ends, we can predict 139 that all (or most) stations will consist in local upwelling zones, fed by deep groundwater (in gaining 140 sections) or by surface water after a short flow path inside the sediment (in losing sections, H2, Hof, 141 1963). Secondly, at the scale of the studied sector, gaining and losing sections can be predicted by the 142 geological context: the gaining sections are expected upstream of a canyon narrowing while losing 143 sections are expected in enlarged sections of the valley (H2, Hof, 1963).

The composition of the hyporheic fauna is affected by the origin and the quality of the water (Stanley
& Boulton, 1993). The stygobite fauna, for example, requires a thermic stability and an oligotrophy
generally linked to groundwater inflows. We thus hypothesised that stygobite assemblages will be
more abundant and diversified in local upwelling located in gaining sections where hyporheic water is
of deep origin (H3, Dole-Olivier & Marmonier, 1992).

- In contrast, benthic fauna needs oxygenated water and labile organic matter brought inside the
 sediment by surface water inflows (Dawy-Bower et al., 2006). We hypothesised that benthic
 Ephemeroptera, Plecoptera, Trichoptera and Coleoptera (EPTC) may be more abundant in the losing
 sections (H4, Brunke & Gonser, 1999).

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154 **2-Studied site**

The Cèze River is a 135 km long Mediterranean river of the South-East France with a total difference of level of 773 m. The spring of the Cèze River is located at 800 m a.s.l. in the metamorphic region of the Cevennes (median Stephanien, dominated by Gneiss and Quartzit, Chapuis, 2017). The river flows 158 to the east, crosses the rift valley of Alès (5 km large) and enters the calcareous plateau of Méjeanne-159 le-Clap (Barremian with Urgonian facies, Berger et al. 1978). In this calcareous context, the Cèze River 160 generates a 100m deep canyon of 20km long (Jolivet & Martin, 2008; Chapuis, 2017; Fig. 1). In the 161 canyon, the river is reduced to 20 to 30 m width with a shallow bottom sediment layer (mostly pebbles 162 and gravel of metamorphic origin) and with locally apparent calcareous substratum due to gravel 163 extraction during the 60's and 70's. The surrounding plateau is strongly fractured by several faults due 164 to a rollover anticline structure. These faults favoured an important karstification with losing sectors 165 (noted L1 and L2 on Fig. 1) and lateral springs (noted S1 to S8 in Fig. 1).

166

167 The climate of the region is characterised by a wet season from October to December (mean rainfall 168 of 88 mm/month) and a dry period from June to September (33 mm/month). The hydrology of the 169 Cèze River is thus characterised by extremely changing discharge with three contrasted periods: a low 170 flow period from July to September where the river discharge reaches 0.147 m³/sec (at the entry of 171 the canyon), a winter and spring period with intermediate discharge (mean value of 10.5 m³/sec at the 172 entry of the canyon, varying between years from 3.2 to 16.6 m³/sec) and a period of violent autumn 173 flash floods (called Cévenol episodes, Saulnier & Le Lay, 2009) reaching 2,200 m³/sec in 2002 (Chapuis, 174 2017). During the studied period, the river discharge varied at the entry of the canyon from 1180 175 m³/sec in October 2014 to 0.512 m³/sec in July 2015. In the canyon, the river is partly fed by 176 groundwater that may represent 49% to 68% of the discharge (during intermediate and low flow 177 periods respectively, Chapuis 2017). The inflow of groundwater fed the river through lateral springs 178 (noted S1 to S8 in the Fig. 1) or diffuse upwellings along the banks and gravel bars. Springs are 179 permanent on the left side (S2, S3, S5, S6, S8 in Fig. 1) and temporary on the right (S4, S7, Fig. 1). The 180 diffuse inflow of groundwater through bottom sediment may represent 50% of the total groundwater inputs (e.g. it was estimated to 202 L/sec on a total increase of 394 L/sec in the canyon; Chapuis, 2017). 181 182 In return, the Cèze River fed the aquifer through losing sections (L1 and L2 in Fig. 1). These losses may 183 represent 80% of the annual discharge, inducing summer drought near L1 and significant decrease of 184 the discharge near L2 (Chapuis, 2017).

185

186 **3-Material and methods**

3.1-Longitudinal profiles of physicochemical characteristics of surface water.

Longitudinal profiles of temperature and electrical conductivity were measured with a multiparameter probe (HANNA HI 9829) from a small plastic boat equipped with a GPS to locate each measure along the river. Two longitudinal profiles were performed during the spring season. The first one was realised in two distinct sessions on the 14th of May and on the 7th of July 2013 (with a rather similar discharge of 0.5 m³/sec at the entry of the canyon). Measurements made in July were corrected 193 and reported to a similar upstream level measured in May. The second longitudinal profile was realised 194 on two successive days in early June 2015 (1.19 m³/sec at the entry of the canyon) a period where all 195 springs were supplied by groundwater except S7. Each campaign of measurements took two days, this 196 duration introduced temporal changes inside the longitudinal profiles. For temperature, the 197 combination of variations between the two successive days and variations during the day length made 198 any corrections very complex. Thermic profiles were thus presented uncorrected with a combination 199 of spatial and temporal trends. Electrical conductivity corrected to 25°C was roughly stable over the 200 day length and the two successive days, because of stable hydrological conditions during the sampling 201 period. All measurements of the profiles were expressed as a distance from an upstream point (Pont 202 de Rochegude) and noted as kilometric points (kp), the studied sector was thus located between kp 203 4.8 and kp 17.5 (Fig. 1).

