1 **Does sand content in spawning substrate result in early larval emergence? Evidence from**

2 **a lithophilic cyprinid fish.**

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ABSTRACT

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

1 INTRODUCTION

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

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

72   fines in substrates can result from premature or delayed emergence which are driven by

73   reductions in space and oxygen concentration and subsequent changes in metabolic and growth

74   rates (Bloomer et al. 2016; Chapman et al., 2014; Franssen et al., 2012; Sear et al., 2016).

75

76   Early emergence can offer some benefits to individuals, given the opportunity to claim high

77   quality territories and switch to exogenous feeding (Einum & Fleming, 2000; Harwood,

78   Griffiths, Metcalfe, & Armstrong, 2003; O'Connor, Metcalfe, & Taylor, 2000). Conversely,

79   early emergers often have small body sizes and a large yolk sac which may increase their

80   susceptibility to predation and downstream displacement (Bloomer et al., 2016; Brännäs, 1995;

81   Franssen et al., 2012; Louhi, Ovaska, Mäki-Petäys, Erkinaro, & Muotka, 2011). Similarly,

82   impaired development due to sustained exposure to sub-optimal environmental conditions in

83   the substratum can increase susceptibility of late emergers to predation (Bloomer et al., 2016;

84   Brännäs, 1995; Einum & Fleming, 2000; Louhi et al., 2011; Roussel 2007) and intraspecific

85   competition (Cutts, Metcalfe, & Taylor, 1999; Einum & Fleming, 2000).

86

87   Most of the work on fines accrual and implications for egg development and larval survival

88   and emergence has focused on salmonid fishes. However, it is important to develop

89   understanding of fines impacts on non-salmonid egg incubation for three reasons. First, within

90   Europe, there are around 75 fish species belonging to the lithophilic group (Noble et al., 2007),

with 85 % of them being non-salmonid and of considerable socio-economic, recreational and ecological importance (FAME Consortium, 2004). Second, spawning mechanisms of salmonid and non-salmonid lithophiles are similar, thus there is potential for knowledge transfer between the groups. Finally, there are only few studies evaluating the impact of fines on non-salmonid fishes (*Leuciscus leuciscus*: Mills, 1981; Kemp et al., 2011; *Petromyzon marinus*: Smith & Marsden, 2009; and *Lampetra fluviatilis*: Silva, Gooderham, Forty, Morland, & Lucas, 2015), emphasising the need for work in this area.

Consequently, the aim of this study was to experimentally test the influence of sand content on egg survival and timing of emergence of an ecologically, recreationally and commercially important non-salmonid lithophilic fish. The lithophile European barbel *Barbus barbus* was selected due to utilisation of spawning habitats that are similar to salmonids (e.g. depth, water flow, substrate characteristics; Table 1). Thus, egg deposition depth, spawning season and incubation period are comparable to other non-salmonid lithophiles (e.g. Acipenser, Barbus, Leuciscus, Chondrostoma lithophilic species; FAME Consortium, 2004; Kottelat & Freyhof, 2007), so results may at some level, be transferable between species. *B. barbus* is also ubiquitous throughout Europe, particularly in the middle and lower reaches of lowland rivers where sedimentation risks are high (Collins & Walling, 2007; Naura et al., 2016). Sand-sized particles were chosen due to their detrimental influence on egg incubation and larval emergence of salmonids (Bryce, Lomnicky, & Kaufmann, 2010; Fudge et al., 2008; Lapointe, Bergeron, Bérubé, Pouliot, & Johnston, 2004; Sear et al., 2016), with the assumption it may influence spawning success of other, un-studied lithophiles. Also, in British lowland rivers where indigenous *B. barbus* populations are present, such as the River Great Ouse in Eastern England, juvenile recruitment tends to be poor where sand content of spawning gravels exceeds 20 % (Bašić, 2016), highlighting a possible link between sand content and reproductive success. The

116   hypothesis tested was that variable subsurface sand content will influence egg-to-emergence

117   survival rates and timing of larval emergence of *B. barbus.* In this study, 'larval emergence'

118   refers to both emergence from the subsurface sediment layer to the water column and

119   emergence to the surface sediment layer, with specific references made to each of these

120   throughout the manuscript.

