1 **Vertical movements through subsurface stream sediments by benthic**

2 **macroinvertebrates during experimental drying are influenced by sediment**

3 **characteristics and species traits.**

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26   **Summary**

1. Streambed drying is becoming more common due to climate change and increasing anthropogenic water resource pressures. Subsurface sediments are a potential refuge for benthic macroinvertebrates during drying events in temporary streams.

2. Sediment characteristics are important controls on the distribution of macroinvertebrates in subsurface habitats, but difficulties making observations impedes quantification of vertical movements. Species traits (e.g., subsurface habitat affinity) also influence vertical movements of macroinvertebrates into the subsurface sediments, but most species-specific responses remain uncharacterized.

3. Transparent artificial mesocosms were used to directly observe the vertical movements of individuals of three aquatic insect nymphs and two crustaceans. Mixtures of three types of transparent sediment of varying particle size were combined to produce six treatments with differing interstitial pore volumes and, hence, differing subsurface porosity. Macroinvertebrate vertical movements were measured during incremental reductions in water level from 5 cm above to 20 cm below the sediment surface. These species comprised a variety of trait categories including feeding group, species affinity to temporary streams and subsurface habitats. Active and passive vertical movements were determined by conducting experiments with both live individuals and their cadavers.

4. Sediment treatment influenced the vertical movements of individuals as reducing subsurface porosity decreased vertical movements for most species. Vertical movement into subsurface sediments in response to water level reduction was the result of active, not passive, movements for all species.

5. Species identity influenced the vertical movements made by individuals. *Nemoura cambrica* had the highest affinity for temporary streams and subsurface habitats and

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51   its vertical movements were unaffected by sediment treatment, generally reaching

52   depths between 20–25 cm. Most individuals of species with a weaker subsurface

53   affinity (i.e. the benthic grazer *Heptagenia sulphurea* and the filter-feeder

54   *Hydropsyche siltalai*) became stranded as water levels were reduced in all sediment

55   treatments. Vertical movements of *Gammarus pulex* and *Asellus aquaticus* were

56   restricted primarily by pore volume, these taxa becoming stranded most commonly in

57   sediments with smaller interstitial volumes.

58   6. Our results highlight the need for the development and implementation of river

59   management strategies that increase streambed porosity, allowing macroinvertebrates

60   to access to the saturated subsurface habitat during stream drying.

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Introduction

Temporary streams experience periods of no flow, often resulting in the loss of longitudinal hydrological connectivity and surface water (e.g. Boulton, 2003; Datry et al., 2014a; Bogan et al., 2015). Surface water loss can be detrimental and, in some instances, fatal to many aquatic species (Extence, 1981; Stanley et al., 1997; Wood et al., 2010). A wide range of species, however, display behavioural adaptations that facilitate their persistence in temporary streams (Lytle & Poff, 2004; Datry, 2012). Temporary streams are widespread and occur in every climatic zone from polar (e.g., McKnight et al., 1999) and temperate regions (Williams & Hynes, 1976; Stubbington et al., 2016) through to tropical and arid zones (Steward et al., 2012; Leigh 2013; Bogan et al., 2015). They, therefore, represent a widespread stream type supporting distinct species assemblages (Westwood et al., 2006; Bogan et al., 2013; Acuña et al., 2014).

Surface flow cessation and streambed drying are the primary drivers of temporary stream community structure (Bogan & Lytle, 2011; Datry et al., 2014a), taxonomic richness (Datry et al., 2014a; Stubbington et al., 2015; Leigh & Datry, 2016), population abundance (Smith & Wood, 2002; Rüegg & Robinson, 2004) and ecosystem functioning (Datry et al., 2011; Magoulick, 2014). Despite an increasing recognition of the wider value of ecosystem services provided by temporary streams and their biota (Acuña et al., 2014; Datry et al., 2014b), few studies have examined the response of individual lotic species to surface water loss, including their survival, and the ability of individuals to access and use subsurface habitats during drying (Imhof & Harrison, 1981; Vadher et al., 2015; Vander Vorste et al., 2016a). Addressing this knowledge gap is important because poor access to subsurface habitats (e.g., due to sedimentation/colmation) during streambed drying is likely to compromise the persistence of many aquatic macroinvertebrate species (Descloux et al., 2013; Jones et al., 2015; Vadher et al., 2015; Leigh et al., 2016).

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87   After benthic sediments dry, subsurface water may persist within the hyporheic zone (Hose et

88   al., 2005; Fenoglio et al., 2006). The potential for these subsurface sediments to function as a

89   refuge has long been recognised (Williams & Hynes, 1974). The hyporheic zone is an

90   important habitat and resource for aquatic fauna during streambed drying in many streams

91   (Dole-Olivier, 2011; Vadher et al., 2015; Vander Vorste, 2016b). If individuals can access

92   and persist in saturated subsurface sediments during periods of surface water loss, they may

93   be able to return to the channel when flow returns (Stubbington, 2012; Vadher et al., 2015),

94   thus facilitating the rapid recolonization and recovery of temporary stream communities

95   (Vander Vorste et al., 2016b). However, not all streams have extensive hyporheic zones and

96   it may be absent in streams where, for example, bedrock dominates the channel (Malard et al.,

97   2002), or in other instances it may be inaccessible due to fine sediment deposition and

98   clogging (Descloux et al., 2013; Vadher et al., 2015).

99   A range of biological traits may enhance species resistance (ability to persist) and resilience

100 (ability to recover) to stream drying, for example body size, locomotion and feeding habits

101 (e.g., Bonada et al., 2007). Sedimentary characteristics that may also affect access to and

102 movement through the hyporheic zone have been explored (e.g., Nogaro et al., 2006;

103 Stubbington et al., 2011; Descloux et al., 2013; Mermillod-Blondin et al., 2015). Field (Duan

104 et al., 2008; Gayraud & Philippe, 2003; Descloux et al., 2013) and laboratory (Nogaro et al.,

105 2006; Navel et al., 2010; Vadher et al., 2015) studies have demonstrated that sediment

106 characteristics including particle size, shape, heterogeneity and porosity can influence the

107 distribution of benthic populations. However, the direct response of individuals to drying and

108 their ability to move into subsurface sediments has rarely been studied in real time

109 (exceptions being Stumpp & Hose 2013; Vadher et al., 2015). This reflects the inherent

110 difficulties of directly observing fauna within subsurface habitats.

In this ex-situ study, we experimentally examined the effect of surface water loss and water level reduction within subsurface sediments of varying sediment characteristics (particle size, heterogeneity and interstitial volume) on the vertical movement of individuals of five benthic macroinvertebrate species. We used artificial sediments of known size and shape within laboratory mesocosms to directly control sediment characteristics. The use of different particle combinations allowed the volume of interstitial space to be quantified and controlled (Mathers et al., 2014). To facilitate direct observation of individuals and their behaviour within the subsurface sediments, transparent artificial sediments were used to allow the precise location of individuals to be observed throughout the experimental period. We hypothesised that the response of lotic benthic macroinvertebrates to water level reduction and their vertical movement through the subsurface would: i) be active rather than passive; ii) depend on subsurface sediment characteristics; and iii) vary between species due to interspecific variation in traits (e.g., mode of locomotion, feeding group, habitat affinities).

Materials and methods

Invertebrate collection and test species

Five species of benthic macroinvertebrate were chosen for examination of their response to surface water loss and water level reduction: a stonefly nymph, *Nemoura cambrica* (Plecoptera: Nemouridae); a caseless caddisfly larvae, *Hydropsyche siltalai* (Trichoptera: Hydropsychidae); a mayfly nymph, *Heptagenia sulphurea* (Ephemeroptera: Heptageniidae); and two crustaceans, *Asellus aquaticus* (Isopoda: Asellidae) and *Gammarus pulex* (Amphipoda: Gammaridae). These species consisted of one widespread inhabitant of seasonally dry headwater streams, *N. cambrica* (Stubbington et al., 2009); two species which have been widely recorded in benthic and hyporheic sediments in both perennial and temporary systems, *A. aquaticus* and *G. pulex* (Stubbington et al., 2015); and two benthic

135   species typically associated with perennially flowing systems, *Hy. siltalai* and *H. sulphurea*

136   (Eyre et al., 2005; Wood et al., 2005a; Datry 2012). These species were selected to represent

137   a range of biological traits (Table 1).