204

205 2.2-Concentration of ²²²Rn in surface and groundwater

206 ²²²Rn is produced by the disintegration of the ²²⁶Ra parent isotope present in the substratum. A granitic or a metamorphic substratum is a consistent source of ²²²Rn (e.g. Cook et al., 2006) as well as in soils 207 208 resulting of limestone degradation (alterites) or in sediment deposits that originate from the 209 metamorphic bedrock (Lamontagne et al., 2007, Bourke et al., 2014). When groundwater is in contact 210 with these substrates, the ²²²Rn activity accumulates. Conversely, ²²²Rn activity rapidly decreases in surface water, because of radon disintegration (the ²²²Rn has a half-life direction of 3.8 days) and 211 degassing to the atmosphere. For surface waters, a ²²²Rn increase is thus a good tracer of groundwater 212 input (Cook et al., 2006) and a ²²²Rn decrease in groundwater is, conversely, a good tracer of surface 213 214 water input (Bourke et al., 2014).

The concentrations of ²²²Rn in the river water was measured along a longitudinal profile from 2-liter samples collected at subsurface every 500 meters. Measurements were performed on air after reaching equilibrium between radon, air and water using two RAD7 portable alpha spectrometers (Kim et al. 2001).

To document the ²²²Rn concentrations in groundwater, supplementary measurements were performed in 4 springs (S1, S3, S4 and S8) at three occasions (March and May 2015 and May 2016) and in the hyporheic water of the river (at kp 10, Fig. 1) in June 2016.

222

223 **3.3-Hyporheic water and fauna**

Hyporheic water and fauna were sampled at 17 stations from kp 4.8 to kp 16.8 (Fig. 1) from the 15th

to the 19th of July 2013, a period of very low discharge (less than 0.5m³/sec at the entry of the canyon).

- 226 The stations were located on side gravel bars. Thirty-four gravel bars were labelled along the studied
- section and 17 of them were randomly selected as sampling stations (Fig. 1). The first station was

228 located just upstream the beginning of the canyon and all the others were located inside the canyon 229 (Fig. 1). At each station, water and fauna were sampled at 3 replicate points, located at the 230 downstream end of a gravel bar (an area predicted to be an upwelling zone) to detect the potential 231 inflow of groundwater. The sampling design resulted in a total of 51 hyporheic samples (17 stations \times 232 3 replicates) and 17 surface water samples. Surface water was directly sampled in the river while the 233 hyporheic water and fauna were sampled at the 51 points at a depth of -40 cm under the sediment 234 surface using the Bou-Rouch technique (Bou and Rouch, 1967; Bou, 1974). A perforated metal pipe of 235 23 mm internal diameter (9 rows of 5 mm diameter holes in a 13 cm band, 4 cm from the distal end of 236 the pipe) was driven into the sediment at each point.

237

Water temperature (in °C), electrical conductivity (in µS/cm), and dissolved oxygen (mg/L; Hach HQ-40d Multiparameter) were measured directly in the surface water and in the hyporheic water pumped inside the Bou-Rouch pipe using a peristaltic hand pump (Willy A. Bachofen type). Vertical Hydraulic Gradient (in %) was measured as the difference between surface and hyporheic levels. Additional water samples were taken, filtered on GF/F Whatman filters, stored in 40 mL plastic bottles, and kept in insulated containers for the return to the laboratory to measure Na, K, Ca, Mg, Sulphate and Nitrate concentrations (± 0.1 %) with an ionic chromatography (Dionex 1100, conductivity detector).

245

246 Hyporheic invertebrates were collected at the same 51 sampling points using the Bou-Rouch sampler; 247 10 L of water and sediment were pumped and filtered through a 160 μ m mesh net. Samples were 248 preserved in the field in 96% alcohol. Invertebrates were sorted in the laboratory under a 249 stereomicroscope (x20) after sieving. Clitellata and most crustaceans (Ostracoda, Cladocera, Isopoda, 250 and Amphipoda) were identified to species level. Planaria, Acheta, Mollusca and most Insects 251 (Ephemeroptera, Plecoptera, Trichoptera, Heteroptera and Coleoptera) were identified to genus level 252 or group of species, while Diptera remained at the family or sub-familly levels. Finally, Nematoda, 253 Hydracarina, Cyclopids and Harpacticids were not further identified (except for Parastenocaris sp.). For 254 Clitellata, Isopoda and the stygobite Amphipoda of the genus Niphargus, the morphological 255 identification was completed by DNA barcoding (mitochondrial COI for Clitellata, 16S mitochondrial 256 rDNA for Isopoda and 28S nuclear rDNA for Niphargus; see details in Marmonier et al., 2019) to ensure 257 tricky morphological identifications and to identify damaged/young individuals.