121

122   2 MATERIALS AND METHODS

123   2.1 Experimental setup

124   The experiment tested differences in the number and timing of emerged larvae from a range of

125   sediment mixtures containing different concentrations (0 to 40 %) of sand (0.064 to 2.000 mm).

126   Sediments utilised in this experiment were collected from 6 spawning sites of *B. barbus* in the

127   River Great Ouse using a McNeil sampler (core volume $\approx$ 0.005 m^3; McNeil & Ahnell, 1964)

128   and Koski plunger. Subsurface sediment samples (n = 10 per site) were dried and sieved into

129   half phi size fractions (0.064 to 45 mm) using an electronic sieve shaker and sieve stacks. The

130   mass of sediment within each discrete size fraction was determined and used to produce grain-

131   size distributions for each site (see Bašić, Britton, Rice, & Pledger, 2017 for detailed

132   methodology). River-averaged values were calculated from these data and used to inform

133   selection of sand (0.064 - 2 mm) and gravel (2 - 45 mm) components that were combined to

134   form experimental sediment mixtures. In each case, gravel and sand components were

135   combined to obtain experimental sediment mixtures with 0 (control), 10, 20, 30 and 40% (4

136   treatments) sand (Table 2). Particles < 0.064 mm (silt) were excluded from each of the

137   experimental mixtures as silt impacts on incubation were not the focus of this study.

138

139   In Spring 2015, 30 'incubator' boxes (14 L; external dimensions: 0.5 x 0.3 x 0.095 m) were

140   installed within a recirculating system consisting of two 500-litre water tanks (one header tank,

141   one sump) and five 200-litre troughs, connected via a series of pipes (Figure 1; Figure S1).

142   These boxes were filled with the different sediment mixtures to an approximate depth of 150

143   mm and the total mass of sediment within each box was 14 kg. Incubator boxes were distributed

144   among the 5 troughs so that each trough contained 6 boxes (replicates; Figure 1; Figure S1).

145   Sediment mixtures were disinfected with Virkon S (Antec International Ltd., Sudbury, UK),

146   rinsed, dried and mixed on site, prior to insertion into the incubator boxes.

147

148   Water flowed through the system from the header tank via gravity, through the UV filter and

149   into the return pipe where water either entered the sump directly or via the troughs, incubator

150   boxes and drain. Before recirculation, sump water was heated using an Elecro 2kW S/S electric

151   heater (230v 1ph) and pumped back to the header tank via the delivery pipe (Figure 1). Water

152   entered each trough through an inflow pipe before being pumped by a small submersible pump,

153   through a manifold and into the incubator boxes. A gate valve on each of the manifold branches

154   allowed for the regulation of flows within incubator boxes. Flow rates within each incubation

155   box were approximately 7 L min^{-1}, as per Fudge et al. (2008), and consistent with those

156   measured in the field at *B. barbus* spawning sites (Bašić et al., 2017). The excess water that

157   overflowed the boxes was collected in the troughs and transported away via outflow pipes

158   (Figure 1). To ensure emerged larvae did not escape from each box with the overflowing water,

159   fine mesh (1 mm) was placed around the outer edges of each box. We observed no fine

160   sediment loss from either the inflow pipes of overflows during the experiment.

161

162   Following initial set-up, the system was allowed to run for 7 days before adding fertilized *B.*

163   *barbus* eggs. Flow velocity was measured three times in each box before sediment was added,

164   just above the inflow outlet using a side-facing Nortek Vectrino, sampling at 100Hz for 60

165   seconds. This was to ensure flow conditions were consistent between the different treatments

166   and control. Importantly, no significant differences in the vertical component of velocity were

167   detected (one-way ANOVA; $F_{(4,25)} = 1.07$, $P > 0.05$). Additionally, water velocity within each

168   of the incubator boxes was measured after the addition of sediment. Measurements were made

169   just above the sediment surface (10 - 20 mm) at three locations per incubator box, using the

170   equipment and procedure described above. Mean values of the vertical component of velocity

171   from each trough were used as a proxy for interstitial water velocity at the start of the

172   experiment. Mean velocity was 0.01 ± 0.002 ms^{-1}, well above the minimal interstitial flow

173   velocity associated with high salmon embryo survival (4.17e^{-05} ms^{-1}; Franssen et al., 2012;

174   Greig, Sear, & Carling, 2007). Therefore, it was assumed that initial conditions within

175   incubation boxes were suitable for egg incubation.

