

1 **Does sand content in spawning substrate result in early larval emergence? Evidence**
2 **from a lithophilic cyprinid fish.**

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22 **ABSTRACT**

23 The spawning success of lithophilic salmonids is strongly influenced by the fine sediment
24 content ('fines') of spawning substrates, yet knowledge on the impacts of fines on the
25 spawning of non-salmonid lithophiles remains limited, despite their ecological and socio-
26 economic importance in European rivers. Consequently, the aim here was to use an *ex-situ*
27 experiment to investigate the impact of sand content on egg survival and timing of larval
28 emergence of the surface-spawning cyprinid European barbel *Barbus barbus*. Thirty
29 incubator boxes within a recirculating system were filled with one of five experimental
30 sediment mixtures (0 to 40 % sand by mass) that each contained 300 fertilised eggs at a depth
31 of 50 mm. Emerged, free-swimming larvae were captured and counted daily to assess grain
32 size effects on larval survival and emergence. Specifically, total proportion of emerged
33 larvae, cumulative daily proportion of emerged larvae and time required to reach 50 %
34 emergence were measured during the study. Whilst the proportion of sand in the sediments
35 did not have a significant impact on egg-to-emergence survival (mean survival per treatment
36 75 % to 79 %), it significantly affected the timing of larval emergence to the water column;
37 early emergence was detected in treatments with elevated sand content (on average, 50 %
38 emergence after 12 - 13 days versus 19 days in the control). Similar to findings from
39 salmonid studies, these results suggest high sand content in spawning gravels can influence
40 timing of larval emergence and potentially cyprinid lithophilic fish survival.

41 **1 INTRODUCTION**

42 Availability and suitability of spawning habitats are important determinants of fish
43 population viability in freshwater ecosystems (Bond & Lake, 2003; Goldstein, D'Alessandro,
44 Reed, & Sponaugle, 2016; Kondolf, 2000; Parsons, Middleton, Smith, & Cole, 2014). This is
45 true for lithophilic fish species, whose reproductive success is strongly related to
46 environmental conditions experienced in the substrate during the period of egg incubation
47 and larval development (Balon, 1975; Louhi, Mäki-Petäys, & Erkinaro, 2008; Mann, 1996;
48 Noble, Cowx, Goffaux, & Kestemont, 2007). Local hydraulic conditions, sediment
49 composition and oxygen content are all factors which influence egg and larval development
50 and survival in salmonids (Bloomer, Sear, Dutey-Magni, & Kemp, 2016; Casas-Mulet,
51 Alfredsen, Brabrand, & Saltveit, 2015; Sear et al., 2016). Specifically, fine sediment content
52 ('fines', ≤ 2 mm) and composition (e.g. organic matter content) influence bed porosity and
53 permeability, and oxygen demand in the substratum (e.g. Kemp, Sear, Collins, Naden, &
54 Jones, 2011; Sear et al., 2014, 2016; Wharton, Mohajeri, & Righetti, 2017). As such, fines are
55 important in determining reproductive success in lithophilic fishes and there is a strong
56 connection between fine sediment loadings in rivers and anthropogenic activities, primarily
57 land use changes, such as deforestation and agricultural practices (Kemp et al., 2011;
58 Wharton et al., 2017; Wood & Armitage, 1997).

59

60 The direct and indirect impacts of fines on egg and larval survival rates, larval development
61 and emergence have been widely documented for salmonid fishes (e.g. Franssen et al., 2012;
62 Levasseur, Bergeron, Lapointe, & Bérubé, 2006; Sear et al., 2014, 2016). Direct influences
63 occur pre-hatching when asphyxiation results from river bed sedimentation (Franssen et al.,
64 2012) and the subsequent alterations of flow and oxygen supply to incubating eggs (Greig,
65 Sear, & Carling, 2005a; Pattison, Sear, Collins, Jones, & Naden, 2014; Sear et al., 2014;

66 Soulsby, Malcolm, & Youngson, 2001). Fines which infiltrate chorion micropores can inhibit
67 oxygen permeation and metabolic waste removal across the egg membrane (Greig, Sear,
68 Smallman, & Carling, 2005b; Kemp et al., 2011; Sear et al., 2014). In contrast, post-hatching
69 survival and larval emergence time is strongly dependent on both asphyxiation and
70 entombment mechanisms, which can lead to increased larval mortality (Franssen et al., 2012;
71 Fudge, Wautier, Evans, & Palace, 2008; Sternecker & Geist 2010). Indirect impacts of
72 elevated fines in substrates can result from premature or delayed emergence which are driven
73 by reductions in space and oxygen concentration and subsequent changes in metabolic and
74 growth rates (Bloomer et al. 2016; Chapman et al., 2014; Franssen et al., 2012; Sear et al.,
75 2016).

76

77 Early emergence can offer some benefits to individuals, given the opportunity to claim high
78 quality territories and switch to exogenous feeding (Einum & Fleming, 2000; Harwood,
79 Griffiths, Metcalfe, & Armstrong, 2003; O'Connor, Metcalfe, & Taylor, 2000). Conversely,
80 early emergers often have small body sizes and a large yolk sac which may increase their
81 susceptibility to predation and downstream displacement (Bloomer et al., 2016; Brännäs,
82 1995; Franssen et al., 2012; Louhi, Ovaska, Mäki-Petäys, Erkinaro, & Muotka, 2011).
83 Similarly, impaired development due to sustained exposure to sub-optimal environmental
84 conditions in the substratum can increase susceptibility of late emergers to predation
85 (Bloomer et al., 2016; Brännäs, 1995; Einum & Fleming, 2000; Louhi et al., 2011; Roussel
86 2007) and intraspecific competition (Cutts, Metcalfe, & Taylor, 1999; Einum & Fleming,
87 2000).