258

259 3.4-Statistical analyses

Principal Component Analysis (PCA) was used to assess the spatial variation in physicochemical
 characteristics of hyporheic water with 10 parameters (i.e. temperature, Vertical Hydraulic Gradient,
 electrical conductivity, Mg, Ca, K, Na, SO₄, NO₃ and dissolved oxygen). To avoid a mix between spatial

and temporal changes (i.e. along the week for solutes or along the day for temperature), measurements in the hyporheic water were expressed as a difference with surface water. Differences between the 17 stations (n=51, using replicates inside each station) and between section based on hydrology (i.e. losing or gaining sections, n=17, using stations as replicates inside sections) were tested using one-way ANOVA after log(x+1) transformation when necessary.

268

269 Total taxonomic richness and total abundances of the hyporheic fauna, stygobite organisms, micro-270 crustacean and benthic fauna (sum of Ephemeroptera, Plecoptera, Trichoptera and Coleoptera, noted 271 EPTC) were compared between the 17 stations using one-way ANOVAs (n=51) and between river 272 sections based on their hydrology (i.e. losing or gaining sections) using one-way ANOVAs with stations 273 as replicate measurements in each section (n=17). Three types of river sections were tested: (1) 274 sections defined by surface water profile of electrical conductivity obtained in July 2013 and June 2015, (2) sections using ²²²Rn concentrations in the surface water in June 2015 and (3) sections defined by 275 276 the hyporheic water chemistry measured in July 2013. Changes in faunal characteristics observed in 277 July 2013 were related to the ²²²Rn concentrations measured in June 2015, because similar patterns were observed for electrical conductivity at these two dates. The data were log(x+1) transformed when 278 279 necessary and tests were performed using ExcelStat 2014, the limit of significance was a p-value of 280 0.05.

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282

283 **4-Results**

284 4.1-Longitudinal profiles of temperature and electrical conductivity in the surface water

285

286 Similar spatial patterns were observed in July 2013 and June 2015 (Fig. 2). The temperature was high 287 upstream of the canyon (i.e. 27.3°C in July 2013 and 28°C in June 2015) and strongly modified just downstream of the S1 spring (with a decrease of 1.0°C in July 2013 and 4°C in June 2015). These 288 289 decreases were linked to the influx of water from the S1 spring that was colder than the river at both 290 dates (14.8°C and 13.8°C in July 2013 and June 2015, respectively). The temperature irregularly 291 decreased all along the first part of the studied sector until Kp 10 (to 25.8°C in July 2013 and to 21.8°C 292 in June 2015). In the second part of the canyon, different trends were observed between dates. In July 293 2013, the temperature still decreased to 25.02°C at Kp 16.2. In June 2015, a 2.5°C decrease was observed at Kp 10.2 due to a stop followed by a progressive increase until 24.8°C at Kp 16.8. At both 294 dates, the temperature profiles were strongly disturbed downstream of the S8 spring, with a decrease 295 296 of 1.1°C and 5.3°C in July 2013 and June 2015 respectively. The temperatures measured in the S8 spring 297 (14.4°C and 14.9°C in July 2013 and June 2015, respectively) were effectively lower than the temperature of the river. The resulting longitudinal patterns in water temperature were not associated to changes in valley width (Fig. 1) but with diffuse inflows of cold water in the first 4 km of the canyon and some very rapid changes at 100m-scale, just downstream of the S1 and S8 springs in July 2013 and June 2015. These rapid changes correspond to groundwater plume downstream of springs (see aerial infrared photographs in Fig. S2). A similar effect was observed for the S5 spring (June 2005) and at a lesser extent near the S7 spring, but no effect was observed for the four other springs

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305 The pattern in electrical conductivity follows a more repeatable trend in July 2013 and June 2015 (Fig. 306 2). It was low upstream of the canyon (380 μ S/cm and 375 μ S/cm in July 2013 and June 2015, 307 respectively), but strongly changed just downstream of the S1 spring (increase to 420 or 450 μ S/cm) 308 because of the input of highly conductive groundwater (547 and 615 μ S/cm in July 2013 and June 2015, 309 respectively). All along the first part of the canyon, from Kp5 to Kp 9, the electrical conductivity 310 regularly increased to a relatively stable value (around 420 μ S/cm at both dates) from Kp 10 to Kp 17 311 (noted in blue in the Fig. 2). Few rare and rapid changes were observed (generally less than 20% of the 312 values) just downstream of the S5 and S7 springs. Finally, the electrical conductivity was strongly 313 modified just downstream of the S8 spring, with an increase to 447 μ S/cm in July 2013 and to 458 314 μ S/cm in June 2015, when the water of the S8 spring reached 442 μ S/cm and 510 μ S/cm in July 2013 315 and June 2015, respectively (noted in blue in Fig. 2).

316

The longitudinal patterns in electrical conductivity were not associated to changes in valley width: no significant inflow of groundwater, with associated low temperature or high electrical conductivity, were measured upstream of the canyon entry (pk 4.8, noted in white in Fig. 2). Conversely, the first part of the canyon (pk 5 to 10) or the downstream end of the studied sector (pk 17) did not show any consistent change in river width, but a decrease in the temperature and an increase in electric conductivity (gaining sections noted in blue in Fig. 2).