176

2.2 Collection and seeding of *Barbus barbus* eggs

178   Fertilized *B. barbus* eggs were provided by the Environment Agency of England, a

179   government-funded organisation responsible for fisheries management and regulation. Eggs

180   and milt were extracted from one female (fork length: 690 mm; mass: 4.5 kg) and 2 males (fork

181   lengths: 490 and 530 mm) under anaesthetic, following two rounds of hormone injections (carp

182   pituitary extract; 0.1 ml/kg) over a 24-hour period. A single female was used to ensure

183   consistent levels of fertilisation between experimental replicates. Following fertilization in the

184   hatchery (Figure S1), eggs were immediately transferred to the experimental recirculating

185   system located on site (Figure 1; Figure S1). Approximately 300 eggs were deposited inside

186   each spawning box (Table 3) at a depth of 100 mm and covered with a 50 mm layer of

187   additional sediment. The exact number of eggs per box was determined using image analysis

188   in Image J (Schneider, Rasband, & Eliceiri, 2012; Figure S1) of photographs of the eggs prior

189   to their deposition in the incubator boxes. Eggs were not counted manually due to time

190   constraints and high sensitivity of *B. barbus* eggs to handling (personal observation by Bašić).

191

192   All eggs were deposited 24/05/15 at 06:00, marking the start of the experiment. Water

193   temperature was initially set at 16 °C but increased to 17.5 °C five days later when hatching

194   started to ensure optimal conditions for egg and larvae development (Wijmans, 2007). The

195   experiment utilized a 14:10 h light: dark photoperiod (Policar, Podhorec, Stejskal, Hamackova,

196   & Hadi Alavi, 2010, 2011), controlled by timer-operated lamps above each of the incubator

197   boxes. Water temperature, pH, conductivity, dissolved oxygen and unionized ammonia

198   concentration were monitored at least two times per day per replicate using a YSI probe,

199   ensuring physico-chemical water conditions were suitable (*cf* Policar et al., 2010, 2011;

200   Wijmans, 2007), relatively constant and importantly, consistent between replicates of the

201   different treatments and control (temperature: 17.54 ± 0.11 °C; dissolved oxygen

202   concentration: 8.25 ± 0.05 mgl^{-1}; pH: 8.04 ± 0.01; conductivity: 738.38 ± 3.27 μScm^{-1};

203   unionized nitrogen ammonia concentration: 0.03 ± 0.001 mgl^{-1}). Our monitoring tested for

204   differences in environmental conditions as a function of the experimental design and found

205   none, with measured parameters consistent between each of the troughs and so, treatments and

206   the control (Linear mixed effects models; temperature: $\chi^2_{(4)} = 0.06$, $P > 0.05$; dissolved oxygen

207   concentration: $\chi^2_{(4)} = 2.83$, $P > 0.05$; pH: $\chi^2_{(4)} = 0.31$, $P > 0.05$; conductivity: $\chi^2_{(4)} = 0.85$, $P >$

208   0.05; unionized nitrogen ammonia concentration: $\chi^2_{(4)} = 7.7$, $P > 0.05$). It is therefore

209   reasonable to assume any differences in environment (specifically, flows and water chemistry)

210   and so, emergence, are a result of the different treatment/control sediment mixtures, rather than

211   experimental design. Furthermore, environmental conditions were consistent with those

212   described in literature and mimicked natural conditions in UK lowland rivers around the time

213   of spawning.