138   All five species were collected from sites on Wood Brook (52°46'07.5"N 1°12'34.6"W) and

139   Burleigh Brook (52°45'50.5"N 1°14'28.6"W) in Loughborough (Leicestershire, UK).

140   *Gammarus pulex*, *Hy. siltalai, H. sulphurea* and *N. cambrica* were collected from shallow

141   riffles and *A. aquaticus* was collected from a slow-flowing pool adjacent to Wood Brook.

142   Individuals were collected using a gentle kick-sampling technique with a standard kick-net

143   (900 µm mesh, 23 cm × 25.5 cm frame, 27.5 cm bag depth). Captured specimens were

144   removed from the net individually and transferred to a 5-L container of stream water and

145   transported to the laboratory for use in experiments on the same day.

146   *Sediment-column mesocosms*

147   Twelve sediment-column mesocosms were constructed from transparent acrylic pipes (35 cm

148   × 4.6 cm internal diameter; Fig. 1a) to allow direct observation of individuals. Columns were

149   sealed at the base by a rubber bung with a 5-mm glass tube (3 mm internal diameter) in the

150   centre to allow drainage. A silicon tube was secured over the glass tube and a Hoffman clip

151   allowed control of the water level to within 0.5 mm (Fig. 1a).

152   Columns were mounted onto retort stands (Fig. 1b) within an environmental cabinet (108 cm

153   × 27 cm × 68 cm). The front wall of the cabinet was covered with a black cloth to maintain

154   darkness and provide lighting conditions analogous to the subsurface streambed whilst

155   allowing an observer to inspect the columns inside.

156   *Sediment treatments*

157   Mixtures of three transparent particle types were used to create six sediment treatments of

158   varying pore-size volumes: small (10 – 15 mm) angular particles; large (20 – 25 mm) angular

159   particles; and large (14 – 20 mm) smooth particles (Table 2). The difference in interstitial

160   volume between sediment treatments allowed examination of the effect of sediment porosity

161   on the vertical movement by species. The interstitial volume was calculated by measuring the

162   amount of free water within the sediment-filled columns five times to provide a mean and

163   standard error for each sediment treatment (Table 2). Sediment treatments containing

164   multiple particle types were thoroughly mixed prior to use, randomly distributed throughout

165   the columns and filled to a depth of 25 cm (Fig. 1b).

166   *Water treatment and depth control*

167   Tap water was pre-treated with AquaSafe® (Tetra®, Virginia) to neutralise any residual

168   chemicals and cooled to 11°C over a 24-h period prior to the commencement of experiments.

169   Dissolved oxygen was measured directly in the surface water at the start of experiments and

170   oxygen saturation was maintained throughout each experiment using oxygen tablets, widely

171   used in domestic aquaria.

172   Water was added to each column to 5 cm above the sediment surface (Fig. 1b). Water level

173   was then reduced in 12.5 mm increments every 15 min over a 5 h experimental period until a

174   5 cm depth of water (i.e., 20 cm below the sediment surface) was retained in each column at

175   the end of each trial as a refuge. This rapid rate of drying is analogous to that experienced on

176   topographic high points (riffles and marginal gravel bars) in streams with permeable

177   sediments where upstream anthropogenic structures (e.g., weirs and spillways) control the

178   volume of discharge and flow can be effectively cut off when a low flow threshold is crossed.

179   *Experimental procedure*

180   One individual from the same species was introduced into each water-filled column.

181   Individuals were left to acclimatise within the columns and environmental cabinet prior to the

182   start of an experiment until they ceased active exploration and burrowing behaviour

183   (preliminary experiments indicated approximately 20 min were sufficient). Following

184   acclimatisation, macroinvertebrates were observed within the cabinet using an LED light to

185   minimise disturbance to each column during the experiment. Initial vertical movement from

186   the sediment surface (depth = 0) into the subsurface was recorded (1 mm accuracy) at this

187   time (time 0 = 5 cm surface water). Individuals within the water column or on the sediment

188   surface were recorded as having a vertical movement of 0 mm. Prior to each water level

189   reduction, the vertical position of the individual in each column (mm below depth 0) was

190   recorded.

191   Once the water level had been reduced to 20 cm below the sediment surface for 15 min, the

192   final depth reached by the macroinvertebrate beneath the substrate surface was recorded and

193   the experiment terminated. The difference between the depth of individuals at the start of the

194   experiment (time = 0) and the final depth reached beneath the substrate surface at the end of

195   the experiment (time = 5 h) determined their absolute depth moved (i.e., total vertical

196   movement during experiment). The sediment columns were deconstructed and their

197   sediments removed and washed thoroughly to remove live test macroinvertebrates, debris and

198   biological waste. Macroinvertebrates were then euthanized and preserved using 70%

199   industrial methylated spirit (IMS).

200   To distinguish between active and passive movements associated with water level reduction,

201   experiments were repeated using the cadavers of the same individuals as used in live

202   experiments, which were rinsed thoroughly in tap water prior to reintroduction into

203   reconstructed sediment treatments. If a cadaver became stranded above the water-line during

204   the water level reduction, the depth was recorded, the experiment terminated, and the

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205   individual retrieved from the column and preserved in IMS. Each experiment (live and

206   cadaver) was replicated 20 times for each of the six sediment treatments, providing 240

207   experimental trials using 120 individuals of each species.

208   Head widths of individuals were measured and calibrated using Motic® Images Plus 2.0

209   software (Motic®, Hong Kong), as a surrogate for body size (Smock, 1980). Head width

210   measurements were standardised within species, being taken from the base of the antenna to

211   the posterior margin of the head carapace for *G. pulex* and *A. aquaticus*, and as the widest

212   cross section of the head capsule for *Hy. siltalai*, *N. cambrica* and *H. sulphurea*.

213   *Data analysis*

214   We tested our first hypothesis, that movement into the subsurface would be active rather than

215   passive, using paired sample t-tests to compare the absolute depth moved (difference

216   between depth of an individual at time = 0 and at the end of the experiment) by live and

217   cadavers of the same individuals for each species and sediment treatment.

218   We tested our second and third hypotheses, that vertical movements through the subsurface

219   would be influenced by sediment characteristics and would vary between species, using a

220   General Linear Model (GLM) and a Binary Logistic Regression (BLR, using the logit link

221   function). We defined macroinvertebrate responses to surface water loss and water level

222   reduction in three ways: final depth reached, absolute depth moved, and likelihood of

223   becoming stranded. A GLM was used to examine the effect of sediment treatment, species

224   identity, and their interaction, on the final depth reached and absolute depth moved by live

225   individuals. To examine and account for the potential influence of individual body size on

226   final depth reached, head width was included in the model as a covariate, nested within

227   species. The model was fitted using Maximum Likelihood Estimates (MLE). Fisher's LSD

228   *post-hoc* tests were used where significant effects of sediment treatment (hypothesis ii) or

229   species (hypothesis iii) were detected.

230   BLR analysis was used to determine the effect of sediment treatment and species identity on

231   the likelihood of live individuals becoming stranded above the water-line. Individuals were

232   classified into two groups: stranded (coded as '0') or below the water-line (not stranded;

233   coded as '1'). In all cases, the BLR model had a good predictive capacity (correct

234   classification rate 84.4%) and fit (Nagelkerke $R^2 = 0.672$). The BLR model was run multiple

235   times to create a pairwise BLR model. *Nemoura cambrica* was excluded from the BLR

236   analysis because no individual of this species became stranded during the live experiments,

237   resulting in no variation in the response for the BLR to model. All analyses were performed

238   using IBM SPSS Statistics (version 23, IBM Corporation, New York).