323

324 **4.2-Longitudinal pattern of** ²²²**Rn concentrations**

The concentrations of ²²²Rn were low at the entrance of the canyon (less than 500 Bq/m³) and slowly increased all along the first part of the section until Kp 9 where ²²²Rn reached a maximal value (close to 1000 Bq/m³, Fig. 3). Downstream, the ²²²Rn concentrations decreased irregularly until Kp 15 to similar values to those measured upstream of the canyon (close to 400 Bq/m³, Fig. 3). Finally, the concentrations increased again downstream of the Kp 15 to the end of the studied sector.

330

The 222 Rn concentrations were high in the karstic groundwater of the region, varying between 1120 and 2560 Bq/m³ in the spring water (S1, S3, S4 and S8). In the same way, the 222 Rn concentrations

- measured inside the sediment at Kp 10 reached very high values (i.e. ca 14 kBq/m³ in June 2016). The changes in ²²²Rn concentrations may be used to locate gaining and losing sections along the studied sector (noted in blue and in white in Fig. 3).
- 336

337 4.3-Hyporheic water chemistry

The PCA performed on the 17 stations (Fig. 4) highlights differences in hyporheic water chemistry that 338 339 mirror differences in geologic characteristics of the watershed: when entering the canyon, the surface 340 water of the Cèze River is characterised by high concentrations in Mg, K, and Na (Table 1) a 341 consequence of the metamorphic rocks dominating the upper part of the watershed. These 342 parameters have positive coordinates on the PC1 axis (Fig. 4a). In contrast, the groundwater sampled 343 in the karstic springs were characterised by high electrical conductivity and high concentrations in Ca 344 (Table 1) linked to the calcareous substratum. These two parameters have clearly negative coordinates 345 on the PC1 Axis (Fig. 4a). The PC2 axis highlights a reverse pattern between Dissolved Oxygen, Nitrate 346 and Sulphate, with a poor link with the origin of water.

The differences in chemical characteristics of the hyporheic water sampled in the 17 stations result in a gradient of stations along the PC1 axis (Fig. 4b). All sampling stations have positive Vertical Hydraulic Gradients, ranging from a minimum value of +0.4% at stations 24, 27 and 32, to +6.6% at the station 3 and +7.5% at station 34 (Fig. 4e), but there were two types of waters that upwell in the different stations:

- the hyporheic water sampled upstream of the canyon (at station 1, Kp 5), in station 12 and 14 (Kp7.7
and 8.8) and in the second part of the canyon (stations 19 to 33, from Kp 11 to 16.5) was similar to
surface water for temperature, K, Mg, Na, and sulphate concentrations (Fig. 4d, f, h), all characteristics
of surface water that follow short flow path inside the hyporheic zone before to return to the river
(noted in white in Fig. 4b).

In contrast, the water sampled in the first part of the canyon (stations 3 to 11, 13 and 16, from pk 5
to 10) and at the end of the studied sector (station 34) was cold and with a high electrical conductivity
(Fig. 4c, f) thus similar to karstic groundwater or, at least, with a hyporheic water that follows a long
flow path inside sediment (noted in blue in Fig. 4b).

361

362 4.4-Hyporheic biodiversity

A total of 65,860 individuals and 116 taxa were sampled in the 51 hyporheic samples. For the surface water organisms (63,830 individual and 99 taxa), the dominant organisms were the Naididae (*N. communis* and *N. barbata*), *Alona guttata* and *Vestalenula* sp. for micro-crustaceans, the insect Leuctridae (*L. major* and *L. nigra*), three species of Ephemeroptera (*Procloeon bifidum*, *Caenis* sp. and *Choroterpes picteti*), three species of Coleoptera (*Esolus* sp., *Hydroscapha* sp. and *Yola* sp.) and three groups of Diptera (Ceratopogonidae, Chironomidae Orthocladiinae and Tanytarsini). The stygobite
fauna was less abundant (only 2030 individuals for the 51 samples), but well diversified with 17 species.
The most abundant stygobites were the Phallodrilinae *Gianius aquaedulcis* and the Lumbriculidae (cf *Trichodrilus*), the microcrustaceans *Parastenocaris* sp., *Phreatalona phreatica*, *Marmocandona zschokkei* and *Cryptocandona kieferi*, the Amphipoda *Niphargus casparyi* and *N. kochianus* and the
Isopoda *Proasellus walteri*.

374

375 The total abundance and taxonomic richness of the hyporheic fauna varied significantly with the stations ($F_{(17,36)}$ =10.83, p-value=1.7 10⁻⁹ and $F_{(17,36)}$ =4.36, p-value=0.0001 respectively; Fig. 5) with 376 377 maximum abundances in stations 20 and 24 (with a mean of 2143 and 5760 ind./10L respectively). 378 When stations were grouped into hydrologic sections (i.e. gaining versus losing) the abundances did 379 not significantly vary, whatever the indicator chosen (i.e. longitudinal profile of electrical conductivity, 380 ²²²Rn concentrations or hyporheic water chemistry; in all cases p-values > 0.05), because of high 381 variability inside each group of stations. On the contrary, the taxonomic richness was significantly 382 higher in gaining sections (23.8 \pm 3.8 taxa) than in losing sections (17.2 \pm 6.7 taxa) based on ²²²Rn 383 changes (F_(1,16)=6.01, p-value=0.027).