214

215   Each egg box was inspected twice daily (morning and evening) for emerged larvae. Direct egg

216   hatching success could not be assessed as *B. barbus* larvae are photophobic after hatching and

217   remained in the sediment until yolk sac absorption (Balon, 1975; Vilizzi & Copp, 2013). Thus,

218   pre-emergence survival was assessed as the proportion of eggs that resulted in an emerged

219   larva. The timing of emergence was assessed when larvae emerged from the substrate into the

220   surface water column, allowing their capture with an aquarium net without disturbing the

221   sediments. We observed no attempts by free-swimming larvae to re-enter the sediment during

222   collection although some did stay close to the bed, presumably to avoid detection and so,

223   capture. A variety of capture techniques were considered, including use of pipettes, but these

224   methods were rejected due to time constraints and high sensitivity of *B. barbus* larvae to

225   handling (personal observation by Bašić). Emergence to the surface water column began on

226   day 12 of the experiment and typically coincided with yolk sac exhaustion (personal

227   observation by Bašić). However, emergent larvae with the yolk sac intact were observed on the

228   sediment surface of treatments with high sand content (30 and 40 %) from day 5 of the

229   experiment. These larvae could not be removed without sediment disruption, so were left and

230   recovered following emergence to the water column. Consequently, body length and size of

231   the yolk sac immediately after emergence from the gravels could not be assessed during the

232   experiment.

233

234   Upon emergence to the water surface, larvae were captured daily from treatment and control

235   incubation boxes, counted and transferred to separate holding cages (0.17 x 0.13 x 0.13 m).

236   Daily enumeration and removal of emergent larvae continued through the emergence period

237 and after 3 consecutive days of no emergence from any treatment/control, the experiment

238 concluded. Upon experiment completion fish were stocked into a nursery pond but no

239 subsequent measurements of physiology or fate were made.

240

241 **2.3 Data analysis**

242 The effect of substrate composition on egg to emergence survival was assessed using a

243 generalized linear mixed model (GLMM) with the proportion of eggs that resulted in an

244 emerged larva in each replicate (as a value between 0 and 1) and treatment specified as a

245 response variable and fixed effect, respectively (Table 4). In addition, each sample was fitted

246 as a random effect on the intercept to correct for over-dispersion and validated accordingly post

247 fit (Bolker et al., 2009; Harrison, 2014). The impact of treatment on time (in days) required to

248 reach 50 % emergence was quantified using a linear model (LM) (Table 4).

249

250 The difference in emergence timing across treatment was assessed using a GLMM where the

251 cumulative proportion of daily emerged larvae to the water column (each daily proportion value

252 per replicate was added to previous available proportions to establish total proportion of

253 emerged larvae for a certain day and treatment) was specified as a response variable and the

254 interaction of treatment and time a fixed effect. Each incubator box was specified as a nested

255 random effect on the intercept to account for temporal dependency of data (Table 5).

256

257 GLMMs were fitted by maximum likelihood using a Laplace approximation (family-binomial;

258 link-logit; Zuur, Ieno, Walker, Saveliev, & Smith, 2009) in R (R Development Core Team,

259 2011) package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Model assumptions were

260 checked using standard graphical validation for GLMM and LM in R (Zurr et al., 2009). The

261 significance of fixed effect/s for each model was assessed using an F test (LM) or Wald test

262   (GLMMs) in the R car package (Fox & Weisberg 2011). Following a significant effect of

263   treatment on egg to emergence survival, time to 50 % emergence or proportion of emerged

264   larvae, comparisons of covariate adjusted means were conducted via least-squares means with

265   Dunnett adjustments for *P* values for multiple independent comparisons using multcomp

266   package in R (Hothorn, Bretz, & Westfall, 2008).

267

268   **3 RESULTS**

269   Over the 21-day experimental period, the proportion of eggs that survived to larval emergence

270   was similar across treatments (Wald $\chi^2_{(4)} = 1.37$; $P = 0.85$; Table 5; Figure 2) and average egg

271   survival to larval emergence never exceeded 80 % (Table 3; Figure 2).

272

273   Time required to reach 50 % emergence was significantly affected by Treatment ($F_{(4,\ 25)} =$

274   45.19; $P < 0.01$; Table 4; Figure 3) with significant differences detected when comparing

275   control vs. treatment data (Table 4; Figure 3). On average, more than 50 % of larvae emerged

276   from the 40 and 30 % sand treatments on day 12 and 13 of the experiment, respectively.

277   However, for 10 and 20 % treatments and the control, 50 % emergence was reached on average

278   on day 16, 17 and 19 of the experiment, respectively (Figure 3 and 4).