88

89 Most of the work on fines accrual and implications for egg development and larval survival
90 and emergence has focused on salmonid fishes. However, it is important to develop

91 understanding of fines impacts on non-salmonid egg incubation for three reasons. First,
92 within Europe, there are around 75 fish species belonging to the lithophilic group (Noble et
93 al., 2007), with 85 % of them being non-salmonid and of considerable socio-economic,
94 recreational and ecological importance (FAME Consortium, 2004). Second, spawning
95 mechanisms of salmonid and non-salmonid lithophiles are similar, thus there is potential for
96 knowledge transfer between the groups. Finally, there are only few studies evaluating the
97 impact of fines on non-salmonid fishes (*Leuciscus leuciscus*: Mills, 1981; Kemp et al., 2011;
98 *Petromyzon marinus*: Smith & Marsden, 2009; and *Lampetra fluviatilis*: Silva, Gooderham,
99 Forty, Morland, & Lucas, 2015), emphasising the need for work in this area.

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101 Consequently, the aim of this study was to experimentally test the influence of sand content
102 on egg survival and timing of emergence of an ecologically, recreationally and commercially
103 important non-salmonid lithophilic fish. The lithophile European barbel *Barbus barbus* was
104 selected due to utilisation of spawning habitats that are similar to salmonids (e.g. depth, water
105 flow, substrate characteristics; Table 1). Thus, egg deposition depth, spawning season and
106 incubation period are comparable to other non-salmonid lithophiles (e.g. *Acipenser*, *Barbus*,
107 *Leuciscus*, *Chondrostoma* lithophilic species; FAME Consortium, 2004; Kottelat & Freyhof,
108 2007), so results may at some level, be transferable between species. *B. barbus* is also
109 ubiquitous throughout Europe, particularly in the middle and lower reaches of lowland rivers
110 where sedimentation risks are high (Collins & Walling, 2007; Naura et al., 2016). Sand-sized
111 particles were chosen due to their detrimental influence on egg incubation and larval
112 emergence of salmonids (Bryce, Lomnický, & Kaufmann, 2010; Fudge et al., 2008; Lapointe,
113 Bergeron, Bérubé, Pouliot, & Johnston, 2004; Sear et al., 2016), with the assumption it may
114 influence spawning success of other, un-studied lithophiles. Also, in British lowland rivers
115 where indigenous *B. barbus* populations are present, such as the River Great Ouse in Eastern

116 England, juvenile recruitment tends to be poor where sand content of spawning gravels
117 exceeds 20 % (Bašić, 2016), highlighting a possible link between sand content and
118 reproductive success. The hypothesis tested was that variable subsurface sand content will
119 influence egg-to-emergence survival rates and timing of larval emergence of *B. barbuis*. In
120 this study, ‘larval emergence’ refers to both emergence from the subsurface sediment layer to
121 the water column and emergence to the surface sediment layer, with specific references made
122 to each of these throughout the manuscript.

123

124 **2 MATERIALS AND METHODS**

125 **2.1 Experimental setup**

126 The experiment tested differences in the number and timing of emerged larvae from a range
127 of sediment mixtures containing different concentrations (0 to 40 %) of sand (0.064 to 2.000
128 mm). Sediments utilised in this experiment were collected from 6 spawning sites of *B. barbuis*
129 in the River Great Ouse using a McNeil sampler (core volume $\approx 0.005 \text{ m}^3$; McNeil & Ahnell,
130 1964) and Koski plunger. Subsurface sediment samples (n = 10 per site) were dried and
131 sieved into half phi size fractions (0.064 to 45 mm) using an electronic sieve shaker and sieve
132 stacks. The mass of sediment within each discrete size fraction was determined and used to
133 produce grain-size distributions for each site (see Bašić, Britton, Rice, & Pledger, 2017 for
134 detailed methodology). River-averaged values were calculated from these data and used to
135 inform selection of sand (0.064 - 2 mm) and gravel (2 - 45 mm) components that were
136 combined to form experimental sediment mixtures. In each case, gravel and sand components
137 were combined to obtain experimental sediment mixtures with 0 (control), 10, 20, 30 and
138 40% (4 treatments) sand (Table 2). Particles < 0.064 mm (silt) were excluded from each of
139 the experimental mixtures as silt impacts on incubation were not the focus of this study.

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141 In Spring 2015, 30 ‘incubator’ boxes (14 L; external dimensions: 0.5 x 0.3 x 0.095 m) were
142 installed within a recirculating system consisting of two 500-litre water tanks (one header
143 tank, one sump) and five 200-litre troughs, connected via a series of pipes (Figure 1; Figure
144 S1). These boxes were filled with the different sediment mixtures to an approximate depth of
145 150 mm and the total mass of sediment within each box was 14 kg. Incubator boxes were
146 distributed among the 5 troughs so that each trough contained 6 boxes (replicates; Figure 1;
147 Figure S1). Sediment mixtures were disinfected with Virkon S (Antec International Ltd.,
148 Sudbury, UK), rinsed, dried and mixed on site, prior to insertion into the incubator boxes.

149

150 Water flowed through the system from the header tank via gravity, through the UV filter and
151 into the return pipe where water either entered the sump directly or via the troughs, incubator
152 boxes and drain. Before recirculation, sump water was heated using an Electro 2kW S/S
153 electric heater (230v 1ph) and pumped back to the header tank via the delivery pipe (Figure
154 1). Water entered each trough through an inflow pipe before being pumped by a small
155 submersible pump, through a manifold and into the incubator boxes. A gate valve on each of
156 the manifold branches allowed for the regulation of flows within incubator boxes. Flow rates
157 within each incubation box were approximately 7 L min^{-1} , as per Fudge et al. (2008), and
158 consistent with those measured in the field at *B. barbatus* spawning sites (Bašić et al., 2017).
159 The excess water that overflowed the boxes was collected in the troughs and transported
160 away via outflow pipes (Figure 1). To ensure emerged larvae did not escape from each box
161 with the overflowing water, fine mesh (1 mm) was placed around the outer edges of each
162 box. We observed no fine sediment loss from either the inflow pipes or overflows during the
163 experiment.