239   **Results**

240   *Active vs passive movement of benthic macroinvertebrates into the subsurface sediments in*

241   *response to water level reduction*

242   Live individuals of each species reached greater absolute depths than their cadavers, which

243   remained close to the sediment surface (Table 3). Live individuals within each sediment

244   treatment reached greater absolute depths compared to their cadavers (Table 3).

245   *Effect of sediment treatment, species identity and body size on the final depth reached by live*

246   *individuals*

247   The effect of sediment treatment on the final depth reached beneath the substrate surface by

248   individuals varied significantly between species (i.e. the interaction term was significant;

249   GLM, $P < 0.001$). The final depth reached in each sediment treatment was dependent on

250   species identity. The pattern of final depth reached for each species within each sediment

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251 treatment generally increased from sediment treatment 1-6 with increasing interstitial volume

252 (Table 2), however, final depth reached in each sediment treatment decreased for each

253 species from *N. cambrica*, *A. aquaticus*, *G. pulex*, *Hy. siltalai* to *H. sulphurea*, respectively

254 (Fig. 2). Within each species, body size had no effect on final depth reached (GLM, $P =$

255 0.179).

Effect of sediment treatment and species identity on the absolute depth moved

257 The effect of sediment treatment on the absolute depth moved by individuals varied between

258 species (i.e. the interaction term was significant; GLM, $P < 0.01$). Specifically, the absolute

259 depth moved by *N. cambrica* individuals were significantly greater than: *A. aquaticus*

260 individuals in sediment treatments 1-4 and 6; *Hy. siltalai* individuals in sediment treatments 1,

261 2 and 4; and *H. sulphurea* individuals in sediment treatments 1-4 (see Table S1 in Supporting

262 Information). The absolute depth moved by *G. pulex* individuals were significantly greater

263 than: *A. aquaticus* individuals in sediment treatments 4-6; *Hy. siltalai* individuals in sediment

264 treatments 3 and 4; and *H. sulphurea* individuals in sediment treatments 1-4 (see Table S1 in

265 Supporting Information). Body size had no effect on the absolute depth moved (GLM, $P =$

266 0.401).

Effect of sediment treatment and species identity on the stranding of live individuals

268 Sediment treatment affected the likelihood of individuals being stranded (BLR, $P < 0.001$).

269 Individuals were most likely to become stranded in sediment treatment 1 followed by 2-4 (not

270 significantly different), then 5, and finally treatment 6 (Fig. 3a). More specifically, the

271 likelihood of individuals becoming stranded differed between sediment treatments 1, 5 and 6

272 (pairwise BLR, $P < 0.05$). In addition, the likelihood of stranding in sediment treatments 1, 5

273 and 6 differed (pairwise BLR, $P < 0.05$) from the likelihood of stranding in treatments 2, 3 or

274 4 (for which the likelihood of stranding was comparable; pairwise BLR, $P > 0.05$).

275 Species identity also had a significant effect on the likelihood of individuals becoming

276 stranded (BLR, $P < 0.001$). Individuals of *H. sulphurea* were most likely to become stranded

277 followed by *Hy. siltalai*, *G. pulex* and finally *A. aquaticus* (pairwise $P < 0.05$; Fig. 3b); no *N.*

278 *cambrica* individuals became stranded in any treatment (Table 4 and Fig. 2a). The majority of

279 *G. pulex* individuals stranded in sediment treatment 1 (70%) and < 50% were stranded in

280 treatments 2 and 3 (Table 4 and Fig. 2b). Over half of *A. aquaticus* individuals were stranded

281 in sediment treatment 1 (Table 4 and Fig. 2c) and ≤ 10% became stranded in treatments 2 and

282 3. The majority of *Hy. siltalai* individuals became stranded during water level reduction

283 across all sediment treatments (except treatment 6 = 45%; Fig. 2d) and all *H. sulphurea*

284 became stranded in sediment treatments 1-3 with ≤ 60% stranded in treatments 4-6 (Table 4

285 and Fig. 2e).

286 **Discussion**

287 *Benthic macroinvertebrates actively move into the subsurface during dewatering*

288 We found support for our first hypothesis, that faunal movement into the subsurface in

289 response to dewatering would be active. Studies have recorded benthic macroinvertebrates

290 relatively deep within the hyporheic zone when surface sediments dry (e.g., Fenoglio et al.,

291 2006; Young et al., 2011). *Agabus paludosus* (Coleoptera: Dytiscidae), for example, has been

292 recorded at 70 – 90 cm below the streambed surface in response to drying (Fenoglio et al.,

293 2006). It has been suggested that individuals recorded deep within riverbed sediments have

294 moved there (actively or passively), via interstitial pore spaces. However, in the absence of

295 direct observation and being able to track individuals, it has not previously been possible to

296 determine if these movements were active (macroinvertebrates moving vertically to remain

297 submerged) or passive (being drawn down with the receding water-line). Comparison of our

298 direct observations of live individuals and cadavers in response to water level reduction in

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299 transparent sediment-column mesocosms provide the first definitive evidence that vertical

300 movements are active, not passive.

301 *Effect of sediment treatment on the vertical movements of benthic macroinvertebrates*

302 We found support for our second hypothesis, that subsurface sediment characteristics would

303 influence the ability of individuals to move vertically in response to water level reduction.

304 Sediments with lower interstitial volume (sediment porosity) due to smaller particle sizes

305 reduced the vertical movements of individuals of all species except *N. cambrica*. This

306 supports previous studies which found that sediment characteristics influence the use of

307 subsurface sediments by a range of benthic macroinvertebrate species (Stubbington et al.,

308 2011; Descloux et al., 2013; Vadher et al., 2015).

309 Previous studies have indicated that sediment characteristics such as interstitial pore volume

310 influence the ability of macroinvertebrate taxa to move into subsurface sediments and that

311 movement would reflect species traits (Boulton et al., 1998; Gayraud & Philippe, 2003;

312 Vadher et al., 2015; Mathers & Wood, 2016). Small particles with reduced sediment porosity

313 (i.e. fine sediment deposited on the sediment surface or those subject to sedimentation within

314 the sediment matrix) potentially limit the function of the subsurface as a refuge for

315 macroinvertebrates following surface water loss (Navel et al., 2010; Descloux et al., 2013;

316 Vadher et al., 2015) and studies have reported the absence or reduced use of subsurface

317 sediments due to the limited interstitial pore spaces available (Boulton 1989; Richards &

318 Bacon, 1994; Smock et al., 1994; James et al., 2008). Our observations provide direct

319 evidence to support studies which have inferred that sediment characteristics limit the

320 movement of benthic macroinvertebrates into the subsurface during adverse conditions (e.g.

321 Stanley et al., 1994; Smock et al., 1994; Olsen & Townsend, 2005; Stubbington et al., 2011).

322 *Species-specific vertical movements of individuals through sediments*

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323 Our results demonstrate that vertical movement varies between species, reflecting differences

324 in traits and habitat affinities. This supports our third hypothesis, that the vertical movement

325 of species through the subsurface would be influenced by their traits. The response to

326 sediment porosity of the species examined was similar; however, the absolute vertical

327 distance moved by the five species differed. The family-level trait designation of *Nemoura*

328 suggests that species in this genus have no affinity with the subsurface (Table 1; Tachet et al.,

329 2010), but our results suggest that this species *is* able to move into the sediments in response

330 to water level reduction. In our study *N. cambrica* was able to freely move into the subsurface,

331 in response to water level reduction and has also been recorded in temporary streams

332 (Stubbington et al., 2009). *Nemoura cambrica* has a small body size compared to the other

333 species used in our experiments and can burrow and excavate itself from fine sediment

334 deposits (Wood et al., 2005b). A reduced size of mature nymphs can promote invertebrate

335 resistance in subsurface habitats (Gayraud & Philippe, 2001; Navel et al., 2010; Vander

336 Vorste et al., 2016b) and may explain why *N. cambrica* did not become stranded above the

337 water-line and moved freely through interstitial pore spaces in all experimental treatments.