384

385 Microcrustaceans represented a large proportion of the total abundances of the hyporheic fauna (76 386 ± 16% of the specimens). Their abundances and taxonomic richness varied significantly with stations $(F_{(17,36)}=11.87, p-value=5.10^{-10} \text{ and } F_{(17,36)}=3.66, p-value=0.0005, respectively; Fig. 5), with highest$ 387 388 values measured at the same stations 20 and 24, just upstream or inside the second losing section (L2 389 in Fig. 1). Some species were more abundant in losing sections (such as the Vestalenula sp. restricted 390 to the stations 1, 24, 25, and 27; Supplementary material Fig. S3), but when all microcrustaceans are 391 considered together, neither the species richness nor the abundances significantly changed between 392 sections defined by their hydrology (i.e. losing vs gaining) whatever the indicator chosen (i.e. longitudinal profiles of electrical conductivity, ²²²Rn concentrations, hyporheic water chemistry; in all 393 394 cases p-value > 0.05).

395

The abundances and richness of benthic EPTC (Fig. 5) did not vary significantly with stations (p-value > 0.05), but with sections based on ²²²Rn concentrations. The EPTC were two times more abundant in the gaining sectors (mean of 48.5 individuals/10L) than in losing (17.7 individuals/10L; $F_{(1,16)} = 4.92$, p-value = 0.042) and showed higher taxonomic richness in gaining (mean of 3.9 taxa) than in losing sections (2.4 taxa; $F_{(1,16)} = 5.09$, p-value = 0.039). These patterns were explained by a clear preference for gaining stations of the Plecoptera *Leuctra* spp. (L. *major* and L. *nigra*), the Ephemeroptera *Choroterpes picteti* and the Coleoptera *Esolus* sp. (Supplementary material Fig. S3). The differences in

- 403 EPTC abundances and taxonomic richness disappeared when the delineation of hydrological sections
- 404 was based on the electrical conductivity profile or the hyporheic water chemistry.
- 405

406 Similarly, the abundances and species richness of the stygobite fauna (Fig. 5) did not vary between 407 stations, but abundances significantly changed when stations were grouped according to their 408 hydrology (i.e. gaining vs losing). Stygobites were more abundant in gaining sections (i.e. 55.4 ± 48.7 409 individuals/10L) than in losing sections (16.9 \pm 11.9 individuals/10L) when sections were based on ²²²Rn 410 changes ($F_{(1,15)} = 7.002$, p-values = 0.0018) or electric conductivity profiles ($F_{(1,15)} = 6.797$, p-values = 411 0.019). Some stygobite species showed a clear preference for gaining sections. For example, the 412 ostracods Marmocandona zschokkei and Cryptocandona kieferi, the Amphipods Niphargus kochianus 413 and N. fontanus were all restricted or more abundant in the gaining sections of the river 414 (Supplementary material Fig. S3) and the karstic Amphipod N. virei only occurred at the station 33. 415 When the group of stations were built using hyporheic water characteristics, neither the stygobite 416 abundances nor the stygobite taxonomic richness varied significantly between gaining and losing 417 sections (p-value>0.05).

418

419 **5-Discussion**

420 The location of gaining and losing sections along a 13-km section of a Mediterranean karstic river, such 421 as the Cèze River, is difficult because of the complexity of interactions between the geological 422 heterogeneities of the substratum, the complex tectonics, the local geomorphologic features and the 423 hydrology that constitute altogether a single karst-river system comparable to the stygoscape 424 described for alluvial rivers by Ward (1997) or Datry et al. (2008). When a general model of the spatial 425 distribution of river - groundwater exchanges (based on an alluvial river and a porous aquifer; Toth, 426 1963; Dole-Olivier et al., 1993; Capderrey et al., 2013) is applied to this karstic river, only a part of the 427 hypotheses proposed at the beginning of the study was supported by the results. In this study, we 428 observed that the inflow of karstic groundwater modified the surface water chemistry (H1 validated), 429 the gravel bar tails were upwelling zones with positive Vertical Hydraulic Gradient (first part of H2 430 validated), and the link between groundwater inflows and stygobite abundances in the hyporheic zone was effectively observed in the karstic canyon of the Cèze River (H3 validated). Another original result 431 of this study is the link between the ²²²Rn concentration profiles in surface water and the occurrence 432 433 of groundwater fauna in the river sediment. Two hypotheses were rejected (i.e. the gaining sections 434 located upstream of valley narrowing -second part of H2 - and the occurrence of benthic EPTC fauna 435 in the losing stations -H4). These rejections highlight some specificities of karstic rivers and 436 Mediterranean rivers in their relation with surrounding groundwater that must be accounted for in 437 river management.

438 **5.1- Groundwater inflow and surface water chemistry**

As predicted by the first hypothesis, the inflows of groundwater from the karstic systems were strong 439 440 enough to modify the chemical characteristics of the Cèze surface water. This influence was not always 441 clear for the longitudinal changes in temperature. Thermal profiles may be modified by several 442 environmental changes without relation to groundwater dynamics (e.g. shaded area, temporal 443 changes along the day cycle..., Wawrzyniak et al., 2017). In contrast, the electrical conductivity 444 appeared to be a very consistent indicator of groundwater inflow in the Cèze River, with regular 445 increase along the first part of the canyon and close to the spring S8 and a relative stability elsewhere. 446 These types of longitudinal profiles of electrical conductivity were frequently observed in rivers, for 447 example in the Murray River (Simpson & Herczeg, 1991) or the Cockburn River (Cook et al., 2006). In 448 the case of the Cèze River, the electrical conductivity was particularly relevant because of the 449 contrasted origin of the surface water (linked to the upstream metamorphic watershed) and of the 450 groundwater (linked to the karstic area).