279

280   The interaction of treatment and experimental time had a significant effect (Wald $\chi^2_{(49)} =$

281   113921; $P < 0.01$) on the proportion of emerged larvae to the surface water column between

282   treatments (Table 5). Dunnett's pairwise comparison revealed significant differences between

283   proportions of larvae emerged to the surface water in the control and 10% sand treatment from

284   the thirteenth day of the experiment (z = 2.55, $P = 0.04$; Table 5) until the nineteenth day of

285   the experiment (z = 1.33, $P = 0.47$; Table 5). The daily proportions of emerged larvae to the

286   surface water varied significantly between the control and 20% sand treatment from day 12 (z

287 = 4.58, *P* = 0.01; Table 5) to day 18 of the experiment (z = 2.42, *P* = 0.05; Table 4), but the

288 overall rate of emergence equalized thereafter (Table 5; Figure 4).

289

290 Treatments with the highest amounts of sand (30 and 40 %) differed significantly in the rate of

291 daily emergence from the control, with a general pattern of more rapid emergence rates to the

292 surface water column (Table 4). Most of these larvae emerged from the sediment 5 days after

293 the start of the experiment in 30 and 40 % sand treatments, but their capture and enumeration

294 were not possible prior to their emergence to the water column. Consequently, proportion of

295 emerged larvae to the surface water column between treatments with 30 and 40 % sand and

296 control differed significantly from day 12 (z = 8.02, *P* < 0.01 and z = 9.46, *P* < 0.01,

297 respectively) until day 19 of the experiment (z = 2.117 , *P* = 0.10 and z = 1.62, *P* = 0.30,

298 respectively) (Table 5; Figure 4).

299

4 DISCUSSION

301 Sand content did not significantly impact upon *B. barbus* egg to emergence survival rates in

302 this experiment. However, timing of larval emergence differed between treatments with high

303 sand content (30 and 40 %) and control conditions. Most larvae from the high sand treatments

304 moved to the sediment surface on day 5 and appeared to have their yolk sacs intact and were

305 unable to swim, but enumeration was not possible until emergence to the surface water column.

306 Correspondingly, larvae from treatments with 30 and 40 % sand emerged to the water surface

307 earlier than control larvae, which took 8 additional days to reach 50 % larval emergence.

308

309 There are three plausible reasons for the early emergence detected during this experiment. First,

310 smaller gaps between grains in the 30 and 40 % sand treatments may have limited the body

311 size at which larvae could emerge (Sear et al. 2016), meaning larvae left the substrate earlier

13

312 to avoid entombment. Second, it is reasonable to assume larvae exposed to the 30 and 40 %

313 sand treatments were at increased risk of abrasion due to increased availability of fines. Thus,

314 fish exposed to these sediment mixtures may have emerged prematurely to avoid risk of

315 damage, which may have influenced survival. Third, low oxygen levels within sediments with

316 high fines may have caused premature emergence as shown in several studies (e.g. Bloomer et

317 al., 2014; Chapman et al., 2014; Sear et al., 2016). Regardless of the driver of early emergence,

318 our observations suggest the timing of emergence had a significant impact on larval physiology

319 immediately post-emergence, with larvae emerging early from substrates with 30 and 40 %

320 sand appearing smaller and with a larger yolk sack. These observations are broadly supported

321 by quantitative data from other studies. Franssen et al. (2012) showed premature emergence of

322 *Salvelinus fontinalis* in fines-rich sediment (< 0.5 mm) under controlled conditions. The body

323 size and weight of larvae was smaller in earlier emerged individuals and the yolk sack was

324 larger. Similarly, prematurely emerged larvae of wild *Salvelinus confluentus* had a larger yolk

325 sac at a site with high fine sand content (< 1 mm: > 18 %) in subsurface sediments (Bowerman,

326 Neilson, & Budy, 2014). This is comparable to our treatments with 30% and 40 % sand (< 1

327 mm component: 21.4 - 28.5 %), where earlier emergence of larvae was observed (Day 5 of the

328 experiment). However, this did not correspond with higher mortality rates, as the numbers of

329 emerged larvae equalized after 16 days (Day 21 of the experiment) across all treatments.

