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2 ***The role of fine sediment characteristics and body size on the vertical***
3 ***movement of a freshwater amphipod***

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33 **Running title: Sediment deposition and hyporheic body size**

34 **The role of fine sediment characteristics and body size on the vertical**
35 **movement of a freshwater amphipod**

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38 **Abstract**

- 39 1. Sedimentation and clogging (colmation) of interstitial pore spaces with fine
40 sediment particles is widely considered to be one of the most significant
41 threats to lotic ecosystem functioning. This paper presents the results of a
42 running water mesocosm study examining the effect of benthic and hyporheic
43 fine sediment loading and particle size on the vertical movement and
44 distribution of the freshwater amphipod *Gammarus pulex*.
- 45 2. A gradient of fine sediment loading and different particle sizes were used to
46 examine the ability of *G. pulex* from two body size classes to access and
47 migrate vertically within subsurface sediments.
- 48 3. We tested three hypotheses: i) sediment loading would modify the distribution
49 of *G. pulex* by limiting vertical movement; ii) the deposition of large particles
50 and heterogenous sediments would limit the vertical movement of individuals
51 more than homogeneous fine grained sediments; and iii) large bodied
52 individuals would be prevented from migrating vertically with increasing
53 sediment loading and particle size / heterogeneity.
- 54 4. Sediment loading, particle size and heterogeneity of deposited sediment had
55 a significant effect on the vertical movement of individuals, with
56 heterogeneous sand (0.125 - 4 mm) acting as the strongest barrier to the
57 vertical movement of individuals through the infilling and clogging of interstitial
58 spaces followed by coarse (1 - 4mm) and fine sand (0.125 - 4 mm).
- 59 5. Fine sediment loading and particle size acted as a filter on body size and
60 limited the ability of large bodied individuals to migrate vertically to a greater
61 extent than small bodied individuals.
- 62 6. This study demonstrates that the effects of fine sediment on habitat
63 availability and faunal movement is dependent on both sedimentological
64 characteristics and an individual's body size. The results illustrate the
65 importance of both abiotic and biotic factors when evaluating the ecological
66 effects of fine sediment deposition.

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69 **Keywords:** colmation, particle size, substrate composition, hyporheic zone,
70 invertebrate.

71

72 **Introduction**

73 Fine sediment transport and deposition is a natural component of healthy river
74 systems, but in many regions across the globe fine sediment inputs have been
75 increasing and are now far in excess of historic background levels (Foster et al.,
76 2011; Collins and Zhang, 2016). In excessive quantities, fine sediment (defined here
77 as particles $\leq 4\text{mm}$; Sear 1993) is widely recognised to be a major contributor of
78 ecosystem degradation, modifying biological, geomorphological and hydrological
79 processes (Wood and Armitage, 1997; Church, 2002; Stewardson et al., 2016).
80 Substrates comprising high fine sediment content typically have reduced porosity
81 and hydraulic permeability, limiting the exchange of nutrients and oxygen between
82 the surface and subsurface sediments (Bo et al., 2007; Datry et al., 2015; Hartwig
83 and Borchardt, 2015). The infiltration of fine sediments into the river bed, commonly
84 referred to as 'clogging' (Blaschke et al., 2003) or 'colmation' (Wharton et al., 2017),
85 can also lead to a disconnection of surface substrates from the subsurface hyporheic
86 zone, reducing the availability of interstitial habitat (Brunke, 1999; Descloux et al.,
87 2013; Mathers et al., 2014).

88 The sediments immediately below the active riverbed and the hyporheic zone are
89 widely recognised as integral to lotic ecosystem functioning (Stanford and Ward,
90 1988; Bo et al., 2007; Krause et al., 2011), serving as the ecotone between surface
91 and groundwater ecosystems (Hancock, 2002) and frequently acting as a refugium
92 for benthic fauna during adverse conditions in surface water habitats (Dole-Olivier,
93 Marmonier and Buffy, 1997; Wood et al., 2010; Maazouzi et al., 2017). Typically, the
94 hyporheic zone is limited in spatial extent to around a metre vertically below the
95 riverbed (Williams and Hynes, 1974), although some lotic fauna have been recorded
96 up to 10m below the riverbed and several kilometres laterally (Stanford and Ward,
97 1988).

98 Fine sediment deposition and infiltration into riverbeds can lead to habitat
99 homogenisation, altering both the structure and function of instream communities
100 (Kaller and Hartman, 2004; Descloux et al., 2013; Jones et al., 2015; Doretto et al.,
101 2017). Substrates dominated by fine sediment typically support a greater proportion
102 of taxa tolerant of low dissolved oxygen concentrations and that are capable of
103 burrowing into the substrate (Rabeni et al., 2005; Cover et al., 2008). Moreover, the
104 reduction of interstitial space may preclude large bodied organisms from accessing
105 subsurface hyporheic sediments (Boulton, 2007). There have been a limited number
106 of field-based studies which have investigated the role of body size on the
107 distribution of macroinvertebrates. Those which have considered body size have
108 reported a reduction in the maximum body size of organisms within substrates
109 dominated by fine sediment (Buendia et al., 2013; Descloux et al., 2014; Mathers,
110 Rice & Wood, 2017). However, direct evidence demonstrating and quantifying the
111 role of body size on the ability of taxa to access subsurface substrates under varying
112 fine sediment loads is lacking.

113 The extent of fine sediment clogging is dependent on a number of hydraulic and
114 sedimentological parameters (Dudill et al., 2017). The direction and strength of
115 hydrological exchange exerts a strong control over sediment ingress, with upwelling
116 water limiting the infiltration of fine sediments and where sufficiently strong it can
117 flush and clear interstitial pore spaces of fine sediment (Huettel et al., 1996; Ren and
118 Packman, 2007). In contrast, downwelling water typically transports fine sediment
119 and associated nutrients into the hyporheic zone, facilitating the process of clogging
120 (Boulton, 1993; Mathers, Hill and Wood, 2017). Lateral hydraulic exchange may also
121 be an important pathway for fine sediment transport within subsurface sediments of
122 gravel-bed rivers (Pettricrew et al., 2007; Mathers and Wood, 2016; Harper et al.,
123 2017; Casas-Mulet et al., 2017).