338 Although we did not detect an effect of body size on the vertical movement within individual

339 species, the body size differences between species probably influences vertical movements.

340 Most *G. pulex* and *A. aquaticus* individuals moved vertically in all sediment treatments

341 except the smallest particle size treatment. This observation advances the experimental

342 findings of Vander Vorste et al. (2016a), who observed that *G. pulex* used the subsurface as a

343 refuge in response to water level reduction, and Vadher et al. (2015), who found that *G. pulex*

344 were unable to use the subsurface when sediment porosity was reduced. In marked contrast,

345 *Hy. siltalai* and *H. sulphurea* displayed limited ability to move vertically into the subsurface

346 in response to water level reduction. Both these taxa are primarily associated with benthic

347 habitats in perennial streams, and a low affinity to intermittence (Eyre et al., 2005; Wood et

348   al., 2005a, Table 1) may mean that *Hy. siltalai* and *H. sulphurea* lack behavioural adaptations

349   to move strongly into the subsurface sediments in response to drying.

350   The net-spinning caseless caddisfly larvae *Hy. siltalai* and the free-living mayfly nymph *H.*

351   *sulphurea*, became stranded in most sediment treatments during water level reduction.

352   *Hydropsyche siltalai* larvae are benthic filter-feeders and *H. sulphurea* larvae are benthic

353   grazers (Table 1). These feeding traits mean that these taxa typically occupy microhabitats

354   close to, or on, the sediment surface where algal growth is most abundant and filter-feeding is

355   most efficient. As a result, they may not typically move vertically into the subsurface as

356   trophic resources would be reduced. However, even for these species, around half of *Hy.*

357   *siltalai* individuals moved to the column base in the coarsest sediments with the largest

358   interstitial pore space (treatments 5 and 6) and 60% of *H. sulphurea* remained submerged in

359   the largest sediments (treatment 6) suggesting that in rivers with coarse sediments and open

360   gravel frameworks, stranding may be reduced and vertical movement possible to enhance the

361   use of the subsurface refuge during streambed drying. These results provide evidence to

362   support studies indicating reduced invertebrate species diversity within streams which have

363   experienced surface water loss and drying (Extence, 1981; Feminella, 1996; Datry, 2012;

364   Bogan et al., 2013) and clearly highlights the reduced vertical movement of some species

365   typically associated with benthic habitats.

366   *Conclusions and future directions*

367   Hydrological extremes within streams may become increasingly common as climate change

368   (Ledger & Milner, 2015; Pyne & Poff, 2017) and water resource pressures interact to increase

369   the duration of dry phases in some regions (Datry et al., 2014b). This study highlights the

370   variation in species responses to simulated water level reduction in sediments with different

371   characteristics. We highlight the need to understand species-specific responses in relation to

differences in sediment characteristics among streams. Although the subsurface sediments of the hyporheic zone can be an extensive refuge in gravel-bed rivers (Vander Vorste et al., 2016c), they are naturally heterogeneous and can also be a patchy refuge (Dole-Olivier et al., 1997). In some places, the hyporheic zone can be limited in spatial extent due to reduced interstitial habitat availability as a result of small particle sizes limiting access to the subsurface for many taxa (Gayraud & Philippe, 2003). Furthermore, anthropogenic activity (e.g., mining and flow regulation) may result in enhanced fine sediment deposition, further reducing the ability of subsurface sediments to function as a refuge (Descloux et al., 2013; Vadher et al., 2015).

Our results also highlight the need for effective refuge management and maintenance of sediment porosity in streams as active movements made by macroinvertebrates into the subsurface sediments could potentially enhance recovery from drying events and may maintain species abundance and diversity. Such management strategies should include measures of reduce fine sediment inputs to river channels via the use of sediment detention ponds/wetlands and more effective planting of riparian vegetation (buffer strips) to reduce sediment transport and help stabilize river banks, especially in agricultural areas (Verstraeten & Poesen, 2000; Hughes, 2016). In some instances, where fine sediment inputs are high and river flows are insufficient to flush fines from the interstices of the riverbed, gravel cleaning may be required to reconnect benthic and hyporheic habitats, improve subsurface water quality and ultimately increase sediment porosity and hydrological connectivity (Meyer et al., 2008). Developing effective management strategies is essential if the future of 'drying refuges', such as the hyporheic zone, is to increase community resistance and resilience to stream drying. However, the ability of macroinvertebrates to migrate back to the surface and recolonize benthic habitats as water levels rise remains uncharacterized. Future research should, where possible, combine field and laboratory mesocosm-based approaches to validate

397 observations and facilitate a greater understanding of community and individual responses to

398 the processes of streambed drying and flow resumption.

406 References

407 Acuña, V., Datry, T., Marshall, J., Barceló, D., Dahm, C. N., Ginebreda A., … Palmer M. A.
408   (2014). Why should we care about temporary waterways? *Science*, 343, 1080–1081.

409 Bogan, M. T., & Lytle, D. A. (2011). Severe drought drives novel community trajectories in
410   desert stream pools. *Freshwater Biology*, 56, 2070–2081.

411 Bogan, M. T., Boersma, K. S., & Lytle, D. A. (2013). Flow intermittency alters longitudinal
412   patters of invertebrate diversity and assemblage composition in an arid-land stream
413   network. *Freshwater Biology*, 58, 1016–1028.

414 Bogan, M. T., Boersma, K. S., & Lytle D. A. (2015). Resistance and resilience of invertebrate
415   communities to seasonal and supraseasonal drought in arid-land headwater streams.
416   *Freshwater Science*, 60, 2547–2558.

417 Bonada, N., Dolédec, S., & Statzner, B. (2007). Taxonomic and biological trait differences of
418   stream macroinvertebrate communities between mediterranean and temperate regions:
419   implications for future climatic scenarios. *Global Change Biology*, 13, 1658–1671.

420 Boulton, A. J. (1989). Over-summering refuges of aquatic macroinvertebrates in two
421   intermittent streams in central Victoria. *Transactions of the Royal Society of South
422   Australia*, 113, 23–34.

423   Boulton, A. J. (2003). Parallels and contrasts in the effects of drought on stream
424   macroinvertebrate assemblages. *Freshwater Biology*, 48, 1173–1185.

425   Boulton, A. J., Findlay, S., Marmonier, P., Stanley, E. H., & Valett, M. (1998). The
426   functional significant of the hyporheic zone in streams and rivers. *Annual Review of*
427   *Ecology and Systematics*, 29, 59–81.

428   Datry, T. (2012). Benthic and hyporheic invertebrate assemblages along a flow intermittence
429   gradient: effects of duration of dry events. *Freshwater Biology*, 57, 563–574.

430   Datry, T., Corti, R., Claret, C., & Philippe, M. (2011). Flow intermittence controls leaf litter
431   breakdown in a French temporary alluvial river: the "drying memory". *Aquatic*
432   *Sciences*, 73, 471–483.

433   Datry, T., Larned, S. T., Fritz, K. M., Bogan, M. T., Wood, P. J., Meyer, E. I., & Santos, A. N.
434   (2014a). Broad-scale patterns of invertebrate richness and community composition in
435   temporary rivers: effects of flow intermittence. *Ecography*, 37, 94–104.

436   Datry, T., Larned, S. T., & Tockner, K. (2014b). Intermittent rivers: a challenge for
437   freshwater ecology. *Bioscience*, 64, 229–235.