451

452 Another surface water characteristic that changed along the studied sector was the concentration of 453 ²²²Rn. This radioactive isotope was already used to locate groundwater upwelling in rivers (Ellins et al., 1990; Wu et al., 2004; Cook et al., 2006). The Cèze River offered a particularly interesting geological 454 455 context for the use of ²²²Rn: the bed sediments are of metamorphic origin (mostly dominated by 456 gneisses and quartzites, Chapuis, 2017) surrounded by a karstic system with alterite deposits. The water flowing in karstic systems was enriched in ²²²Rn that ranged from 1120 to 2560 Beq/m³ 457 458 (according to measurements in springs). In the same way, the hyporheic water was also strongly enriched in ²²²Rn (until 14 kBq/m³ at station 16) certainly because of the metamorphic origin of the 459 460 river sediment. The mix of karstic groundwater and deep hyporheic water that upwells in the river may explain the changes in ²²²Rn concentration in gaining sections. In contrast, the ²²²Rn in the losing 461 462 sections decreased through disintegration and release to the atmosphere and the concentration in surface water returns to upstream concentrations (Cook, 2013). The ²²²Rn concentration turned out as 463 464 a possible indicator of deep groundwater reaching the river, at least in this geological context with 465 metamorphic sediment layer in a calcareous context.

466

467 **5.2-Location of river-groundwater exchange zones**

The location of large-scale gaining and losing sections is an increasing concern for alluvial rivers, where several methods were proposed based on hydraulic head measurements in several mini-piezometers (i.e. measurement of relative groundwater level, Kasahara & Wondzell, 2003), longitudinal profiles of water chemistry (Cook, 2013; Guida et al., 2013) or thermal infra-red images (Wawrzyniak et al., 2013, Longard 1, 2016). In karstic rivers, few studies focus on the location of diffuse karstic inflows to river compared to large karstic springs (e.g. Rugel et al., 2016). The present study confirms the changes in the discharge
observed by Chapuis (2017): the karstic inflows were not limited to large springs, but also occurred
through diffusive inflows along large sections of the river, at least along a 4km-long section in the
upstream part of the canyon.

477

478 At the scale of the geomorphological units, all downstream ends of the studied gravel bars were 479 upwelling zones (in all cases Vertical Hydraulic Gradient had positive values, the first part of H2 480 validated), even if upwelling zones were fed by different types of water in gaining or losing sections. 481 At the scale of the geological context, the predicted influence of valley width reduction on 482 groundwater inflow (the second part of H2) was not verified. The most significant reduction in valley 483 width is located just upstream of the canyon entrance, where a hydrological study highlighted a losing 484 section (noted L1 in Fig. 1; Chapuis, 2017). In these sections (where station 1 is located), the hyporheic 485 water that upwells into the river was extremely similar to river water suggesting short flowpaths inside 486 the sediment.

487

488 The sections with the most intense groundwater inflow were not linked to changes in canyon width, 489 but were associated to karstic spring (see electrical conductivity changes close to S1 and S8, Fig. 2), but 490 also to hidden diffuse groundwater inputs (see electrical conductivity and ²²²Rn concentration changes 491 in the upstream part of the canyon, Fig. 3). Chapuis (2017) evaluated groundwater contribution to the 492 Cèze River discharge to 49 to 68% (for intermediate and low discharge, respectively), but only the half 493 of this contribution was explained by the spring discharge, the other half may have originated from 494 diffuse inflow through the bed sediment (Chapuis, 2017). This result is important for river management 495 because large springs are generally the only groundwater inflows taken into account in management 496 plans (e.g. protection of groundwater against excessive pumping or pollution), while the gaining 497 sections of diffuse groundwater are frequently ignored and may represent a crucial objective for 498 resource protection.

499

500 **5.3-The response of the hyporheic fauna**

The total taxonomic richness of hyporheic assemblages varied between sections, with higher richness in the gaining area, while no differences were observed for total abundances because of very high differences between stations, resulting in highly variable microcrustacean abundances. Some stations (i.e. 20 and 24) were highly populated by a large set of species, especially by microcrustaceans. In these two stations, the abundances of the ostracod *Vestalenula* sp. were the highest. This Darwinulidae genus pertains to a group of subtropical Ostracoda (Artheau, 2007) and is certainly thermophilous. These stations 20 and 24 were located close to a losing section of the river and showed very high 508 temperatures in their hyporheic water (e.g. 27°C in station 24) that may explain this strong 509 development of hyporheic microcrustaceans (Dole-Olivier et al., 2000).