124 The sedimentological characteristics of both the coarse grained structural framework
125 (Frostick et al., 1984) and the fine sediment matrix (Franssen et al., 2014) of a gravel
126 deposit strongly influences the availability of pore spaces and the potential for fine
127 sediment infiltration. The ratio between the diameter of coarse particles (forming the
128 framework) and the infiltrating fine sediment (termed the grain size ratio) has been
129 the focus of experimental work, in an effort to understand and quantify infiltration
130 processes (e.g., Frings et al., 2008; Gibson et al., 2009a, 2009b; Herrero and Berni,

131 2016). In riverbeds where interstitial space permits, fine sediment typically infiltrates
132 unhindered with pore spaces being filled from the base of the deposit upwards
133 (Diplas and Parker, 1992), termed unimpeded static percolation. In gravel beds
134 where this infiltration process dominates, interstitial space may be maintained in the
135 presence of flushing flows. Larger grains in contrast, may be too large to infiltrate or
136 pass through interstitial spaces and may become lodged in the gravel pore opening,
137 impeding subsequent infiltration of fine sediment in a process termed bridging
138 (Beschta and Jackson, 1979). This process can be ecologically significant as it may
139 prohibit the transfer of resources below the clog and reduce the movement of
140 organisms within and between interstitial spaces. Despite the potential ecological
141 significance of characterising the dominant infiltration process occurring during
142 sediment loading, relatively few studies to date have considered the distribution of
143 infiltrating sediments when evaluating the effect of sedimentation in the field due to
144 the inability to make direct observations. Consequently, a mechanistic understanding
145 of the implications of fine sediment deposition as a function of the particle grain size
146 for biota is limited.

147 In this paper, we examine the response of the freshwater amphipod, *Gammarus*
148 *pulex* (L.) (Amphipoda: Crustacea) to different fine sediment loadings and particle
149 sizes using *ex-situ* running water mesocosms specifically designed to allow the
150 vertical location and distribution of individuals to be determined. The influence of
151 body size on the ability of amphipods to access subsurface sediments was also
152 examined. Amphipod crustacea occur widely in benthic, hyporheic and subterranean
153 aquifers and caves, often dominating the biomass where they occur (MacNeil et al.,
154 1997; Wood et al., 2010; Johns et al., 2015). *G. pulex* is rheophilic with a fully
155 aquatic life history resulting in both adult and juveniles of different sizes being
156 present within waterbodies throughout the year (Gledhill et al., 1993). *G. pulex* is
157 moderately sensitive to sedimentation but is capable of burrowing through fine
158 sediment deposits (Mathers et al., 2014), therefore making it an ideal model
159 organism to examine the effect of fine sediment loading on its vertical movement
160 patterns. We hypothesised that increasing sediment loading, particle size and body
161 size of *G. pulex*, would influence the vertical distribution of individuals within the
162 mesocosms. Specifically, we predicted that: 1) increasing levels of sediment loading
163 would modify the vertical distribution of *G. pulex* within the experimental columns by

164 limiting and / or preventing their movement to greater depths; 2) increasing particle
165 size and heterogeneity of fine sediment would enhance the process of interstitial
166 clogging and limit movement to greater depths and; 3) greater body size would limit
167 an individual's ability to access deeper substrate layers under increasing sediment
168 loading and particle size / heterogeneity.

169 **Methods**

170 *Experimental sediment columns*

171 Experiments were undertaken within two identical sediment columns comprising five
172 interlocking sections as outlined in Mathers et al., (2014) (Figure 1 – sections A-E).
173 Sections were 22 cm in diameter and contained 50 mm depth of coarse riverine
174 sediments (gravel particles 20-64 mm in diameter). Each section was stacked
175 vertically to provide a total sediment column depth of 250 mm. Ten holes (10 mm
176 diameter) in the base of the top four sections (0 - 200 mm depth) permitted the
177 transfer of water and organisms between sections. The final section (200-250 mm
178 depth) was perforated with smaller holes (2 mm diameter) to allow the vertical
179 exchange of water but prevent the movement of individuals outside of the
180 experimental column. In addition, 0.25 mm mesh sieves were placed over the base
181 and the top of the sediment columns for the duration of each experiment, and a 5
182 mm rubber seal was created around the base of each section to prevent the
183 migration of individuals outside the column.

184 The sediment columns were placed inside separate large cylindrical black plastic
185 water containers (90 x 40 cm, volume = 100 L). Two external pumps delivered
186 flowing water to the columns (4.5-4.8 L min⁻¹) at a rate which was sufficient to
187 maintain interstitial exchange through the sediments but which did not initiate
188 sediment transport and consequently, any vertical movement of fine sediment during
189 the experimental period was primarily a function of gravity or the direct activity of *G.*
190 *pulex* movement. Experiments were conducted under upwelling hydraulic exchange
191 conditions. Previous experiments have demonstrated the affinity of *G. pulex* for
192 subsurface substrates under upwelling hydrological exchange and for surface
193 substrates under downwelling conditions (Mathers et al., 2014), reflecting their
194 rheophilic nature. The use of upwelling flow conditions in the absence of any fine
195 sediment therefore provided a baseline distribution pattern of *G. pulex* for the

196 experiments and allowed the effect of fine sediment clogging to be detected as a
197 reduction in the number of individuals located beneath fine sediment treatments.

198 To simulate upwelling hydraulic conditions, water was pumped through a 200 mm
199 funnel / diffuser on which the base of the experimental column was placed. Water
200 rose through the column and overflowed from the top section of the column. Each
201 experiment was conducted with a minimum of 10 cm water depth over the substrate
202 and experimental containers were aerated via an aquaria pump and held at a
203 constant water temperature (15°C +/- 0.4°C) by an external water-cooler (Aqua
204 medic, Titan 150).

205 Three particle size treatments of pre-washed fine sediment were used in the
206 experiments; i) fine sand (0.125 - 1 mm in diameter), ii) coarse sand (1 - 4 mm) and
207 iii) heterogeneous sand which consisted of a 50 / 50 mixture of the coarse and fine
208 sands (0.125 – 4 mm). The two size fractions were selected to include grains with a
209 low propensity to clog interstitial spaces (0.125 – 1 mm) and grains with a high
210 propensity to bridge between framework clasts thereby preventing further infiltration
211 (1 – 4 mm). These particular grain sizes were determined using calculations based
212 on studies by Gibson et al., (2009b) and Frings et al., (2008) who provide ratios to
213 discriminate between pore filling loads and bed structure loads. Silt and clay
214 fractions (<0.125 mm) were removed via wet sieving to ensure that turbidity did not
215 vary between experiments. Prior to each experiment, dry fine sediment was applied
216 evenly to the surface of each wet gravel section using a 4 mm sieve to enable fines
217 to infiltrate under gravity. The same clean gravel framework was retained within each
218 mesocosm layer throughout the experimental period for consistency.