438   Descloux, S., Datry, T., & Marmonier, P. (2013). Interactions between fauna and sediment
439   control the breakdown of plant matter in river sediments. *Aquatic Sciences*, 75, 493–
440   507.

441   Dole-Olivier, M. J. (2011). The hyporheic refuge hypothesis reconsidered: a review of
442   hydrological aspects. *Marine and Freshwater Research*, 62, 1281–1302.

443   Dole-Olivier, M. J., Marmonier, P., & Beffy, J. L. (1997). Response of invertebrates to lotic
444   disturbance: is the hyporheic zone a patch refugium? *Freshwater Biology*, 37, 257–276.

445   Duan, X., Wang, Z., & Tian, S. (2008). Effect of streambed substrate on macroinvertebrate
446   biodiversity. *Frontiers of Environmental Science & Engineering in China*, 2, 122–128.

447   Extence, C. A. (1981). The effect of drought on benthic invertebrate communities in a
448   lowland river. *Hydrobiologia*, 83, 217–224.

449   Eyre, M. D., Pilkington, J. G., McBlane, R. P., & Rushton, S. P. (2005). Macroinvertebrate
450   species and assemblages in the headwater streams of the River Tyne, northern England

451   in relation to land cover and other environmental variables. *Hydrobiologia*, 544, 229–
452   240.

453   Feminella, J. W. (1996). Comparison of benthic macroinvertebrate assemblages in small
454   streams along a gradient of flow permanence. *Journal of the North American*
455   *Benthological Society*, 15, 651–669.

456   Fenoglio, S., Bo, T., & Bosi, G. (2006). Deep interstitial habitat as a refuge for *Agabus*
457   *paludosus* (Fabricus) (Coleoptera: Dytiscidae) during summer droughts. *The*
458   *Coleopterists Bulletin*, 60, 37–41.

459   Gayraud, S., & Philippe, M. (2001). Does subsurface interstitial space influence general
460   features and morphological traits of the benthic macroinvertebrate community in
461   streams? *Archiv für Hydrobiologie*, 151, 667–686.

462   Gayraud, S., & Philippe, M. (2003). Influence of bed-sediment features on the interstitial
463   habitat available for macroinvertebrates in 15 French streams. *International Review of*
464   *Hydrobiology*, 88, 77–93.

465   Hose, G. C., Jones, P., & Lim, R. P. (2005). Hyporheic macroinvertebrates in riffle and pool
466   areas of temporary streams in south eastern Australia. *Hydrobiologia*, 532, 81–90.

467   Hughes, A. O. (2016). Riparian management and stream bank erosion in New Zealand. *New*
468   *Zealand Journal of Marine and Freshwater Research*, 50, 277–290.

469   Imhof, J. G. A., & Harrison, A. D. (1981). Survival of *Diplectrona modesta* Banks
470   (Trichoptera: Hydropsychidae) during short periods of desiccation. *Hydrobiologia*, 77,
471   61-63.

472   James, A. B. W., Dewson, Z. S., & Death, R. G. (2008). Do stream macroinvertebrates use
473   instream refugia in response to severe short-term flow reduction in New Zealand
474   streams? *Freshwater Biology*, 53, 1316–1334.

475   Jones, I., Growns, I., Arnold, A., McCall, S., & Bowes, M. (2015). The effects of increased
476   flow and fine sediment on hyporheic invertebrates and nutrients in stream mesocosms.
477   *Freshwater Biology*, 60, 813–826.

478   Ledger, M. E., & Milner, A. M. (2015). Extreme events in running waters. *Freshwater*
479   *Biology*, 60, 2455–2460.

480 Leigh, C. (2013). Dry season changes in macroinvertebrate assemblages of highly seasonal
481   rivers: responses to low flow, no flow and antecedent hydrology. *Hydrobiologia*, 703,
482   95–112.

483 Leigh, C. & Datry, T. (2016). Drying as a primary hydrological determinant of biodiversity in
484   river systems: a broad-scale analysis. *Ecography*, 39, 1–13.

485 Leigh, C., Bonada, N., Boulton, A. J., Hugueny, B., Larned, S. T., Vander Vorste R., & Datry,
486   T. (2016). Invertebrate assemblage responses and the dual roles of resistance and
487   resilience to drying in intermittent rivers. *Aquatic Sciences*, 78, 291–301.

488 Lytle, D. A., & Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends in Ecology*
489   *and Evolution*, 19, 94–100.

490 Magoulick, D. D. (2014). Impacts of drought and crayfish invasion on stream ecosystem
491   structure and function. *River Research and Applications*, 30, 1309–1317.

492 Malard, F., Tockner, K., Dole-Olivier, M. J., &Ward, J. V. (2002). A landscape perspective
493   of surface-subsurface hydrological exchanges in river corridors. *Freshwater Biology*,
494   47, 621–640.

495 Mathers, K. L., & Wood, P. J. (2016). Fine sediment deposition and interstitial flow effects
496   on macroinvertebrate community composition within riffle heads and tails.
497   *Hydrobiologia*, 776, 147–160.

498 Mathers, K. L. Millett, J., Robertson, A. L., Stubbington, R., & Wood, P. J. (2014). Faunal
499   response to benthic and hyporheic sedimentation varies with direction of vertical
500   hydrological exchange. *Freshwater Biology*, 59, 2278–2289.

501 McKnight, D. M., Niyogi, D. K., Alger, A. S., Bomblies, A., Conovitz, P. A,. & Tate, C. M.
502   (1999). Dry valley streams in Antarctica: ecosystems waiting for water. *BioScience*, 49,
503   985–995.

504 Mermillod-Blondin, F., Winiarski, T., Foulquier, A., Perrissin, A., & Marmonier, P. (2015).
505   Links between sediment structures and ecological processes in the hyporheic zone:
506   ground-penetrating radar as a non-invasive tool to detect subsurface biologically active
507   zones. *Ecohydrology*, 8, 626–641.

508 Meyer, E. I., Niepagenkemper, O., Molls, F., & Spänhoff, B. (2008). An experimental
509   assessment of the effectiveness of gravel cleaning operations in improving hyporheic
510   water quality in potential salmonid spawning areas. *River Research and Applications*,
511   24, 119–131.

512 Navel, S., Mermillod-Blondin, F., Montuelle, B., Chauvet, E., Simon, L., Piscart, C., &
513   Marmonier, P. (2010). Interactions between fauna and sediment control the breakdown
514   of plant matter in river sediments. *Freshwater Biology*, 55, 753–766.

515 Nogaro G., Mermillod-Blondin F., François-Carcaillet F., Gaudet J.P., LaFont M. & Gibert J.
516   (2006) Invertebrate bioturbation can reduce the clogging of sediment: an experimental
517   study using infiltration sediment columns. *Freshwater Biology*, **51**, 1458–1473.

518 Olsen, D. A., & Townsend, C. R. (2005). Flood effects on invertebrates, sediment and
519   particulate organic matter in the hyporheic zone of a gravel-bed stream. *Freshwater
520   Biology*, 50, 839–853.

521 Pyne, M. I., & Poff, N. L. (2017). Vulnerability of stream community composition and
522   function of projected thermal warming and hydrologic change across ecoregions in the
523   western United States. *Global Change Biology*, 23, 77–93.

524 Richards, C., & Bacon, K. L. (1994). Influence of fine sediment on macroinvertebrate
525   colonization of surface and hyporheic stream substrates. *Great Basin Naturalist*, 54,
526   106–113.

527 Rüegg, J., & Robinson, C. T. (2004). Comparison of macroinvertebrate assemblages of
528   permanent and temporary streams in an Alpine flood plain, Switzerland. *Archiv für
529   Hydrobiologie*, 161, 489–510.

530 Smith, H., & Wood, P. J. (2002). Flow permanence and macroinvertebrate community
531   variability in limestone spring systems. *Hydrobiologia*, 487, 45–58.