164

165 Following initial set-up, the system was allowed to run for 7 days before adding fertilized *B.*
166 *barbus* eggs. Flow velocity was measured three times in each box before sediment was
167 added, just above the inflow outlet using a side-facing Nortek Vectrino, sampling at 100Hz
168 for 60 seconds. This was to ensure flow conditions were consistent between the different
169 treatments and control. Importantly, no significant differences in the vertical component of
170 velocity were detected (one-way ANOVA; $F_{(4,25)} = 1.07$, $P > 0.05$). Additionally, water
171 velocity within each of the incubator boxes was measured after the addition of sediment.
172 Measurements were made just above the sediment surface (10 - 20 mm) at three locations per
173 incubator box, using the equipment and procedure described above. Mean values of the
174 vertical component of velocity from each trough were used as a proxy for interstitial water
175 velocity at the start of the experiment. Mean velocity was $0.01 \pm 0.002 \text{ ms}^{-1}$, well above the
176 minimal interstitial flow velocity associated with high salmon embryo survival ($4.17e^{-05} \text{ ms}^{-1}$;
177 Franssen et al., 2012; Greig, Sear, & Carling, 2007). Therefore, it was assumed that initial
178 conditions within incubation boxes were suitable for egg incubation.

179

180 **2.2 Collection and seeding of *Barbus barbus* eggs**

181 Fertilized *B. barbus* eggs were provided by the Environment Agency of England, a
182 government-funded organisation responsible for fisheries management and regulation. Eggs
183 and milt were extracted from one female (fork length: 690 mm; mass: 4.5 kg) and 2 males
184 (fork lengths: 490 and 530 mm) under anaesthetic, following two rounds of hormone
185 injections (carp pituitary extract; 0.1 ml/kg) over a 24-hour period. A single female was used
186 to ensure consistent levels of fertilisation between experimental replicates. Following
187 fertilization in the hatchery (Figure S1), eggs were immediately transferred to the
188 experimental recirculating system located on site (Figure 1; Figure S1). Approximately 300

189 eggs were deposited inside each spawning box (Table 3) at a depth of 100 mm and covered
190 with a 50 mm layer of additional sediment. The exact number of eggs per box was
191 determined using image analysis in Image J (Schneider, Rasband, & Eliceiri, 2012; Figure
192 S1) of photographs of the eggs prior to their deposition in the incubator boxes. Eggs were not
193 counted manually due to time constraints and high sensitivity of *B. barbuis* eggs to handling
194 (personal observation by Bašić).

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196 All eggs were deposited 24/05/15 at 06:00, marking the start of the experiment. Water
197 temperature was initially set at 16 °C but increased to 17.5 °C five days later when hatching
198 started to ensure optimal conditions for egg and larvae development (Wijmans, 2007). The
199 experiment utilized a 14:10 h light: dark photoperiod (Policar, Podhorec, Stejskal,
200 Hamackova, & Hadi Alavi, 2010, 2011), controlled by timer-operated lamps above each of
201 the incubator boxes. Water temperature, pH, conductivity, dissolved oxygen and unionized
202 ammonia concentration were monitored at least two times per day per replicate using a YSI
203 probe, ensuring physico-chemical water conditions were suitable (*cf* Policar et al., 2010,
204 2011; Wijmans, 2007), relatively constant and importantly, consistent between replicates of
205 the different treatments and control (temperature: 17.54 ± 0.11 °C; dissolved oxygen
206 concentration: 8.25 ± 0.05 mgl⁻¹; pH: 8.04 ± 0.01 ; conductivity: 738.38 ± 3.27 μScm⁻¹;
207 unionized nitrogen ammonia concentration: 0.03 ± 0.001 mgl⁻¹). Our monitoring tested for
208 differences in environmental conditions as a function of the experimental design and found
209 none, with measured parameters consistent between each of the troughs and so, treatments
210 and the control (Linear mixed effects models; temperature: $\chi^2_{(4)} = 0.06$, $P > 0.05$; dissolved
211 oxygen concentration: $\chi^2_{(4)} = 2.83$, $P > 0.05$; pH: $\chi^2_{(4)} = 0.31$, $P > 0.05$; conductivity: $\chi^2_{(4)} =$
212 0.85 , $P > 0.05$; unionized nitrogen ammonia concentration: $\chi^2_{(4)} = 7.7$, $P > 0.05$). It is
213 therefore reasonable to assume any differences in environment (specifically, flows and water

214 chemistry) and so, emergence, are a result of the different treatment/control sediment
215 mixtures, rather than experimental design. Furthermore, environmental conditions were
216 consistent with those described in literature and mimicked natural conditions in UK lowland
217 rivers around the time of spawning.

218

219 Each egg box was inspected twice daily (morning and evening) for emerged larvae. Direct
220 egg hatching success could not be assessed as *B. barbus* larvae are photophobic after
221 hatching and remained in the sediment until yolk sac absorption (Balon, 1975; Vilizzi &
222 Copp, 2013). Thus, pre-emergence survival was assessed as the proportion of eggs that
223 resulted in an emerged larva. The timing of emergence was assessed when larvae emerged
224 from the substrate into the surface water column, allowing their capture with an aquarium net
225 without disturbing the sediments. We observed no attempts by free-swimming larvae to re-
226 enter the sediment during collection although some did stay close to the bed, presumably to
227 avoid detection and so, capture. A variety of capture techniques were considered, including
228 use of pipettes, but these methods were rejected due to time constraints and high sensitivity of
229 *B. barbus* larvae to handling (personal observation by Bašić). Emergence to the surface water
230 column began on day 12 of the experiment and typically coincided with yolk sac exhaustion
231 (personal observation by Bašić). However, emergent larvae with the yolk sac intact were
232 observed on the sediment surface of treatments with high sand content (30 and 40 %) from
233 day 5 of the experiment. These larvae could not be removed without sediment disruption, so
234 were left and recovered following emergence to the water column. Consequently, body length
235 and size of the yolk sac immediately after emergence from the gravels could not be assessed
236 during the experiment.