219 Preliminary tests indicated that the application of an equivalent of 5 kg m⁻² of the fine
220 sand fraction (0.125 - 4 mm) filled all available interstices (100% of interstitial
221 volume) of each section and covered the surface of all gravel particles. In addition to
222 this heavy sediment loading which filled all interstitial spaces and covered all
223 particles in the section of the column, a moderate sediment loading of 3 kg m⁻² was
224 applied in other treatments. This treatment filled the interstitial spaces of the lower
225 half of the treated layer (when the 0.125 - 1 mm fraction was applied) but gravel
226 particles remained visible at the surface, thereby representing conditions comparable
227 to those observed in the field. Five sediment treatments were examined which were

228 adapted from Mathers et al., (2014): 1. Heavy surface (benthic) sedimentation: the
229 equivalent of 5 kg m⁻² fine sediment applied to the top section (section A); 2.
230 Subsurface (hyporheic) sedimentation of one section: the equivalent of 3 kg m⁻² fine
231 sediment applied to section C (100-150 mm depth); 3. Hyporheic sedimentation of
232 three sub-surface sections (simulating hyporheic clogging): the equivalent of 3 kg m⁻²
233 applied to sections B, C and D (50-100 mm, 100-150 mm and 150-200 mm); 4.
234 Benthic and subsurface-sedimentation (simulating benthic and hyporheic clogging) –
235 the equivalent of 3 kg m⁻² applied to all five layers (sections A, B, C, D and E); and
236 5. Control experiments (O – Figure 1) in which no fine sediment was applied and
237 which consisted of an open gravel framework: 50 mm depth of gravel in all sections
238 of the column. For all treatments, 50 mm gravel was retained in each section prior to
239 the fine sediment treatment (Figure 1).

240 The sediment treatments (4 applications and 1 control, n = 5) and sediment grain
241 sizes (n = 3) were combined in a full-factorial design giving 15 treatment
242 combinations and one set of control experiments. Each combination was replicated
243 six times to give a total of 90 individual experiments. Treatments were randomly
244 allocated to an experimental trial. All *G. pulex* specimens were collected from a local
245 stream (Burleigh Brook, Loughborough; 52°76'20"N., -1°24'18"W.) where they
246 occurred in high abundances. To assess the influence of body size on the ability of
247 an organism to utilize subsurface sediments, two body sizes classes were
248 distinguished i) < 2mm head width and ii) > 2mm head width. Individuals were placed
249 onto a 2 mm sieve allowing those small enough to pass through freely and thereby
250 separating the two size classes. Fifteen individuals from the two sizes classes (total
251 = 30 individuals) were released onto the top section of the prepared column (0-50
252 mm) and left for 24-hours to allow individuals to redistribute within the column. A
253 single pre-conditioned horse chestnut leaf (*Aesculus hippocastanum*) was placed in
254 each section for food (Joyce et al., 2007). At the end of each experiment (24-hours),
255 individuals were collected from each section by washing the contents of each section
256 through a nest of sieve sizes 4 – 0.125 mm. All fine sediments were removed from
257 the column and retained for use in subsequent experimental trials.

258 **Statistical analysis**

259 *Abundance*

260 Differences in the abundance of *G. pulex* in each section as a function of sediment
261 grain size and sediment loading were tested via a linear mixed effects model (LME)
262 using the *lme* function from the package nlme (Pinheiro et al., 2018). Section (n = 5),
263 sediment loading (n = 5) and sediment grain size (n = 3) were specified as fixed
264 effects and section was nested within the experimental replicate (column) as a
265 random factor (reflecting the fact that sections within individual columns were not
266 independent from each other). Models were fitted using the restricted maximum
267 likelihood (REML) estimation. Differences between sections within each sediment
268 combination were tested using a Tukey *post hoc* test using the *glht* function in the
269 multcomp package (Hothorn et al., 2008). *Post hoc* tests between the same section
270 for each sediment grain size and sediment loading are provided in supplementary
271 material (Tables S1-S3).

272 *Body size*

273 To assess the influence of body size on the ability of individuals to migrate vertically,
274 data was coded so that the abundance of large and small bodied individuals above
275 and below the section(s) in columns where the sediment treatment was applied
276 could be analysed separately. This allowed us to examine the effect of both
277 sediment grain size and sediment loading on the distribution of the two body size
278 classes. For example, for sediment loading in the third layer, location above the
279 sediment clog was calculated as the total abundance of individuals in sections 1-3 (0
280 – 150 mm), and sections 4-5 (150 - 250 mm) for below the sediment treatment.

281 Differences were statistically tested using a linear mixed effects model with a similar
282 structure to that employed in the abundance tests with location in column (n = 2),
283 sediment loading (n = 4), sediment grain size (n = 3) and body size (n = 2) specified
284 as fixed effects, and location nested within the experimental replicate (column) as a
285 random factor. To assess the influence of body size on the vertical distribution of
286 individuals as a function of sediment loading and sediment grain size, abundances
287 were converted to the proportion of individuals above and below the sediment
288 treatment for the respective size classes (small and large). An arcsine square root
289 transformation was applied to the data, and differences in the proportion of
290 individuals above the clog by sediment loading and sediment grain size were tested
291 using a Tukey *post hoc* test using the *glht* function in the multcomp package. These
292 tests were conducted for small and large bodied organisms separately and are

293 presented as supplementary material (Tables S3 & S4). All statistical analyses were
294 undertaken in the R environment (R Development Core Team, 2017).

295 **Results**

296 Recapture rates of amphipods for all experiments were high (average = 95.6%,
297 range = 90 – 100%) and did not differ significantly between experiments. The
298 distribution of *G. pulex* between the sediment layers was dependent on the sediment
299 loading, fine sediment particle size and the interaction of these factors (all $p < 0.001$;
300 Table 1). When amphipod body size was considered, the distribution of *G. pulex*
301 above and below the sediment clog was dependent on sediment loading, fine
302 sediment particle size, amphipod body size and the interaction of these factors
303 (Table 2). Consequently, patterns in the vertical distribution of *G. pulex* vary in
304 relation to sediment loading and sediment particle size (Tables S1 – S4).

305 *Control conditions*

306 Under upwelling conditions, the distribution of *G. pulex* was characterised by the
307 greatest number of individuals being recorded in the bottom section (Section E,
308 average 17 individuals, range = 19 - 16) and on average two individuals being
309 recorded in the surface section (range = 4 – 0; Figure 2).