330

331 Other investigations into emergence of salmonid species found limited impacts of fine

332 sediment on larval survival. For example, no significant differences in survival and total

333 emergence were detected for *Salmo salar* and *Oncorhynchus mykiss* larvae between treatments

334 with variable fine sediment content (Fudge et al., 2008; MacCrimmon & Gots, 1985).

335 However, emergence patterns changed with sediment treatment such that in MacCrimmon and

336 Gots (1985), mean time to initial emergence to the water column and time to median emergence

14

were shorter in sand-rich substrates (< 4 mm). Specifically, 90.9 % of larvae migrated towards the surface immediately after hatching in treatments with 60 - 100 % fines, with delayed emergence observed for treatments with 20 and 0 % fines (MacCrimmon & Gots, 1985). In addition, and in line with our observations, early emergers were significantly smaller and had a larger yolk sac in comparison to larvae from low fines treatments (MacCrimmon & Gots, 1985). Fudge et al. (2008) also observed changes in temporal patterns of emergence from sand-rich substrates (< 4 mm). He identified greater emergence in sand-rich substrates (> 25 %) initially as a result of unsuitable conditions in the hyporheic layer, with emergence rates declining with sand seal formation. However, larvae condition and yolk sac did not significantly differ between treatments (Fudge et al., 2008). Longer residence times in the substratum could be advantageous in the wild because it can provide sufficient nourishment and protection from predation and downstream drift until a size is reached at which that individual has higher competence to avoid sub-optimal conditions (Bowerman et al., 2014; Chapman et al., 2014; Sear et al., 2016). However, this can be offset by longer exposure to unsuitable conditions, resulting in impaired development and survival post-emergence (Brännäs, 1995; Einum & Fleming, 2000; Roussel, 2007). Furthermore, longer residence in the substratum increases the risk of predation by egg-eating predators (Chotkowski & Marsden, 1999; Edmonds, Riley, & Maxwell, 2011; Johnson & Ringler, 1979) and entrainment by high flows (Lisle 1989; Montgomery, Beamer, Pess, & Quinn, 1999; Montgomery, Buffington, Peterson, Schuett-Hames, & Quinn, 1996), with the latter two particularly relevant for shallow spawners such as *B. barbus*.

Given lack of information on the factors influencing reproductive success in *B. barbus*. it is important to compare risks associated with the life history and spawning strategies of the species vs. those of other, better-studied lithophiles. Sand content had no recognisable

362   influence on the survival of *B. barbus* eggs or larvae during the experiment. For salmonid

363   species, there is typically an inverse relationship between sand content and recruitment

364   (Lapointe et al., 2004; Sear et al., 2016; Zimmerman & Lapointe, 2005), with mortalities

365   expected for UK salmonids (e.g. *Salmo salar*, *Salmo trutta*) where < 1 mm and < 2mm particles

366   make up over 5.4 - 15 and 10 - 20 % of the bed, respectively (Table 1). Pacific Salmon are also

367   less tolerant of substrates with fines exceeding 7.5 - 21 (< 1 mm) and 11 % (< 2 mm) (Table

368   1). Longer incubation time of salmonid eggs and larvae (four to six months) in the gravel

369   (Hendry, Hensleigh, & Reisenbichler, 1998; Malcolm, Middlemas, Soulsby, Middlemas, &

370   Youngson, 2010; Murray & McPhail, 1988) can increase risk of entombment (Franssen et al.,

371   2012; Fudge et al., 2008; Sternecker & Geist, 2010) and likelihood of asphyxiation resulting

372   from elevated sedimentation rates during early development (e.g. Bowerman et al., 2014;

373   Levasseur et al., 2006; Sear et al., 2016). In contrast, *B. barbus* spawns during late spring in

374   warmer conditions, thus their incubation time is significantly shorter, often less than two

375   weeks, depending on temperature (Wijmans, 2007; Kemp et al., 2011). Salmonid eggs and

376   larvae are typically buried at comparatively greater depths in the substratum (mean bottom egg

377   pocket depth: 28.6 cm; Table 1) as opposed to shallow spawners such as *B. barbus* (around 5

378   cm; personal observation by Bašić), which may impose a higher risk of entombment (Lisle,

379   1989, Montgomery et al., 1996; Wijmans, 2007). However, risks of predation and egg

380   entrainment are presumably higher for shallow-spawning fishes. A shorter incubation time

381   appears to have been advantageous for *B. barbus* in this study, although spring/summer

382   spawning and shallow burial depth could also increase egg and larval predation risk, given

383   elevated metabolic rates and so, food requirements of piscivorous predators around that time.