532 Smock, L. A. (1980). Relationships between body size and biomass of aquatic insects.
533   *Freshwater Biology*, 10, 375–383.

534 Smock, L. A., Smith, L. C., Jones, J. B., & Hooper, S. M. (1994). Effects of drought and a
535   hurricane on a coastal headwater stream. *Archiv für Hydrobiologie*, 131, 25–38.

536   Stanley, E. H., Buschmann, D. L., Boulton, A. J., Grimm, N. B., & Fisher, S. G. (1994).
537   Invertebrate resistance and resilience to intermittency in a desert stream. *American*
538   *Midland Naturalist*, 131, 288–300.

539   Stanley, E. H., Fisher, S. G., & Grimm, N. B. (1997). Ecosystem expansion and contraction
540   in streams. *Bioscience*, 47, 427–425.

541   Steward, A. L., von Schiller, D., Tockner, K., Marshall, J.C., & Bunn, S. E. (2012). When the
542   river runs dry: human and ecological values of dry riverbeds. *Frontiers in Ecology and*
543   *the Environment*, 10, 202–209.

544   Stubbington, R. (2012). The hyporheic zone as an invertebrate refuge: a review of variability
545   in space, time, taxa and behaviour. *Marine and Freshwater Research*, 63, 293–311.

546   Stubbington, R., Boulton, A. J., Little, S., & Wood, P. J. (2015). Changes in invertebrate
547   assemblage composition in benthic and hyporheic zones during a severe supraseasonal
548   drought. *Freshwater Science*, 34, 344–354.

549   Stubbington, R., Greenwood, A. M., Wood, P. J., Armitage, P. D., Gunn, J., & Robertson, A.
550   L. (2009). The response of perennial and temporary headwater stream invertebrate
551   communities to hydrological extremes. *Hydrobiologia*, 630, 299–312.

552   Stubbington, R., Gunn, J., Little, S., Worrall, T. P., & Wood, P. J. (2016). Macroinvertebrate
553   seedbank composition in relation to antecedent duration of drying and multiple wet-dry
554   cycles in a temporary stream. *Freshwater Biology*, 61, 1293–1307.

555   Stubbington, R., Wood, P. J., & Reid, I. (2011). Spatial variability in the hyporheic zone
556   refugium of temporary streams. *Aquatic Sciences*, 73, 499–511.

557   Stumpp, C., & Hose, G. C. (2013). The impact of water table drawdown and drying on
558   subterranean aquatic fauna in in-vitro experiments. *PLoS ONE*, 8, e78502.

559   Tachet, H., Bournaud, M., Richoux, P., & Usseglio-Polatera, P. (2010). *Invertébrés d'eau*
560   *douce: systématique, biologie, écologie* Paris: CNRS Editions.

561   Vadher, A. N., Stubbington, R., & Wood, P. J. (2015). Fine sediment reduces vertical
562   migrations of *Gammarus pulex* (Crustacea: Amphipoda) in response to surface water
563   loss. *Hydrobiologia*, 753, 61–71.

23

564 Vander Vorste, R., Corti, R., Sagouis, A., & Datry, T. (2016c). Invertebrate communities in
565   gravel-bed, braided rivers are highly resilient to flow intermittence. *Freshwater Science*,
566   35, 164–177.

567 Vander Vorste, R., Malard, F., & Datry, T. (2016b). Is drift the primary process promoting
568   the resilience of river invertebrate communities? A manipulative field experiment in an
569   intermittent alluvial river. *Freshwater Biology*, 61, 1276–1292.

570 Vander Vorste, R., Mermillod-Blondin, F., Hervant, F., Mons, R., Forcellini, M., & Datry, T.
571   (2016a). Increased depth to the water table during river drying decreases the resilience
572   of *Gammarus pulex* and alters ecosystem function. *Ecohydrology*, 9, 1177–1186.

573 Verstraeten, G., & Poesen J. (2000). Estimating trap efficiency of small reservoirs and ponds:
574   methods and implications for the assessment of sediment yield. *Progress in Physical
575   Geography*, 24, 219–251.

576 Westwood, C. G., Teeuw, R. M., Wade, P. M., Holmes, N. T. H., & Guyard, P. (2006).
577   Influences of environmental conditions on macrophyte communities in drought-
578   affected headwater streams. *River Research and Applications*, 22, 703–726.

579 Williams, D. D., & Hynes, H. B. N. (1974). The occurrence of benthos deep in the
580   substratum of a stream. *Freshwater Biology*, 4, 233–256.

581 Williams, D. D., & Hynes, H. B. N. (1976). The ecology of temporary streams I. The faunas
582   of two Canadian streams. *International Review of Hydrobiology*, 61, 761–787.

583 Wood, P. J., Boulton, A. J., Little, S., & Stubbington, R. (2010). Is the hyporheic zone a
584   refugium for aquatic macroinvertebrates during severe low flow conditions?
585   *Fundamental and Applied Limnology*, 176, 377–390.

586 Wood, P. J., Gunn, J., Smith, H., & Abas-Kutty, A. (2005a). Flow permanence and
587   macroinvertebrate community diversity within groundwater dominated headwater
588   streams and springs. *Hydrobiologia*, 545, 55–64.

589 Wood, P. J., Toone, J., Greenwood, M. T., & Armitage, P. D. (2005b). The response of four
590   benthic macroinvertebrate taxa to burial by sediments. *Archiv für Hydrobiologie*, 163,
591   145–162.

592   Young, B. A., Norris, R. H., & Sheldon, F. (2011). Is the hyporheic zone a refuge for

593   macroinvertebrates in drying perennial streams? *Marine and Freshwater Research*, 62,

594   1373–1382.

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620 **Fig. 1. Sediment column mesocosms.** (a) Cross-section through a sediment column: i)

621 acrylic pipe; ii) rubber bung; iii) 5 mm glass tube; iv) silicon tubing; v) Hoffman clip to

622 control water drainage. (b) The six sediment treatments at the start of experiments (5 cm

623 surface water). Sediment treatments are as described in Table 2. Not to scale.

624 **Fig. 2. Mean vertical movement of live individuals in response to water level reduction**

625 **in each of six sediment treatments.** (a) *Nemoura cambrica*; (b) *Gammarus pulex*; (c)

626 *Asellus aquaticus*; (d) *Hydropsyche siltalai*; (e) *Heptagenia sulphurea*. Sediment treatments

627 are as described in Table 2.

628 **Fig. 3. Percentage of live individuals stranded (a) in each sediment treatment and (b) by**

629 **species.** a-d indicate statistically different values (Binary Logistic Regression, $P < 0.05$).