310 *Faunal response to sedimentation under fine sand sediment conditions*

311 In fine sand experimental trials, the greatest number of individuals were recorded in
312 the bottom section (Section E; 200 – 250 mm) for the heavy surface sedimentation
313 (Figure 3a; average = 19, range = 22 - 16). Subsurface (hyporheic) sedimentation of
314 one layer (Section C; 150 – 200 mm) resulted in a less marked gradient of increasing
315 abundance by depth (Figure 3b) and subsurface sedimentation of three layers
316 (Sections B, C and D; 50 – 200 mm) resulted in no apparent differences in the
317 abundance of individuals amongst the sections of the column (Figure 3c).
318 Sedimentation of all layers resulted in a reversal of the distribution of individuals
319 (Figure 3d) compared to the open gravel framework (no sediment addition; Figure 2)
320 with greater numbers being recorded in the top layer (Section A; average = 15
321 individuals, range = 18 – 12).

322 *Faunal response to sedimentation under coarse sand sediment conditions*

323 Heavy surface sedimentation (Section A) under coarse sand conditions resulted in a
324 reversal of the distribution of individuals compared to the open gravel framework with

325 significantly greater numbers of *G. pulex* being recorded in the surface layer (Section
326 A, average = 21 individuals, range = 24 – 18; Figure 3a). Subsurface (hyporheic)
327 sedimentation of one layer (Section C) resulted in no statistical difference in the
328 abundance of *G. pulex* between the sections (Figure 3b). Subsurface sedimentation
329 of three layers (Sections B, C and D) also resulted in little variation in the distribution
330 of amphipods between the sections with the greatest number of *G. pulex* recorded
331 directly above the sediment treatment (Section B; average = 12 individuals, range =
332 14- 8; Figure 3c). Sedimentation of all sections resulted in the greatest number of
333 amphipods in the surface layer, with relatively few individuals being recorded in any
334 of the other four sections (Sections B – E; 7% of total abundance; Figure 3d).

335 *Faunal response to sedimentation under heterogeneous sand sediment conditions*
336 *(mixed of fine and coarse sand)*

337 Under heterogeneous sand conditions, the distribution of *G. pulex* was characterised
338 by greater numbers of individuals being recorded within the surface layer under all
339 sediment treatments (Figure 3). The gradient of amphipod distribution by depth was
340 however highly variable as a function of the sediment loading. Heavy surface
341 sedimentation (Section A) and sedimentation of all sections resulted in significantly
342 greater numbers of *G. pulex* being recorded in the surface layer (Figure 3a, d) with
343 only 4.6% and 7% of total amphipod abundance being recorded in the bottom four
344 layers for the two treatments respectively (Sections B – E). Subsurface (hyporheic)
345 sedimentation of one (Section C) and three layers (Sections B, C and D) resulted in
346 a decline in abundance with depth and was most marked under the three section
347 sedimentation treatment (Figure 3b, c).

348 *The influence of amphipod body size on the vertical distribution of G. pulex*

349 Increasing sediment loading and particle size / heterogeneity resulted in an
350 increasing proportion of amphipods being recorded above the deposited fine
351 sediment (Figure 4). This pattern was most marked for large bodied individuals with
352 a greater proportion of smaller individuals being able to migrate through the
353 sediment treatment in all instances. The homogeneous fine sand treatment resulted
354 in the greatest proportion of individuals migrating through the sediment treatment
355 whilst the heterogeneous mixed fraction had the lowest (Figure 4a). With the
356 exception of the fine sand treatment (0.125 – 1 mm) >90 % of large bodied
357 individuals were unable to migrate through the sediment treatment. In contrast, the

358 majority of small bodied individuals (> 50%) were able to migrate through the
359 homogeneous fine sand (0.125 – 1 mm) and coarse sand treatments (1 - 4 mm),
360 with the exception of the coarse sand treatment of all column layers (Sections A-E).
361 Heterogeneous fine sediment (0.125 – 4 mm) resulted in the greatest proportion of
362 large and small bodied individuals being recorded above the sediment treatment;
363 although a greater proportion of small bodied individuals were able to migrate
364 through the fine sediment treatment (Figure 4b). The number of large bodied
365 individuals recorded below the sediment clog, as a function of sediment loading,
366 demonstrated the largest variation for the fine sand treatment and was lowest for the
367 heterogeneous mixed fraction. For small bodied individuals, coarse sand deposition
368 conditions resulted in the greatest variation.

369 **Discussion**

370 Fine sediment deposition and clogging (colmation) of subsurface interstitial habitats
371 potentially plays a significant role in determining the distribution of benthic and
372 hyporheic invertebrates (Weigelhofer and Waringer, 2003; Mermillod-Blondin et al.,
373 2014), although empirical evidence has been limited thus far. This study specifically
374 sought to examine how varying particle size and sediment loading influenced the
375 vertical distribution of *G. pulex* under upwelling hydraulic exchange conditions. Due
376 to their widespread occurrence in benthic and subsurface habitats *G. pulex*, and
377 other amphipod crustacea, are model organisms to examine the influence of particle
378 size variations, fine sediment deposition and vertical movement patterns within
379 alluvial sediments. Within our experiment we also considered how the vertical
380 movement of individuals from different body size classes was affected by the
381 application of different particle sizes and loading.

382 During control experiments, with no fine sediment applied, the majority of *G. pulex*
383 individuals migrated to the deepest section of the column. This reflects the strong
384 rheophilic preferences of *G. pulex* (Gledhill et al., 1993; Mathers et al., 2014) and the
385 open gravel framework, which allowed the unimpeded flow of water and movement
386 of individuals within the subsurface under upwelling conditions. Hydrological
387 exchange exerts a strong influence over the distribution of macroinvertebrates
388 (Pepin and Hauer, 2002; Olsen and Townsend, 2003; Mathers, Hill and Wood,
389 2017), and the deposition of fine sediment (Boulton, 1993; Mathers and Wood,

390 2016). This was the focus of a previous investigation (Mathers et al., 2014) and so is
391 not considered further here.