237

238 Upon emergence to the water surface, larvae were captured daily from treatment and control
239 incubation boxes, counted and transferred to separate holding cages (0.17 x 0.13 x 0.13 m).
240 Daily enumeration and removal of emergent larvae continued through the emergence period
241 and after 3 consecutive days of no emergence from any treatment/control, the experiment
242 concluded. Upon experiment completion fish were stocked into a nursery pond but no
243 subsequent measurements of physiology or fate were made.

244

245 **2.3 Data analysis**

246 The effect of substrate composition on egg to emergence survival was assessed using a
247 generalized linear mixed model (GLMM) with the proportion of eggs that resulted in an
248 emerged larva in each replicate (as a value between 0 and 1) and treatment specified as a
249 response variable and fixed effect, respectively (Table 4). In addition, each sample was fitted
250 as a random effect on the intercept to correct for over-dispersion and validated accordingly
251 post fit (Bolker et al., 2009; Harrison, 2014). The impact of treatment on time (in days)
252 required to reach 50 % emergence was quantified using a linear model (LM) (Table 4).

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254 The difference in emergence timing across treatment was assessed using a GLMM where the
255 cumulative proportion of daily emerged larvae to the water column (each daily proportion
256 value per replicate was added to previous available proportions to establish total proportion
257 of emerged larvae for a certain day and treatment) was specified as a response variable and
258 the interaction of treatment and time a fixed effect. Each incubator box was specified as a
259 nested random effect on the intercept to account for temporal dependency of data (Table 5).

260

261 GLMMs were fitted by maximum likelihood using a Laplace approximation (family-
262 binomial; link-logit; Zuur, Ieno, Walker, Saveliev, & Smith, 2009) in R (R Development

263 Core Team, 2011) package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Model
264 assumptions were checked using standard graphical validation for GLMM and LM in R (Zurr
265 et al., 2009). The significance of fixed effect/s for each model was assessed using an F test
266 (LM) or Wald test (GLMMs) in the R car package (Fox & Weisberg 2011). Following a
267 significant effect of treatment on egg to emergence survival, time to 50 % emergence or
268 proportion of emerged larvae, comparisons of covariate adjusted means were conducted via
269 least-squares means with Dunnett adjustments for P values for multiple independent
270 comparisons using multcomp package in R (Hothorn, Bretz, & Westfall, 2008).

271

272 **3 RESULTS**

273 Over the 21-day experimental period, the proportion of eggs that survived to larval
274 emergence was similar across treatments (Wald $\chi^2_{(4)} = 1.37$; $P = 0.85$; Table 5; Figure 2) and
275 average egg survival to larval emergence never exceeded 80 % (Table 3; Figure 2).

276

277 Time required to reach 50 % emergence was significantly affected by Treatment ($F_{(4, 25)} =$
278 45.19 ; $P < 0.01$; Table 4; Figure 3) with significant differences detected when comparing
279 control vs. treatment data (Table 4; Figure 3). On average, more than 50 % of larvae emerged
280 from the 40 and 30 % sand treatments on day 12 and 13 of the experiment, respectively.
281 However, for 10 and 20 % treatments and the control, 50 % emergence was reached on
282 average on day 16, 17 and 19 of the experiment, respectively (Figure 3 and 4).

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284 The interaction of treatment and experimental time had a significant effect (Wald $\chi^2_{(49)} =$
285 113921 ; $P < 0.01$) on the proportion of emerged larvae to the surface water column between
286 treatments (Table 5). Dunnett's pairwise comparison revealed significant differences between
287 proportions of larvae emerged to the surface water in the control and 10% sand treatment

288 from the thirteenth day of the experiment ($z = 2.55$, $P = 0.04$; Table 5) until the nineteenth
289 day of the experiment ($z = 1.33$, $P = 0.47$; Table 5). The daily proportions of emerged larvae
290 to the surface water varied significantly between the control and 20% sand treatment from
291 day 12 ($z = 4.58$, $P = 0.01$; Table 5) to day 18 of the experiment ($z = 2.42$, $P = 0.05$; Table 4),
292 but the overall rate of emergence equalized thereafter (Table 5; Figure 4).

293

294 Treatments with the highest amounts of sand (30 and 40 %) differed significantly in the rate
295 of daily emergence from the control, with a general pattern of more rapid emergence rates to
296 the surface water column (Table 4). Most of these larvae emerged from the sediment 5 days
297 after the start of the experiment in 30 and 40 % sand treatments, but their capture and
298 enumeration were not possible prior to their emergence to the water column. Consequently,
299 proportion of emerged larvae to the surface water column between treatments with 30 and 40
300 % sand and control differed significantly from day 12 ($z = 8.02$, $P < 0.01$ and $z = 9.46$, $P <$
301 0.01 , respectively) until day 19 of the experiment ($z = 2.117$, $P = 0.10$ and $z = 1.62$, $P =$
302 0.30 , respectively) (Table 5; Figure 4).

303

304 **4 DISCUSSION**

305 Sand content did not significantly impact upon *B. barbuis* egg to emergence survival rates in
306 this experiment. However, timing of larval emergence differed between treatments with high
307 sand content (30 and 40 %) and control conditions. Most larvae from the high sand treatments
308 moved to the sediment surface on day 5 and appeared to have their yolk sacs intact and were
309 unable to swim, but enumeration was not possible until emergence to the surface water
310 column. Correspondingly, larvae from treatments with 30 and 40 % sand emerged to the
311 water surface earlier than control larvae, which took 8 additional days to reach 50 % larval
312 emergence.