510

511 In contrast with microcrustaceans, the abundances of benthic EPTC was not higher in losing stations 512 (H4 rejected). In the Cèze River, the EPTC species colonised preferably the hyporheic zone fed by groundwater in gaining sections defined by changes in ²²²Rn concentrations, both for abundances and 513 514 species richness. This result was not predicted because the benthic fauna generally develops abundant 515 populations in well-oxygenated hyporheic zone rich in labile organic matter (Dole-Olivier & 516 Marmonier, 1992; Capderrey et al., 2013), all characteristics found in losing sections (Claret et al., 517 1998). In a Mediterranean river, such as the Cèze river, the losing sections with high temperatures may 518 be a disadvantage to large benthic fauna like EPTC. In contrast, the low water temperature and the 519 oligotrophy of the groundwater that upwells inside the river in gaining sections may represent a 520 favourable habitat for benthic invertebrates with cold water preferences (e.g. Esolus sp. or Leuctra 521 major, Bertrand, 1965; Berthelemy, 1968).

522

523 In the same way, the abundances of the stygobites were significantly higher in the stations grouped in 524 gaining sections, as predicted in the third hypothesis. The links between hyporheic water 525 characteristics and stygobites are now rather well documented (Dole-Olivier & Marmonier, 1992; 526 Datry et al., 2008): the stygofauna generally colonised areas with a constant temperature and 527 oligotrophic water, with low exigency for dissolved oxygen and fresh organic matter (Malard & 528 Hervant, 1999), they thus develop dense populations in upwelling zones (Dole-Olivier & Marmonier, 529 1992). Here, the stygobite fauna was more abundant or even restricted to the gaining sections (i.e. the 530 first part of the canyon and around the S8 spring) where the hyporheic water had low temperatures 531 (e.g. 15.5 and 17.7°C at stations 3 and 34, respectively) and high dissolved oxygen contents (e.g. 8.6 532 and 6.1 mg/L at stations 3 and 34, respectively).

533

This study highlighted the crucial role of gaining sections in the biological functioning of a Mediterranean river: the river sediment fed by groundwater upwelling represents an attractive habitat for a part of the invertebrates. The total taxonomic richness, the abundances of stygobite fauna and the benthic EPTC were higher in the gaining than in the losing sections. They can be considered as hotspots for invertebrate biodiversity in rivers and must be protected (Boulton, 2000).

539

540 **5.4-Using surface water characteristics to predict hyporheic biodiversity.**

541 Hotspots in hyporheic biodiversity may be endangered by several human activities that modify 542 sediment characteristics and exchanges with groundwater (e.g. groundwater pumping, dam building, change in the river hydrology...; Boulton, 2000). Although they need protection (Gibert & Culver, 2009), the location of these hotspots remains difficult. This study demonstrates that longitudinal profiles in surface water chemistry using simple (e.g. electrical conductivity) or more complex describers (e.g. ²²²Rn) may be used to predict the changes in hyporheic biodiversity. The prediction was based on a segmentation of the river in sections with contrasted hydrology (i.e. gaining and losing sections), but this segmentation may vary according to the indicator used (Table 2).

549

The longitudinal profiles of surface water temperature measured from a canoe using a thermic probe gave poor results. The two profiles presented in this study were difficult to analyse and do not fit with faunal distribution certainly because of the duration of the measurement (at least two successive days) that mixed the temporal changes with the thermic trends linked to groundwater upwelling zones. To avoid this problem, infra-red images took by a ULM or a drone may reduce the duration of the measurement and make more consistent the longitudinal profile (Hare et al., 2015; Wawrzyniak et al., 2013, 2016; Dole-Olivier et al., 2019).

557

558 In the same way, the segmentation of the studied sector using hyporheic water characteristics gave 559 highly fragmented sections (see Fig. 4 and Table 2) that poorly fit with the longitudinal distribution of 560 hyporheic fauna (i.e. the total hyporheic fauna, the EPTC or the stygobionts did not significantly 561 changed with sections defined by hyporheic water chemistry). This discrepancy may be surprising, but 562 already reported for another Mediterranean river, the Drôme River (Marmonier et al., 2019). In these 563 two rivers, the hyporheic assemblages appeared a more integrative and reliable indicator of 564 groundwater upwelling than hyporheic water chemistry that may locally change for light 565 geomorphologic or geologic differences.

566

The longitudinal profiles of electrical conductivities in the surface water were similar in July 2013 and 567 568 June 2015. These profiles were consistent with the profile of ²²²Rn concentrations obtained in June 569 2015: two losing sections were located upstream of the canyon entrance and in its central part, two 570 gaining sections were observed in the first part of the canyon and close to the last S8 spring. The 571 stygobite fauna and the EPTC followed very similar patterns, with significant differences in their abundances in sections where electrical conductivity and ²²²Rn concentrations increased in surface 572 573 water (i.e. gaining sections). This is the first time that these three types of indicators were used in the 574 same sector of a river, even if the hyporheic fauna and the ²²²Rn concentrations were measured at two different dates (i.e. July 2013 and June 2015, respectively). The ²²²Rn concentrations were measured 575 576 at the same time as the second electrical conductivity profile, that gave similar spatial trends with the 577 first one, but this temporal interval remains a limit of this study. The link between hyporheic fauna and

- 578 surface water characteristics look promising for location of potential hotspots of hyporheic biodiversity 579 (Table 2). Future research must focus on the possible use of ²²²Rn indicator in other geological contexts, 580 keeping in mind that each type of indicator brought a complementary image of the functioning of the 581 karst-river system. Altogether, the five indicators used concomitantly in this study showed high 582 efficiency for the location of gaining sections that could help managers to define optimal protection 583 strategies for hyporheic biodiversity and, more globally, for the river integrity.
- 584

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Table 1. Physico-chemical characteristics of the surface water of the Cèze River upstream of the canyon and of four karstic springs in July 2013.