392 Results from the current experiments provide evidence to support our first
393 hypothesis; that increasing fine sediment loading would limit the ability of individuals
394 to access deeper layers of the substrate. The most marked effect of increased
395 loading was recorded for the fine sediment treatments. The application of
396 homogeneous fine sand (0.125-1 mm) to a single section of the column (Section A or
397 C, 0 – 50 mm or 100 – 150 mm) had a relatively limited effect on the movement of
398 individuals due to the large interstitial spaces being maintained and the absence of
399 clog formation (Brunke, 1999; Xu et al., 2012). However, at greater fine sand
400 loadings, pore spaces gradually filled from the base of the column via unimpeded
401 percolation (Diplas and Parker, 1992), limiting faunal access to the deeper sections
402 of the substrate (column). The filling of all available pore space under the greatest
403 sediment loading (3 kg or section A-E) resulted in a reversal in the distribution of *G.*
404 *pulex* compared to control conditions and demonstrates the potential effect that
405 chronic fine sedimentation loading may have on the ability of organisms to utilise
406 subsurface substrates. As a result of increasing sediment loading the majority of
407 individuals were restricted to the layers of the column above the sediment treatment,
408 leading to a disconnection of surface and subsurface sediments. Sedimentation by
409 homogeneous coarse sand (1 – 4 mm) restricted the ability of individuals to access
410 subsurface sediments more than homogeneous fine sand (0.125 – 1 mm). This
411 effect was enhanced through the addition of greater sediment loadings for
412 homogeneous fine and coarse sand but was less marked with the addition of
413 heterogeneous sand (0.125 – 4 mm) because even at the lower loadings the vertical
414 movement of almost all individuals was prevented.

415 In support of our second hypothesis, the results also indicate that increasing particle
416 size and heterogeneity of fine sediment exerts a strong influence on the propensity
417 for clog formation and the ability of individuals to access deeper layers of the
418 column. Coarse sand resulted in the formation of surface clogs associated with
419 particles being unable to percolate into the subsurface and the bridging of pore
420 spaces (Beschta and Jackson, 1979) even at the lowest sediment loadings. This
421 effect was most marked for heterogeneous sand (0.125 – 4 mm) which had the

422 strongest filtering effect and resulted in up to 25 individuals being recorded in the
423 surface (benthic) layer of the column (0 – 50 mm).

424 Finally, we found evidence to support our third hypothesis, that increasing body size
425 would limit an individual's ability to access deeper substrates. We found that both
426 sediment load and particle size acted as a filter on the ability of an individual to
427 migrate vertically, with only smaller bodied individuals being recorded in the deeper
428 layers of the column for the greatest sediment loadings and for increasing
429 heterogeneity. While the ability of an individual to access subsurface interstitial
430 habitat has been reported to be heavily dependent on body size (Gayraud and
431 Philippe, 2001; Descloux et al., 2014; Vadher et al., 2017), this experimental study
432 provides direct evidence for the filtering effect of fine sediment loading and particle
433 size compared to open frameworks as a result of the ability to quantify the number
434 and size of individuals at different depths within the column. The filtering effect was
435 most marked for large bodied amphipods, with a greater proportion being recorded
436 above the sediment clog for all but two of the 12 sediment applications. For fine
437 sand, sediment loading resulted in marked variability in the number of individuals
438 being recorded above the sediment treatment. In contrast, coarse and
439 heterogeneous sand treatments demonstrated less variability associated with
440 sediment loading, due to the bridging of pore space even under surface sediment
441 applications and the confinement of organisms to substrates above the sediment
442 treatment.

443 Small bodied individuals demonstrated a similar, but less marked effect, with more
444 individuals recorded below the sediment application for six of the 12 sediment
445 applications. Homogeneous fine sand (0.125 – 1 mm) had little effect on the ability of
446 small individuals to migrate to deeper substrates even for the greatest loading. In
447 contrast to large bodied individuals, around 50% of small bodied individuals were
448 located below the sediment treatment for the homogeneous coarse sand (1 – 4 mm)
449 for three of the applications (5 kg surface – Section A; 3 kg middle – Section C and 3
450 kg application on three sections B-D). Only with the treatment of all sections of the
451 column (A-E) was the movement of small bodies restricted to the surface layer of the
452 mesocosm. Heterogeneous sand (0.125 – 4 mm) acted as the strongest filter for
453 both size classes; although some small bodied individuals were able to migrate
454 below the sediment treatment in almost every instance. In contrast to large bodied

455 individuals, the effect of different sediment loadings were most marked for the coarse
456 sand treatments. Heterogeneous sand had a consistent effect for small and large
457 bodied individuals regardless of the loading applied. However, it should be noted that
458 the distribution of small bodied individuals may be in part influenced by their
459 reproductory behaviour and the establishment of pre-copulatory pairs (Ward, 1983).
460 Although no pre-copulatory pairs were placed into the mesocosm at the start of
461 experiments, a number were observed at the termination, 24-hours later. As a result,
462 the distribution of some small bodied individuals may be influenced by larger
463 individuals; some in pre-copulatory pairs in the surface layer of the column.

464 The experiments presented here were conducted under controlled laboratory
465 conditions, with sediment deposition being the only factor which was manipulated.
466 Within the natural environment, clogging of interstitial pore space has the potential to
467 modify interstitial flow and the transport of dissolved solutes and resources, resulting
468 in reduced dissolved oxygen concentrations and water quality (Greig, Sear &
469 Carling, 2005, Sear et al., 2017). Consequently, the effects on an organism's ability
470 to utilise the subsurface sediments following fine sediment loading at levels reported
471 in this study may represent an upper estimate. Although individuals may still be able
472 to access subsurface sediments, the associated changes in the physiochemical
473 conditions may make the abiotic environment unfavourable for most taxa /
474 individuals.

475 **Study implications**

476 Deposition of fine sediment, which results in the clogging of substrates and prevents
477 access to subsurface habitats, may have far reaching consequences for benthic and
478 hyporheic communities. Refugium use reduces the predation risk of benthic
479 invertebrates (Gee, 1982). In the case of *G. pulex*, predator avoidance behaviour
480 and refuge seeking behaviour has been widely documented (e.g., Dahl and
481 Greenberg, 1996; Sih, 1997) and the loss of subsurface habitat due to clogging may
482 increase the risk of predation. However, most research examining the role of habitat
483 availability on predator-prey interactions has focussed on the role of hydrological
484 disturbances (Lancaster et al., 1990; Lancaster, 1996) while the effects of sediment
485 deposition are less well documented (see Jones et al., 2012 for review). Given the
486 well-established substrate size selectivity reported for *G. pulex* (e.g., Thompson and
487 Moule, 1982), sedimentation and clogging of the surface of the substrate may lead to

488 a concentration of biotic interactions in the benthic zone and enhanced predation of
489 large bodied individuals (Strommer and Smock, 1989; Covich et al., 2003).
490 Moreover, a reduction of interstitial space may also affect the growth of *G. pulex* over
491 longer time scales, with interstitial pore space availability being reported to reduce
492 the energy requirements and enhance the growth potential of individuals (Franken et
493 al., 2006). Reductions in growth can have cascading effects on populations
494 associated with reduced egg numbers for smaller females (Glazier, 2000).