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There are three plausible reasons for the early emergence detected during this experiment. First, smaller gaps between grains in the 30 and 40 % sand treatments may have limited the body size at which larvae could emerge (Sear et al. 2016), meaning larvae left the substrate earlier to avoid entombment. Second, it is reasonable to assume larvae exposed to the 30 and 40 % sand treatments were at increased risk of abrasion due to increased availability of fines. Thus, fish exposed to these sediment mixtures may have emerged prematurely to avoid risk of damage, which may have influenced survival. Third, low oxygen levels within sediments with high fines may have caused premature emergence as shown in several studies (e.g. Bloomer et al., 2014; Chapman et al., 2014; Sear et al., 2016). Regardless of the driver of early emergence, our observations suggest the timing of emergence had a significant impact on larval physiology immediately post-emergence, with larvae emerging early from substrates with 30 and 40 % sand appearing smaller and with a larger yolk sack. These observations are broadly supported by quantitative data from other studies. Franssen et al. (2012) showed premature emergence of *Salvelinus fontinalis* in fines-rich sediment (< 0.5 mm) under controlled conditions. The body size and weight of larvae was smaller in earlier emerged individuals and the yolk sack was larger. Similarly, prematurely emerged larvae of wild *Salvelinus confluentus* had a larger yolk sac at a site with high fine sand content (< 1 mm: > 18 %) in subsurface sediments (Bowerman, Neilson, & Budy, 2014). This is comparable to our treatments with 30% and 40 % sand (< 1 mm component: 21.4 - 28.5 %), where earlier emergence of larvae was observed (Day 5 of the experiment). However, this did not correspond with higher mortality rates, as the numbers of emerged larvae equalized after 16 days (Day 21 of the experiment) across all treatments.

337 Other investigations into emergence of salmonid species found limited impacts of fine
338 sediment on larval survival. For example, no significant differences in survival and total
339 emergence were detected for *Salmo salar* and *Oncorhynchus mykiss* larvae between
340 treatments with variable fine sediment content (Fudge et al., 2008; MacCrimmon & Gots,
341 1985). However, emergence patterns changed with sediment treatment such that in
342 MacCrimmon and Gots (1985), mean time to initial emergence to the water column and time
343 to median emergence were shorter in sand-rich substrates (< 4 mm). Specifically, 90.9 % of
344 larvae migrated towards the surface immediately after hatching in treatments with 60 - 100 %
345 fines, with delayed emergence observed for treatments with 20 and 0 % fines (MacCrimmon
346 & Gots, 1985). In addition, and in line with our observations, early emergers were
347 significantly smaller and had a larger yolk sac in comparison to larvae from low fines
348 treatments (MacCrimmon & Gots, 1985). Fudge et al. (2008) also observed changes in
349 temporal patterns of emergence from sand-rich substrates (< 4 mm). He identified greater
350 emergence in sand-rich substrates (> 25 %) initially as a result of unsuitable conditions in the
351 hyporheic layer, with emergence rates declining with sand seal formation. However, larvae
352 condition and yolk sac did not significantly differ between treatments (Fudge et al., 2008).
353 Longer residence times in the substratum could be advantageous in the wild because it can
354 provide sufficient nourishment and protection from predation and downstream drift until a
355 size is reached at which that individual has higher competence to avoid sub-optimal
356 conditions (Bowerman et al., 2014; Chapman et al., 2014; Sear et al., 2016). However, this
357 can be offset by longer exposure to unsuitable conditions, resulting in impaired development
358 and survival post-emergence (Brännäs, 1995; Einum & Fleming, 2000; Roussel, 2007).
359 Furthermore, longer residence in the substratum increases the risk of predation by egg-eating
360 predators (Chotkowski & Marsden, 1999; Edmonds, Riley, & Maxwell, 2011; Johnson &
361 Ringler, 1979) and entrainment by high flows (Lisle 1989; Montgomery, Beamer, Pess, &

362 Quinn, 1999; Montgomery, Buffington, Peterson, Schuett-Hames, & Quinn, 1996), with the
363 latter two particularly relevant for shallow spawners such as *B. barbuis*.

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365 Given lack of information on the factors influencing reproductive success in *B. barbuis*, it is
366 important to compare risks associated with the life history and spawning strategies of the
367 species vs. those of other, better-studied lithophiles. Sand content had no recognisable
368 influence on the survival of *B. barbuis* eggs or larvae during the experiment. For salmonid
369 species, there is typically an inverse relationship between sand content and recruitment
370 (Lapointe et al., 2004; Sear et al., 2016; Zimmerman & Lapointe, 2005), with mortalities
371 expected for UK salmonids (e.g. *Salmo salar*, *Salmo trutta*) where < 1 mm and < 2mm
372 particles make up over 5.4 - 15 and 10 - 20 % of the bed, respectively (Table 1). Pacific
373 Salmon are also less tolerant of substrates with fines exceeding 7.5 - 21 (< 1 mm) and 11 %
374 (< 2 mm) (Table 1). Longer incubation time of salmonid eggs and larvae (four to six months)
375 in the gravel (Hendry, Hensleigh, & Reisenbichler, 1998; Malcolm, Middlemas, Soulsby,
376 Middlemas, & Youngson, 2010; Murray & McPhail, 1988) can increase risk of entombment
377 (Franssen et al., 2012; Fudge et al., 2008; Sternecker & Geist, 2010) and likelihood of
378 asphyxiation resulting from elevated sedimentation rates during early development (e.g.
379 Bowerman et al., 2014; Levasseur et al., 2006; Sear et al., 2016). In contrast, *B. barbuis*
380 spawns during late spring in warmer conditions, thus their incubation time is significantly
381 shorter, often less than two weeks, depending on temperature (Wijmans, 2007; Kemp et al.,
382 2011). Salmonid eggs and larvae are typically buried at comparatively greater depths in the
383 substratum (mean bottom egg pocket depth: 28.6 cm; Table 1) as opposed to shallow
384 spawners such as *B. barbuis* (around 5 cm; personal observation by Bašić), which may impose
385 a higher risk of entombment (Lisle, 1989, Montgomery et al., 1996; Wijmans, 2007).
386 However, risks of predation and egg entrainment are presumably higher for shallow-

387 spawning fishes. A shorter incubation time appears to have been advantageous for *B. barbuis*
388 in this study, although spring/summer spawning and shallow burial depth could also increase
389 egg and larval predation risk, given elevated metabolic rates and so, food requirements of
390 piscivorous predators around that time. As incubation time and egg burial depth could have
391 significant implications for reproductive success of different fish species, greater
392 understanding of the nature of spawning and its impact on species resistance to
393 environmental stressors is therefore required to aid conservation efforts.