	Surface water	Spring water	ANOVA test
	Mean ± SD	Mean ± SD	
	n=4	n=4	
Temperature	24.2 ±0.2	15.9 ±1.6	F = 89.6
(°C)			<i>p-value</i> = 0.00008
Electric Conductivity	332.7 ±6.4	492 ±65.4	F = 23.60
(µS/cm)			<i>p-value</i> = 0.0028
Dissolved Oxygen	8.2 ±0.8	7.7 ±4.2	n.s.
(mg/L)			
Sulphate	38.9 ±17.2	38.0 ±18.8	n.s.
(mg/L)			
Nitrate	1.7 ±0.2	29.0 ±42.1	n.s.
(mg/L)			
Na	15.6 ±1.8	6.0 ±3.7	F = 22.56
(mg/L)			<i>p-value</i> = 0.0031
К	1.8 ±0.6	1.0 ±0.5	F = 6.17
(mg/L)			<i>p-value</i> = 0.047
Mg	9.2 ±0.4	3.0 ±2.6	F = 18.71
(mg/L)			<i>p-value</i> = 0.0049
Са	41 ±1.3	62.0 ±15.2	F = 7.55
(mg/L)			<i>p-value</i> = 0.033

Table 2. Suitability of the five describers for the study of groundwater-river exchanges (with usable757 information for location noted by X).

	GW to river	River to GW	Intensity of exchanges	Estimation of the surfaces	Biodiversity hotspot delineation
Surface water temperature	Х		Х		
Surface electric conductivity	Х		Х	Х	х
Surface ²²² Rn concentration	Х		Х	х	х
Hyporheic water characteristics	Х	х	х		
Hyporheic invertebrate composition	х	Х			х





- 767
- 768 Figure 1. Aerial photograph of the studied sector of the Cèze River (modified from Géoportail, IGN).
- 769 With distances from upstream to downstream (kilometric points, triangles), location of the 17
- 770 hyporheic sampling stations (with gravel bar number, yellow arrows), the 8 karstic springs (beige
- arrows) and the 2 loosing sectors (blue arrows).
- 772







electric conductivity (b, for May and July 2013 and d, for June 2015). Blue and white squares

represent the gaining and losing (or no exchange) sections respectively.





Figure 3. Longitudinal profile ²²²Rn in June 2015. Individual measurements (black dots) and lines for
the minimum-maximum values. Blue and white squares represent the gaining and losing (or no
exchange) sections respectively.





782 Figure 4. Chemical characteristics of the hyporheic water in the 17 sampled gravel bars (50cm deep

- inside sediment). Correlation circle of the PCA (a) and location of the sampling station on the PC1 axis
- 784 (b; dots are the 3 replicate samples). Chemical variables expressed as the mean difference (D.)
- 785 between surface water and groundwater for electric conductivity (c), sulphate concentrations (d),
- 786 Vertical Hydraulic Gradient, VHG (e), temperature (f), Sodium (g) and Magnesium (h; Mean ±
- 787 Standard Deviation, n=3). Blue squares highlight the stations with negative coordinates on the PC1
- 788 axis fed by water chemically close to groundwater.



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Figure 5. Hyporheic fauna: total abundances (a), stygobite abundances (b), microcrustacean abundances (c) and benthic species of Ephemeroptera, Plecoptera, Trichoptera and Coleoptera (d) in the 17 stations (from gravel bar 1 to 34). Blue squares represent the gaining sections according to ²²²Rn concentrations in surface water.

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SUPPLEMENTARY MATERIAL





798 Fig. S1 : Interaction between processes controlling exchanges between river and groundwater. At the

- section scale (km), losing and gaining sections are controled by the geographical and geological
- 800 characteristics of the river (A, the Baume River, France). At the local scale, irregularities in the river
- slope (e.g. riffle, gravel bars, logs...) induce local downwellings followed by local upwellings. In a
- 802 losing context (B) the chemical characteristics of the upwelling water are similar to surface water,
- 803 while in a gaining section (C) water chemistry shows influence of deep groundwater.



804

- 805 Figure S2. Aerial infra-red photographs of two sections of the Cèze River close to the Spring S1 (kp 5)
- and the S8 spring (kp 17) with cold water plumes in blue-violet colours (between 16 and 25°C)



Figure S3: longitudinal distribution of 8 hyporheic organisms of the Cèze River with limits of the
gaining sections (blue area, obtained using ²²²Rn concentrations). The ostracods *Vestanlenula* sp. and *Marmocandona zschokkei, Cryptocandona kieferi,* the Coleoptera *Esolus* sp., the Plecoptera *Leuctra*ssp. (sum of *L. major* and *L.* gr *nigra*), the Ephemeroptera *Choroterpes picteti* and the Amphipods *Niphargus kochianus* and *N. fontanus*.