495 This research highlights the importance of characterising both the particle size and
496 heterogeneity of deposited fine sediment when considering the effects on instream
497 invertebrates. To date, only a limited number of studies have specifically considered
498 the grain size distribution of either the gravel-bed matrix or the infiltrating material on
499 instream communities and populations (but see Gayraud and Philippe, 2003;
500 Weigelhofer and Waringer, 2003; Mathers and Wood, 2016; Vadher et al., 2017).
501 However, the current investigation demonstrates that the effect of sedimentation and
502 clogging on faunal distribution of benthic and hyporheic invertebrates is more
503 complex than the gross fine sediment cover. We found that the resultant pattern of
504 faunal distribution is dependent on both the characteristics of the deposited fine
505 sediment (particle size, heterogeneity and loading) and the process of deposition
506 that subsequently takes place (unimpeded percolation or bridging; Dudill et al.,
507 2017). As a result, we recommend that future studies concerned with examining the
508 effects of fine sediment for biota should quantify the grain size distribution of the fine
509 sediment (and the gravel-bed matrix) so that the mechanistic controls of fine
510 sediment on instream ecology can be more clearly understood. Better
511 characterisation of the deposited fine sediments will allow river managers to direct
512 resources more efficiently towards identifying and managing sediment sources within
513 the catchment (Lacey et al., 2017) and where restoration activities are likely to be
514 feasible (Wohl et al., 2015).

515 There have been recent calls for an improved mechanistic understanding of the
516 effects of fine sediment on macroinvertebrates (Wilkes et al., 2017), with some
517 studies documenting variable responses of benthic invertebrates to sedimentation
518 (Descloux et al., 2014; Mathers, Rice & Wood, 2017; Murphy et al., 2017). The
519 results from these experiments provide the first direct evidence that the effects of

520 sedimentation vary as a function of sediment characteristics (particle size,
521 heterogeneity and loading) and the body size of fauna. Fine sand demonstrated the
522 greatest variability in effects associated with loading for large bodied individuals
523 whilst the effects for small bodied individuals were most variable under coarse sand
524 loading. As a result, broad-scale generalisations regarding an individual species'
525 response to sedimentation are unlikely to reflect the subtle effects of intra-specific
526 variations within the morphological characteristics of a population (such as body size
527 which is typically represented as an average value) or fitness. For example, Orlofske
528 and Baird (2010) reported that 55% of measured body sizes of specific taxa were
529 considerably smaller or larger than those documented in existing trait databases. As
530 a result, there is a need for researchers examining the effect of sedimentation to
531 report the body size of the taxa studied (model organism), such as amphipod
532 crustacea which often comprise populations of varying life-stages and body sizes
533 throughout the year. This will enable potential differences in the mean body size of
534 populations to be more readily compared between studies. This is potentially a vital
535 controlling factor given that the effects of fine sediment deposition for many taxa,
536 especially aquatic insects, will vary at different life stages associated with increasing
537 body size. Enhancing this knowledge base will provide mechanistic evidence for
538 studies which have observed the effects of fine sediment loading on benthic
539 communities to vary temporally under field conditions (e.g., Mathers, Rice and
540 Wood, 2017). The implications of fine sediment deposition are therefore complex
541 and reflect a combination of different factors including loading, particle size and an
542 individual's body size.

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550 **Conflict of Interest**

551 The authors declare no conflict of interest.

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814 **Tables**

Table 1. Univariate linear mixed effects model (LME) analysis for *G. pulex* abundance associated with sediment size (fine, coarse, mixed), sediment loading (n=6), section / depth within the sediment column (n=5) and the interactions between these factors. Significant values ($p < 0.05$) are presented in bold.

Factor	d.f	F	p
Section / depth	4, 294	387.10	<0.001
Sediment loading	4, 75	0.53	0.718
Sediment size	2, 75	0.33	0.719
Sediment loading x sediment size	8, 75	0.13	0.998
Section / depth x sediment loading	16, 294	201.95	<0.001
Section / depth x sediment size	8, 294	132.00	<0.001
Section / depth x sediment loading x sediment size	32, 294	29.37	<0.001

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Table 2. Univariate linear mixed effects model (LME) analysis for *G. pulex* abundance associated with sediment size (fine, coarse, mixed), sediment loading (n=6), location within the sediment column (above or below sediment clog), amphipod body size and the interactions between these factors. Significant values ($p < 0.05$) are presented in bold

Factor	d.f	F	p
Location	1, 120	391.06	<0.001
Sediment loading	3, 120	0.79	0.502
Sediment size	2, 120	0.17	0.840
Body size	1, 120	3.16	0.078
Location x sediment loading	3, 120	30.38	<0.001
Location x sediment size	2, 120	435.58	<0.001
Sediment size x sediment loading	6, 120	0.19	0.979
Location x body size	1, 120	312.85	<0.001
Sediment loading x body size	3, 120	0.14	0.935
Sediment size x body size	2, 120	0.67	0.516
Location x sediment loading x sediment size	6, 120	16.75	<0.001
Location x sediment loading x body size	3, 120	5.97	0.001
Location x sediment size x body size	2, 120	15.99	<0.001
Sediment loading x sediment size x body size	6, 120	0.40	0.877
Location x sediment loading x sediment size x body size	6, 120	11.51	<0.001

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821 **List of Figures**

822 **Figure 1.** Fine sediment treatments applied to sections / layers of substratum
823 columns (A – 0-50 mm; B – 50-100 mm; C – 100-150 mm; D – 150-200 mm; and E –
824 200-250 mm) during experiments: O. Open gravel framework for all layers (control
825 conditions); 1. Benthic sedimentation with the equivalent of 5 kg m⁻²; 2. Hyporheic
826 sedimentation of one layer (100-150 mm) with the equivalent of 3 kg m⁻²; 3.
827 Hyporheic sedimentation of three layers (50-200mm) with the equivalent of 3 kg m⁻²
828 applied to each layer; and 4. Benthic and hyporheic sedimentation (all layers) with
829 the equivalent of 3 kg m⁻². Figure adapted from Mathers et al., (2014).

830 **Figure 2.** Mean number of *Gammarus pulex* (\pm 1SE) recorded within each section of
831 the sediment column (0-50mm; 50-100mm; 100-150mm; 150-200mm; 200-250mm)
832 under open gravel framework conditions (control). For post hoc comparisons
833 between the same section for different sediment loading or grain size see Tables S1
834 and S2.