394

395 Comparing experimental conditions to the River Great Ouse, where the mean sand
396 composition of spawning substrates is > 20 % (Bašić, 2016), implies that sand concentrations
397 could be causing early larval emergence in the river. Premature emergence may have
398 implications for post-emergent larval survival. Indeed, several other studies have reported
399 influences of premature emergence on larval survival due to their smaller bodies and larger
400 yolk sacs, which prevents them from avoiding predators and maintaining position without
401 being displaced (Bowerman et al., 2014; Chapman et al., 2014; Franssen et al., 2012; Sear et
402 al., 2016). This could at least partially explain the low natural recruitment of *B. barbuis* in the
403 area despite adults being observed spawning on some gravels on an annual basis (Twine,
404 2013). Moreover, the river suffers high abundances of invasive signal crayfish (Bašić, 2016)
405 that could predate on both *B. barbuis* eggs due to their shallow spawning nature and
406 prematurely emerged larvae on the surface (Copp, Godard, Vilizzi, Ellis, & Riley, 2017;
407 Edmonds, Riley, & Maxwell, 2011).

408

409 Increased sedimentation can reduce the natural resilience of freshwater ecosystems to present
410 and future perturbations, with growing populations and so, demands for food and agriculture,
411 likely to exacerbate ecological impacts. Furthermore, climate change scenarios project

412 increased air temperatures and alterations of precipitation patterns (UKCP09: Murphy et al.,
413 2009), potentially reducing river flows and increasing sedimentation rates. Anthropogenic
414 activities and their impacts on river hydrology and geomorphology can have major
415 implications for lithophilic species, potentially influencing fish recruitment and viability.
416 With scarce knowledge on early development of non-salmonid lithophilic fishes, there is a
417 requirement to investigate impacts of fine sediment on egg survival and larval emergence of a
418 range of species under *in-* and *ex-situ* conditions. Coupled with knowledge of spawning
419 habitat conditions in rivers for a variety of species, these data can inform threshold setting
420 based on the quality of fluvial sediment and the biological impact. Such data could be used to
421 inform managers on the efficiency of different sediment mitigation options in relation to
422 predefined biological targets, as a first step in seeking to appropriately manage fish spawning
423 habitats (Bašić et al., 2017).

424

425 In summary, the experiment revealed that high sand content in the spawning gravels
426 influenced emergence timing of *B. barbus*, but not egg or larval survival. The pattern of early
427 emergence is hypothesised to be important in contributing to observed low recruitment
428 success of *B. barbus* in the river Great Ouse and other similar systems.

429

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435

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699 **7 AUTHORS' CONTRIBUTION STATEMENT**

700 All four authors have been involved throughout the project and contributed significantly to
701 the writing. Specifically:

702 Conceived and designed the investigation: TB, AP, RB;

703 Performed field and/or laboratory work: TB, AP;
704 Analysed the data: TB, AP, RB;
705 Contributed materials, reagents, and/or analysis tools: TB, AP, RB, SR;
706 Wrote the paper: TB, AP, RB, SR.

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727 Table 1. Summary of spawning habitat preferenes of salmonids (Armstrong, Kemp,
 728 Kennedy, Ladle, & Milner, 2003^a; Bowerman et al., 2014^b; Bryce et al., 2010^c; Curry &
 729 Noakes, 1995^d; DeVries, 1997^e; Franssen et al., 2012^f; Hanrahan, Dauble, & Geist,
 730 2004^g; Kondolf, 2000^h; Kondolf & Wolman, 1993ⁱ; Lorenz & Filer, 1989^j; Louhi et al.,
 731 2008^k; Magee, McMahon, & Thurow, 1996^l; Moir, Soulsby, & Youngson, 2002^m;
 732 O'Connor & Andrew, 1998ⁿ).

Species	Egg burial depth (cm)		Water depth (cm)	Water flow (cm/s)	D50 (mm)	Fines threshold (%)			
	Top	Bottom				< 1 mm	< 2 mm	< 3.5 mm	< 6.35 mm
<i>Salmo salar</i>	15 ^e	30 ^e	20–50 ^k 17–76 ^a	35–65 ^k 35–80 ^a	15–16.6 ⁱ 20–30 ^a 16–64 ^k	5.4 ^a 10 ^k 15 ⁿ	10–20 ^m		
<i>Oncorhynchus kisutch</i>	15 ^e	35 ^e			5.4–35 ⁱ	7.5–21 ^h		30–36 ^h	
<i>Oncorhynchus keta</i>	15 ^e	35 ^e			9.6–62 ⁱ			27 ^h	
<i>Oncorhynchus tshawytscha</i>	15 ^e	50 ^e	30–950 ^g	25–225 ^g	10.8–69 ⁱ		11 ^c		15–40 ^h
<i>Oncorhynchus gorbuscha</i>	15 ^e	35 ^e			6.5–11 ⁱ				
<i>Oncorhynchus nerka</i>	10 ^e	25 ^e		10–15 ^j	14.5–48 ⁱ				33 ^h
<i>Salmo trutta</i>	8 ^e	25 ^e	15–45 ^k 6–82 ^a	20–55 ^k 11–80 ^a	5.8–50 ⁱ 8–128 ^a 16–64 ^k	8–12 ^a	10 ^k		
<i>Salvelinus fontinalis</i>	5 ^e	15 ^e	30–70 ^d		7.2–10.7 ⁱ		10–22 ^f		
<i>Oncorhynchus mykiss</i>	10 ^e	25 ^e			10.5–46.3 ⁱ	12 ^h	16 ^c	7.7–24 ^h	30–40 ^h
<i>Salvelinus confluentus</i>	10 ^e	20 ^e					11 ^c		20–30 ^b
<i>Oncorhynchus clarkii</i>	10 ^e	20 ^e	6–27 ^l		3.2–25.4 ^l	3–17.9 ^l	19 ^c		20 ^h 12.1–41.6 ^l