630 Sediment treatments are as described in Table 2.

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641   **Table 1.** Family-level biological traits of the five study taxa relevant to subsurface movement

642   and ecological traits (adapted from Tachet et al., 2010)

Genus	Biological Traits						
	Max. body size (mm)	Locomotion	Habitat type	Flow velocity	Feeding group	Temporary stream affinity	Subsurface affinity
Nemoura	5 – 10	Crawler	Lotic	Medium – fast	Shredder	Moderate	None
Asellus	10 – 20	Crawler	Lentic / Lotic	None – slow	Shredder	None	Low
Gammarus	20 – 40	Swimmer / Crawler	Lotic	Slow - medium	Shredder	Low	Low
Hydropsyche	20 – 40	Crawler	Lotic	Medium	Filter-feeder	None	None
Heptagenia	10 – 20	Crawler	Lotic	Medium – fast	Scraper	None	None

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654 **Table 2.** Description of sediment treatment composition, pore-size volume and the mean

655 depth (± SE) which macroinvertebrates reached at the end of experiments

Sediment treatment	Particle size composition	Pore-size volume (ml)	Mean species depth at experiment end (mm)
1	100% small angular particles	145.6 ± 1.29	129.3 ± 10.5
2	60% small angular and 40% large angular particles	150 ± 0.63	159.5 ± 10.9
3	50% small angular and 50% large angular particles	151.6 ± 1.21	160.2 ± 10.5
4	33% small angular, 33% large angular and 33% large smooth particles	155.2 ± 1.46	173.8 ± 10.3
5	100% large smooth particles	158.6 ± 1.08	195 ± 8.9
6	100% large angular particles	186.4 ± 1.57	216.5 ± 6.8

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Table 3. Paired sample t-test analysis between the absolute depth moved by live individuals and cadavers for each species and within each sediment treatment (see Table 2).

|	Live	Cadaver			
	Mean absolute depth moved (mm)	Mean absolute depth moved (mm)	d.f.	t	*P*
Species					
Nemoura cambrica	158.7 (± 8.2)	1 (± 0.3)	119	19.139	<0.001
Asellus aquaticus	150 (± 8.5)	4 (±1.3)	119	17.038	<0.001
Gammarus pulex	131.7 (± 8.7)	1.8 (± 0.5)	119	14.719	<0.001
Hydropsyche siltalai	62.8 (± 6.3)	2.3 (± 0.5)	119	9.623	<0.001
Heptagenia sulphurea	39.4 (± 5)	1.6 (± 0.4)	119	7.606	<0.001
Sediment treatment					
1	105 (± 9.5)	0.2 (± 0.1)	119	10.999	<0.001
2	130.4 (± 9.9)	1 (± 0.4)	119	12.954	<0.001
3	106.6 (± 9.6)	0.3 (± 0.1)	119	11.094	<0.001
4	118 (± 9.9)	0.6 (± 0.2)	119	11.895	<0.001
5	96.2 (± 9.3)	4 (± 0.7)	119	9.959	<0.001
6	96.1 (± 8.6)	6.8 (± 1.6)	119	10.374	<0.001

670 **Table 4.** Percentage of individuals stranded above the water level during dewatering in each

671 sediment treatment (see Table 2)

Species	% Stranded in sediment treatments						
	1	2	3	4	5	6	All treatments
Nemoura cambrica	0	0	0	0	0	0	0
Asellus aquaticus	55	10	5	0	0	0	12
Gammarus pulex	70	30	40	0	0	0	23
Hydropsyche siltalai	80	75	80	95	55	45	72
Heptagenia sulphurea	100	100	100	95	95	60	92
All species	61	43	45	39	30	21	