835 **Figure 3.** Mean number of *Gammarus pulex* (\pm 1SE) recorded within each section of
836 the sediment column (0-50mm; 50-100mm; 100-150mm; 150-200mm; 200-250mm)
837 under fine sediment loading a) surface (benthic) sedimentation with the equivalent of
838 5kg m⁻²; b) subsurface (hyporheic) sedimentation of one layer (100-150mm) with the
839 equivalent of 3kg m⁻²; c) subsurface (hyporheic) sedimentation of three layers (50-
840 200mm) with the equivalent of 3kg m⁻²; d) benthic and subsurface (hyporheic)
841 sedimentation of all layers (0-200mm) with the equivalent of 3kg m⁻². Shading on the
842 figure represents where the sediment was applied. Solid circles = fine sand (0.125 –
843 1mm); grey circles = coarse sand (1-4mm) and; solid squares = heterogeneous sand
844 (0.125 – 4mm). For post hoc comparisons (i.e. between sections for different
845 sediment loading or grain size) see Tables S1 and S2.

846 **Figure 4.** Proportion (mean \pm 1SE) of a) large bodied and; b) small bodied
847 *Gammarus pulex* individuals recorded above the sediment clog for each sediment
848 grain size (fine sand, coarse sand and mixed) and sediment loading. Rhombus =
849 surface (benthic) sedimentation with the equivalent of 5kg m⁻²; circle = subsurface
850 (hyporheic) sedimentation of one layer (100-150mm) with the equivalent of 3kg m⁻²;
851 square = subsurface (hyporheic) sedimentation of three layers (50-200mm) with the
852 equivalent of 3kg m⁻² and; triangle = benthic and subsurface (hyporheic)

853 sedimentation of all layers (0-200mm) with the equivalent of 3kg m⁻². For post hoc
854 comparisons (i.e. between the same section for different sediment loading or grain
855 size) for each body size category see Tables S3 and S4.

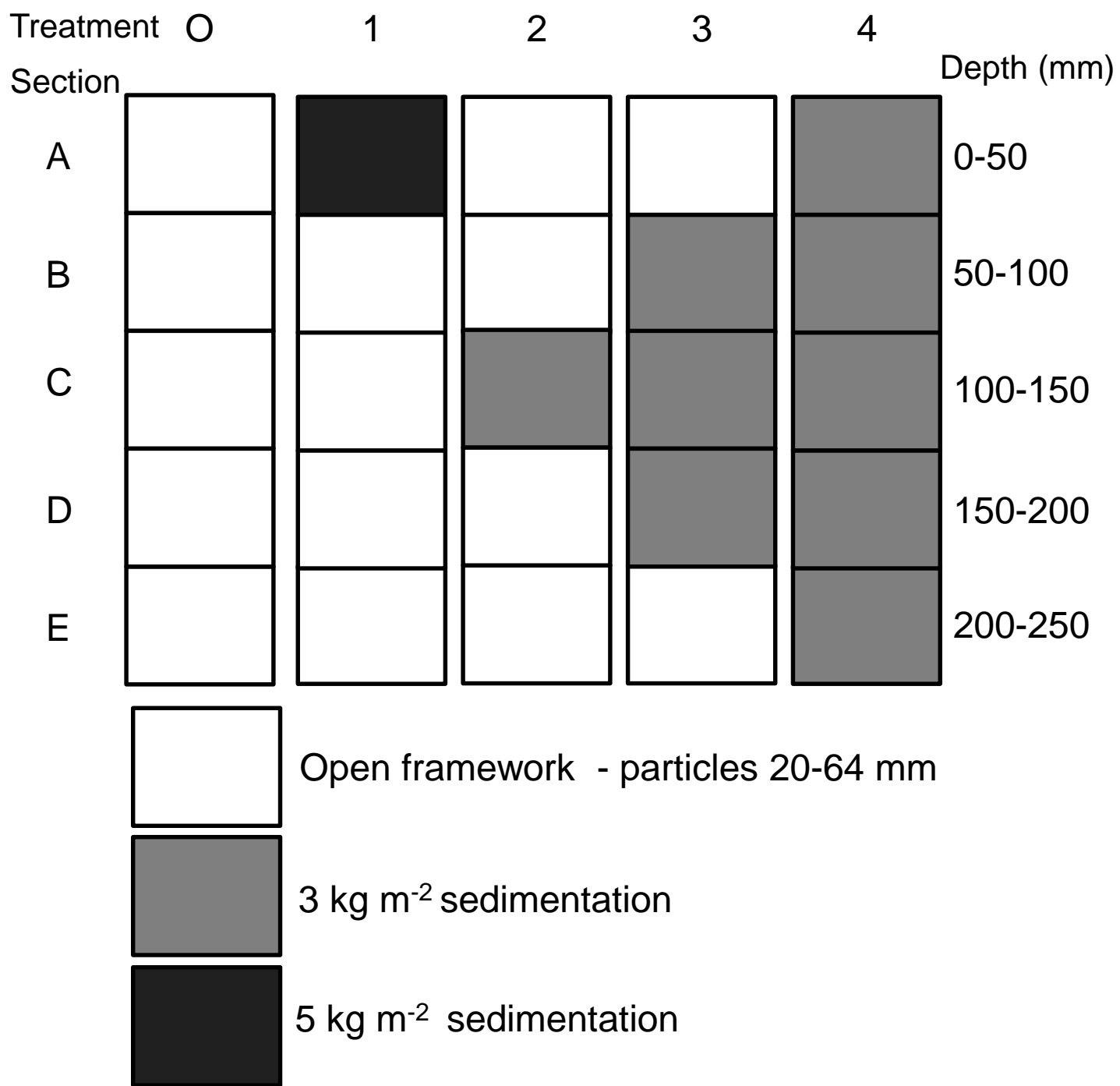


Figure 1.

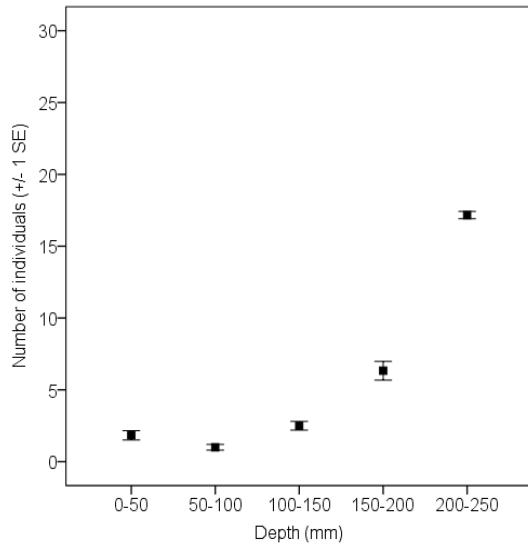


Figure 2.