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736 Table 2. Grain size distributions of sand gravel mixtures used in control and treatment
 737 incubation boxes, expressed in weight percentages. Number of replicates are
 738 represented by n.

Wentworth (1922) class	Grain size (mm)	Treatment				
		Control no	10 %	20 %	30 %	40 %
		sand	sand	sand	sand	sand
		(n=6)	(n=6)	(n=6)	(n=6)	(n=6)
		%	%	%	%	%
Silt	0.06	0.00	0.00	0.00	0.00	0.00
	0.13	0.00	0.13	0.27	0.40	0.53
	0.25	0.00	0.58	1.17	1.75	2.33
Sand	0.50	0.00	3.21	6.41	9.62	12.83
	1.00	0.00	3.19	6.39	9.58	12.77
	2.00	0.00	2.88	5.77	8.65	11.54
	Total	0 %	10 %	20 %	30 %	40 %
	2.80	5.17	4.65	4.13	3.62	3.10
	4.00	7.30	6.57	5.84	5.11	4.38
	5.60	7.84	7.06	6.27	5.49	4.70
	8.00	11.32	10.18	9.05	7.92	6.79
Gravel	11.20	14.45	13.01	11.56	10.12	8.67
	16.00	18.43	16.59	14.75	12.90	11.06
	22.40	17.48	15.73	13.98	12.23	10.49
	31.50	18.02	16.21	14.41	12.61	10.81
	Total	100 %	90 %	80 %	70 %	60 %

739 Table 3. Initial number of *B. barbuis* eggs and percentage survival of eggs to surface water emergence per box and treatment.

Treatment	Control		10 % Sand		20 % Sand		30 % Sand		40 % Sand	
Box	N(eggs)	%Survival	N(eggs)	%Survival	N(eggs)	%Survival	N(eggs)	%Survival	N(eggs)	%Survival
1	226	87	452	91	253	79	258	75	174	66
2	215	99	384	72	245	71	290	65	221	80
3	292	77	273	100	333	89	243	77	348	67
4	308	62	324	70	282	77	269	88	144	86
5	309	78	257	73	349	80	256	79	240	82
6	330	44	427	69	304	74	210	67	224	83
Mean	280.0	74.5	352.8	79.2	294.3	78.3	254.3	75.2	225.2	77.3
(± SE)	(± 19.5)	(± 7.9)	(± 33.0)	(± 5.3)	(± 17.2)	(± 2.5)	(± 11.0)	(± 3.4)	(± 28.6)	(± 3.5)

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742 Table 4. Results of LM testing for the effect of treatment on the time required to 50 %
 743 emergence. Mean differences are from estimated least-square means, significant at * P
 744 < 0.05 and ** $P < 0.01$.

Model:

Time to 50 % emergence ~ Treatment ($F_{(4, 25)} = 45.19; P < 0.01$)

Contrast	z	Mean difference (\pm SE)
Control, day 12 – 10 % sand, day 12	2.96	$1.67 \pm 0.56, P < 0.05^*$
Control, day 12 – 20 % sand, day 12	5.32	$3.00 \pm 0.56, P < 0.01^{**}$
Control, day 12 – 30 % sand, day 12	9.76	$5.50 \pm 0.56, P < 0.01^{**}$
Control, day 12 – 40 % sand, day 12	11.53	$6.50 \pm 0.56, P < 0.01^{**}$

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756 Table 5 Results of GLMMs testing: 1) differences in egg to emergence survival between
 757 treatments, 2) differences in cumulative proportion of daily emerged larvae to the
 758 surface water between treatments. Mean differences are from estimated least-square
 759 means, significant at * $P < 0.05$ and ** $P < 0.01$.

Models:

1. Egg to emergence survival ~ Treatment + (1|Sample), weights=Total number of eggs

(family – binomial (link-logit); Laplace approximation; Wald $\chi^2_{(4)} = 1.37$; $P = 0.85$)

2. Cumulative daily emergence ~ Time x Treatment + (1|Trough/Replicate), weight=Total

number of eggs, (family – binomial (link-logit); Laplace approximation, Wald $\chi^2_{(49)} =$

113921; $P < 0.01$)