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674   Figure 1
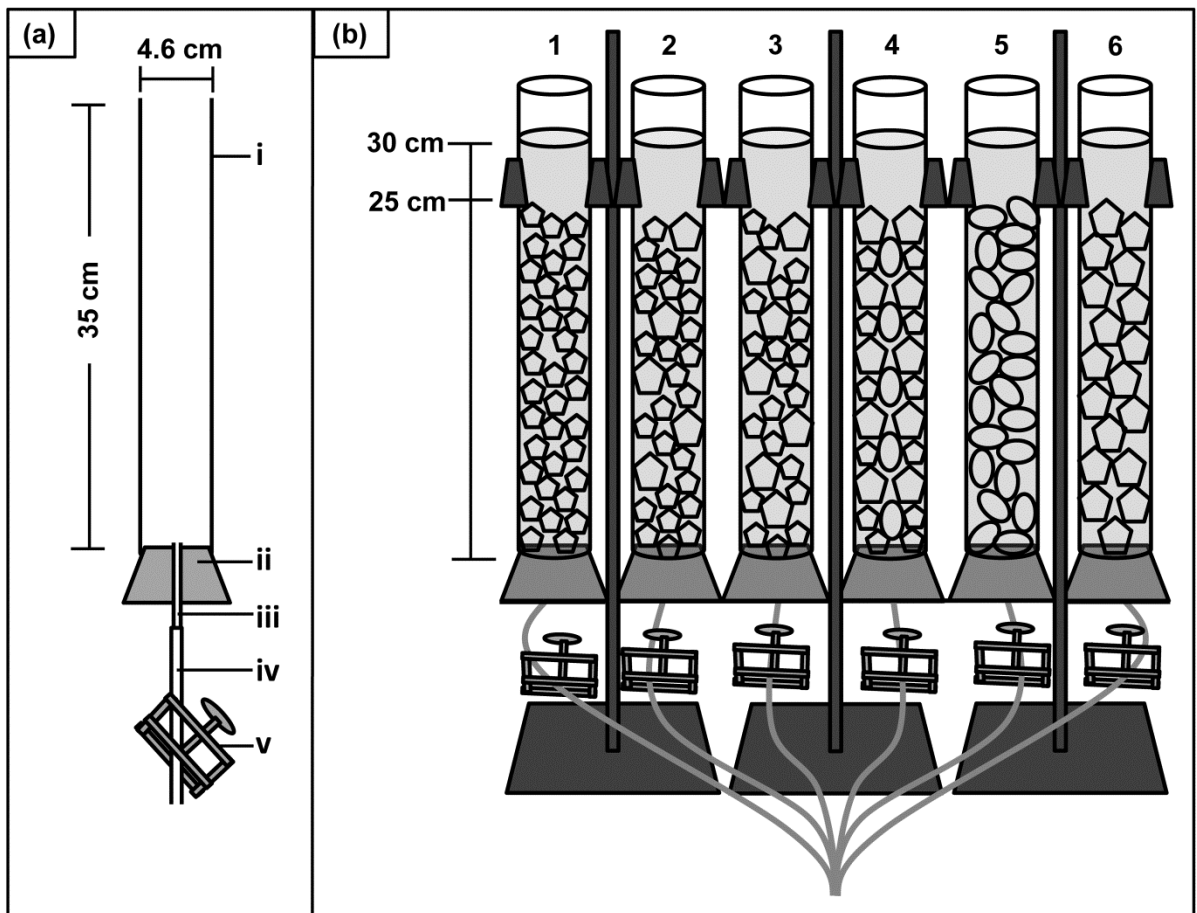
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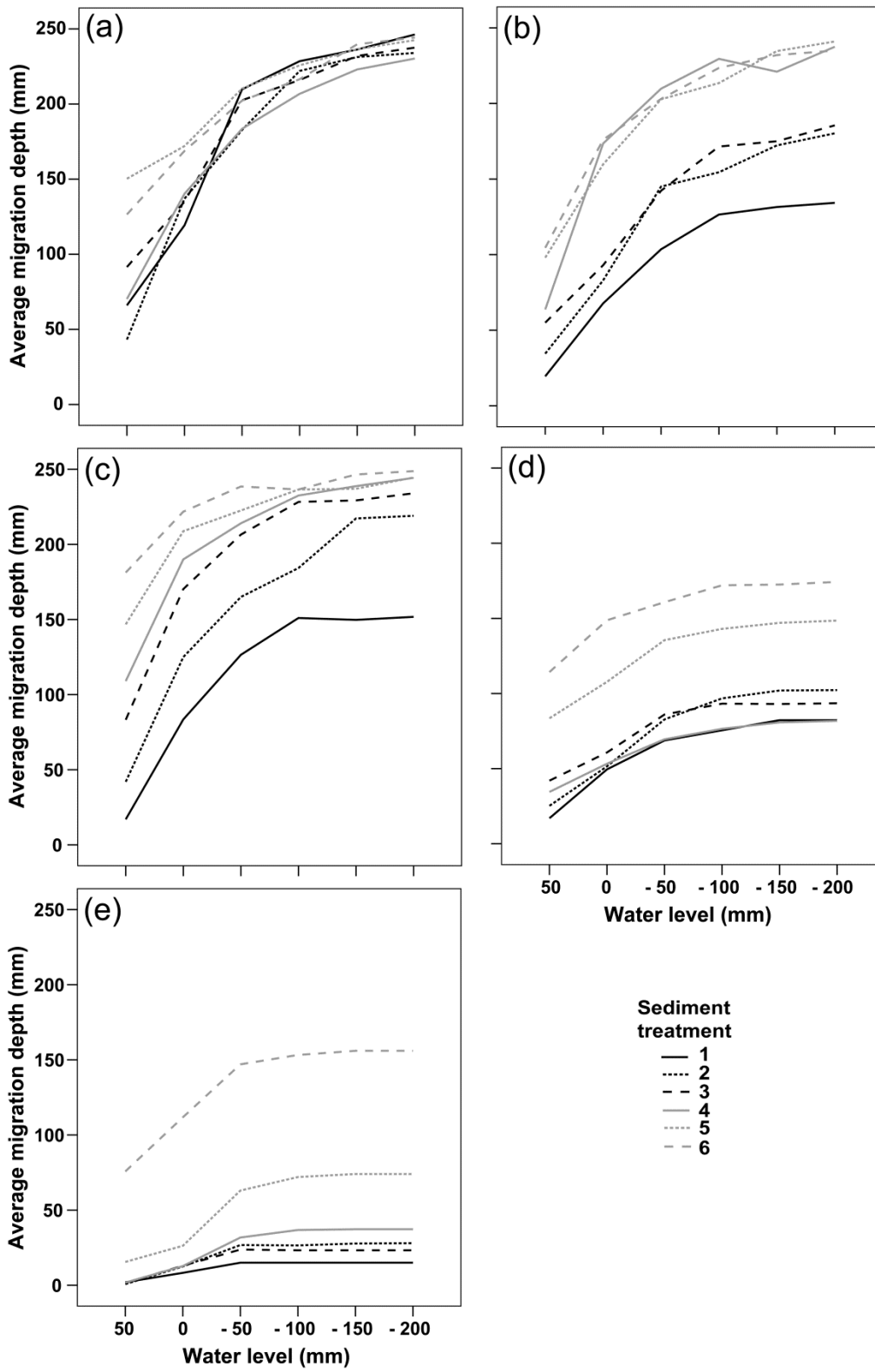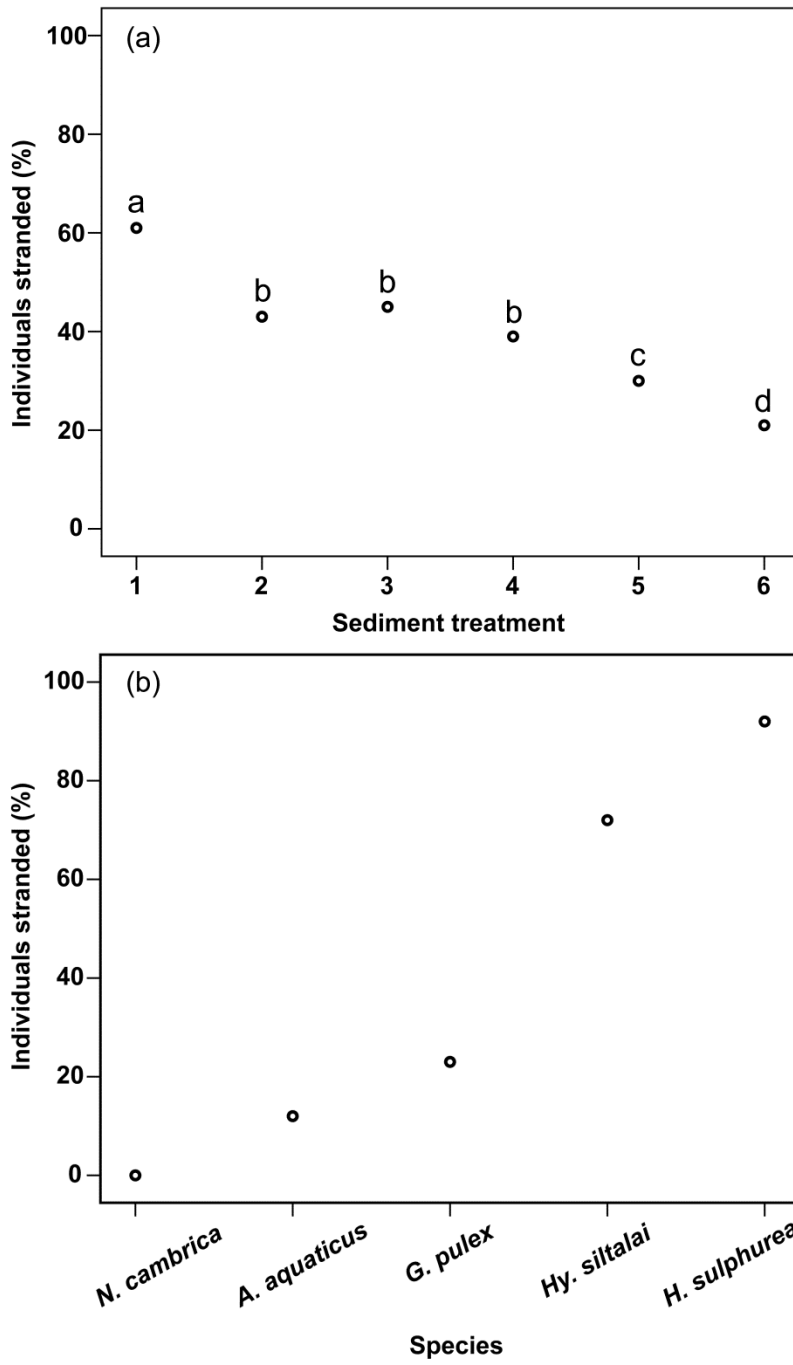
690   Figure 3

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695 **Table S1.** Fisher's LSD post-hoc pairwise comparison of absolute depth moved by live

696 individuals between each species (*Nemoura cambrica*, *Gammarus pulex*, *Asellus aquaticus*,

697 *Hydropsyche siltalai* and *Heptagenia sulphurea*) for each sediment treatment. Significant

698 depths ($P \leq 0.05$) are emboldened. '↑' indicates the taxon listed is significantly higher than

699 the taxa being compared. Sediment treatment 1) 100% small angular 2) 60% small angular 40%

700 large angular 3) 50% small angular 50% large angular 4) 33% small angular, 33% large

701 angular and 33% large rounded 5) 100% large rounded 6) 100% large angular.

Sediment Treatment 1	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.148	↑ **0.016**	↑ **0.050**	↑ **0.009**
G. pulex		0.073	0.261	↑ **0.034**
A. aquaticus			0.256	0.835
Hy. siltalai				0.238

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Sediment Treatment 2	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.197	↑ **0.028**	↑ **0.042**	↑ **0.007**
G. pulex		0.093	0.135	0.016
A. aquaticus			0.430	0.948
Hy. siltalai				0.267

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Sediment Treatment 3	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.446	↑ **0.043**	↑ **0.070**	↑ **0.025**
G. pulex		0.060	↑ **0.050**	↑ **0.019**
A. aquaticus			0.409	0.873
Hy. siltalai				0.388

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Sediment Treatment 4	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.637	↑ **0.019**	↑ **0.032**	↑ **0.021**
G. pulex		↑ **0.011**	↑ **0.003**	↑ **0.006**
A. aquaticus			0.370	0.670
Hy. siltalai				0.581

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Sediment Treatment 5	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.876	0.051	0.369	0.244
G. pulex		↑ **0.010**	0.066	0.064
A. aquaticus			0.121	0.298
Hy. siltalai				0.612

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Sediment Treatment 6	*G. pulex*	*A. aquaticus*	*Hy. siltalai*	*H. sulphurea*
N. cambrica	0.800	↑ **0.013**	0.203	0.236
G. pulex		↑ **0.004**	0.069	0.164
A. aquaticus			0.061	0.112
Hy. siltalai				0.910

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