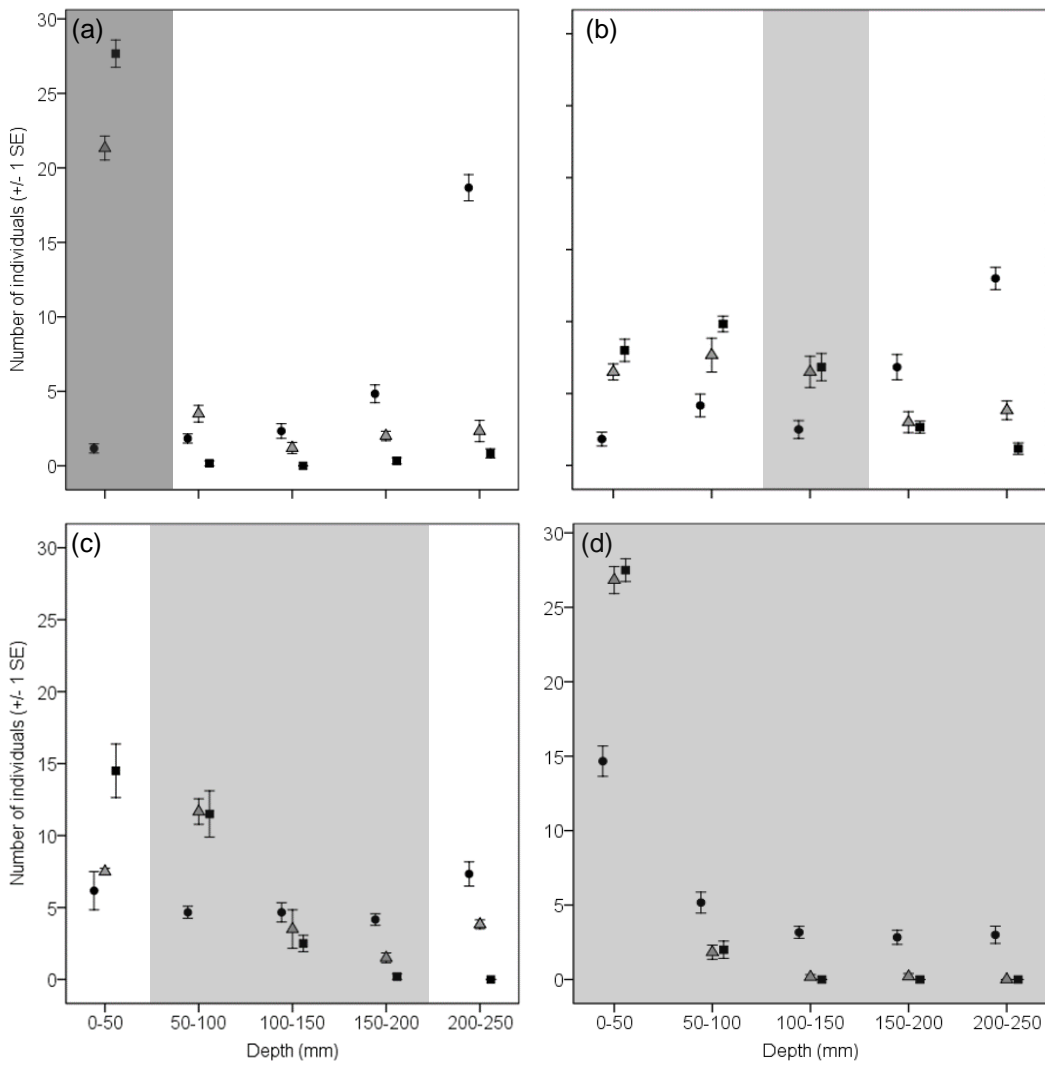


Figure 3.

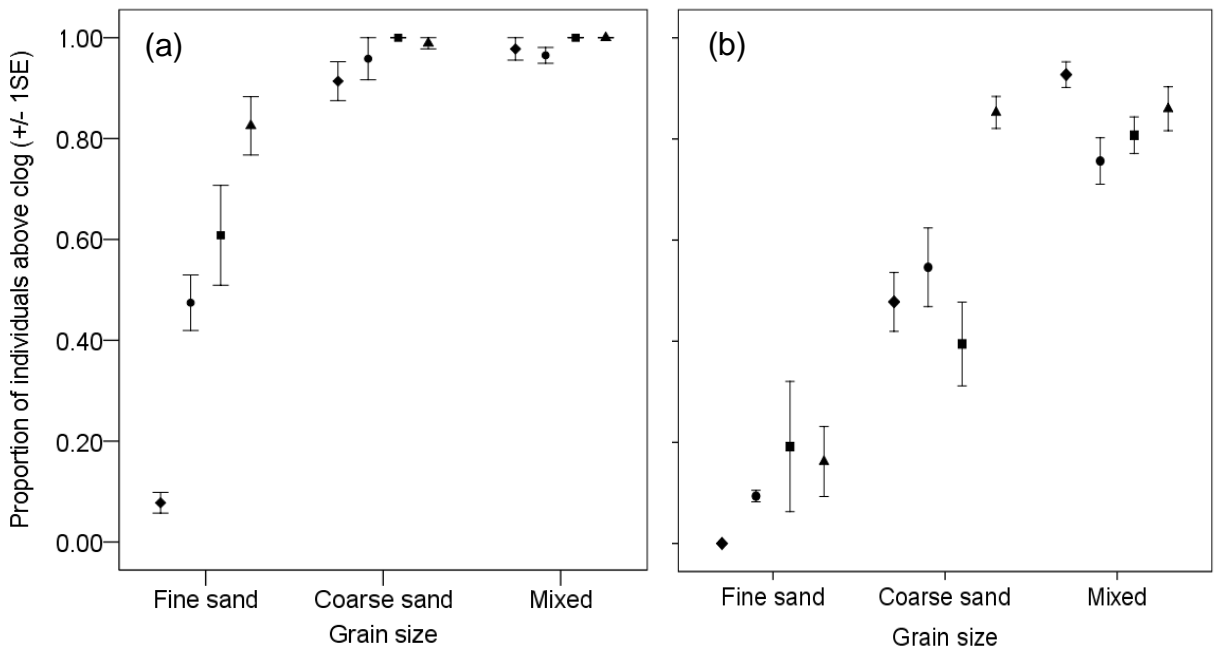


Figure 4.