Contrast	z	Mean difference (\pm SE)
Control, day 12 – 10 % sand, day 12	- 1.48	- 0.46 \pm 0.31, $P = 0.38$
Control, day 12 – 20 % sand, day 12	- 4.58	- 1.40 \pm 0.31, $P < 0.01^{**}$
Control, day 12 – 30 % sand, day 12	- 8.02	- 2.44 \pm 0.30, $P < 0.01^{**}$
Control, day 12 – 40 % sand, day 12	- 9.46	- 2.88 \pm 0.30, $P < 0.01^{**}$
Control, day 13 – 10 % sand, day 13	- 2.55	- 0.77 \pm 0.30, $P < 0.05^*$
Control, day 13 – 20 % sand, day 13	- 6.93	- 2.08 \pm 0.30, $P < 0.01^{**}$
Control, day 13 – 30 % sand, day 13	- 10.74	- 3.24 \pm 0.30, $P < 0.01^{**}$
Control, day 13 – 40 % sand, day 13	- 11.41	- 3.45 \pm 0.30, $P < 0.01^{**}$
Control, day 14 – 10 % sand, day 14	- 3.65	- 1.10 \pm 0.30, $P < 0.01^{**}$
Control, day 14 – 20 % sand, day 14	- 7.47	- 2.25 \pm 0.30, $P < 0.01^{**}$
Control, day 14 – 30 % sand, day 14	- 12.22	- 3.70 \pm 0.30, $P < 0.01^{**}$
Control, day 14 – 40 % sand, day 14	- 11.72	- 3.55 \pm 0.30, $P < 0.01^{**}$
Control, day 15 – 10 % sand, day 15	- 2.79	- 0.82 \pm 0.29, $P < 0.05^*$
Control, day 15 – 20 % sand, day 15	- 6.20	- 1.82 \pm 0.29, $P < 0.01^{**}$
Control, day 15 – 30 % sand, day 15	- 9.97	- 2.95 \pm 0.30, $P < 0.01^{**}$

Contrast	z	Mean difference (\pm SE)
Control, day 15 – 40 % sand, day 15	- 9.36	- 2.77 \pm 0.30, $P < 0.01^{**}$
Control, day 16 – 10 % sand, day 16	- 3.01	- 0.87 \pm 0.29, $P < 0.01^{**}$
Control, day 16 – 20 % sand, day 16	- 4.28	- 1.24 \pm 0.29, $P < 0.01^{**}$
Control, day 16 – 30 % sand, day 16	- 7.31	- 2.14 \pm 0.29, $P < 0.01^{**}$
Control, day 16 – 40 % sand, day 16	- 6.67	- 1.95 \pm 0.29, $P < 0.01^{**}$
Control, day 17 – 10 % sand, day 17	- 2.72	- 0.79 \pm 0.29, $P < 0.05^*$
Control, day 17 – 20 % sand, day 17	- 3.32	- 0.96 \pm 0.29, $P < 0.01^{**}$
Control, day 17 – 30 % sand, day 17	- 5.38	- 1.57 \pm 0.29, $P < 0.01^{**}$
Control, day 17 – 40 % sand, day 17	- 4.71	- 1.38 \pm 0.29, $P < 0.01^{**}$
Control, day 18 – 10 % sand, day 18	- 2.72	- 0.79 \pm 0.29, $P < 0.05^*$
Control, day 18 – 20 % sand, day 18	- 2.42	- 0.70 \pm 0.29, $P = 0.05$
Control, day 18 – 30 % sand, day 18	- 4.08	- 1.19 \pm 0.29, $P < 0.01^{**}$
Control, day 18 – 40 % sand, day 18	- 3.59	- 1.05 \pm 0.29, $P < 0.05^*$
Control, day 19 – 10 % sand, day 19	- 1.33	- 0.38 \pm 0.29, $P = 0.47$
Control, day 19 – 20 % sand, day 19	- 1.01	- 0.29 \pm 0.29, $P = 0.68$
Control, day 19 – 30 % sand, day 19	- 2.12	- 0.62 \pm 0.29, $P = 0.11$
Control, day 19 – 40 % sand, day 19	- 1.62	- 0.48 \pm 0.29, $P = 0.30$
Control, day 20 – 10 % sand, day 20	- 0.70	- 0.20 \pm 0.29, $P = 0.85$
Control, day 20 – 20 % sand, day 20	- 0.47	- 0.14 \pm 0.29, $P = 0.94$
Control, day 20 – 30 % sand, day 20	- 0.81	- 0.24 \pm 0.29, $P = 0.79$
Control, day 20 – 40 % sand, day 20	0.32	- 0.09 \pm 0.29, $P = 0.98$
Control, day 21 – 10 % sand, day 21	- 1.26	- 0.37 \pm 0.29, $P > 0.05$
Control, day 21 – 20 % sand, day 21	- 0.79	- 0.23 \pm 0.29, $P > 0.05$
Control, day 21 – 30 % sand, day 21	- 0.57	- 0.17 \pm 0.29, $P > 0.05$
Control, day 21 – 40 % sand, day 21	- 0.11	- 0.03 \pm 0.29, $P > 0.05$

761 **Figure captions**

762 Figure 1 The set-up of the experimental design, showing the input of water from the
763 borehole, its flow through the UV steriliser and heater and its pumping through the
764 system. The inset image shows detailed view of the experimental set-up inside each
765 trough.

766

767 Figure 2 Average marginal effects and 95 % confidence intervals estimated from the
768 generalized linear mixed-effects model testing the impact of Treatment on egg-to-emergence
769 survival of *B. barbuis*. Letters above bars indicate statistically homogeneous groups.

770

771 Figure 3 Average marginal effects and 95 % confidence intervals estimated from the
772 generalized linear mixed-effects model testing the impact of Treatment on 50 % emergence
773 time of *B. barbuis*. Letters above bars indicate statistically homogeneous groups.

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775 Figure 4 Line plots showing the marginal effects of variables included in interaction terms
776 (Treatment and Time). X - axis is the explanatory variable value, representing cumulative
777 daily emergence of *B. barbuis* larvae to the surface water column. Dots represent mean values
778 of daily larval emergence (\pm 95 % confidence intervals), where (filled circles) control, (filled
779 squares) 10 % sand, (blank triangles) 20 % sand, (blank squares) 30 % sand and (blank
780 circles) 40 % sand. Statistically significant differences between control and each of the
781 treatment in time are presented in Table 5.

Supplementary material

Figure S1 Experimental setup and some of the procedures utilised in the experiment where a) Recirculating system at Calverton fish farm; b) Female *B. barbuis* stripping; and c) An example of the photo used in ImageJ for determining the number of eggs per box and treatment at the start of the experiment.