384   As incubation time and egg burial depth could have significant implications for reproductive

385   success of different fish species, greater understanding of the nature of spawning and its impact

386 on species resistance to environmental stressors is therefore required to aid conservation

387 efforts.

388

389 Comparing experimental conditions to the River Great Ouse, where the mean sand composition

390 of spawning substrates is > 20 % (Bašić, 2016), implies that sand concentrations could be

391 causing early larval emergence in the river. Premature emergence may have implications for

392 post-emergent larval survival. Indeed, several other studies have reported influences of

393 premature emergence on larval survival due to their smaller bodies and larger yolk sacs, which

394 prevents them from avoiding predators and maintaining position without being displaced

395 (Bowerman et al., 2014; Chapman et al., 2014; Franssen et al., 2012; Sear et al., 2016). This

396 could at least partially explain the low natural recruitment of *B. barbus* in the area despite adults

397 being observed spawning on some gravels on an annual basis (Twine, 2013). Moreover, the

398 river suffers high abundances of invasive signal crayfish (Bašić, 2016) that could predate on

399 both *B. barbus* eggs due to their shallow spawning nature and prematurely emerged larvae on

400 the surface (Copp, Godard, Vilizzi, Ellis, & Riley, 2017; Edmonds, Riley, & Maxwell, 2011).

401

402 Increased sedimentation can reduce the natural resilience of freshwater ecosystems to present

403 and future perturbations, with growing populations and so, demands for food and agriculture,

404 likely to exacerbate ecological impacts. Furthermore, climate change scenarios project

405 increased air temperatures and alterations of precipitation patterns (UKCP09: Murphy et al.,

406 2009), potentially reducing river flows and increasing sedimentation rates. Anthropogenic

407 activities and their impacts on river hydrology and geomorphology can have major implications

408 for lithophilic species, potentially influencing fish recruitment and viability. With scarce

409 knowledge on early development of non-salmonid lithophilic fishes, there is a requirement to

410 investigate impacts of fine sediment on egg survival and larval emergence of a range of species

17

411   under *in-* and *ex-situ* conditions. Coupled with knowledge of spawning habitat conditions in

412   rivers for a variety of species, these data can inform threshold setting based on the quality of

413   fluvial sediment and the biological impact. Such data could be used to inform managers on the

414   efficiency of different sediment mitigation options in relation to predefined biological targets,

415   as a first step in seeking to appropriately manage fish spawning habitats (Bašić et al., 2017).

416

417   In summary, the experiment revealed that high sand content in the spawning gravels influenced

418   emergence timing of *B. barbus*, but not egg or larval survival. The pattern of early emergence

419   is hypothesised to be important in contributing to observed low recruitment success of *B.*

420   *barbus* in the river Great Ouse and other similar systems.

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427

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687 7 AUTHORS' CONTRIBUTION STATEMENT

688 All four authors have been involved throughout the project and contributed significantly to the

689 writing. Specifically:

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

691 Performed field and/or laboratory work: TB, AP;

692 Analysed the data: TB, AP, RB;

693 Contributed materials, reagents, and/or analysis tools: TB, AP, RB, SR;

694 Wrote the paper: TB, AP, RB, SR.

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715 Table 1. Summary of spawning habitat preferenes of salmonids (Armstrong, Kemp,

716 Kennedy, Ladle, & Milner, 2003[a]; Bowerman et al., 2014[b]; Bryce et al., 2010[c]; Curry &

717 Noakes, 1995[d]; DeVries, 1997[e]; Franssen et al., 2012[f]; Hanrahan, Dauble, & Geist, 2004[g];

718 Kondolf, 2000[h]; Kondolf & Wolman, 1993[i]; Lorenz & Filer, 1989[j]; Louhi et al., 2008[k];

719 Magee, McMahon, & Thurow, 1996[l]; Moir, Soulsby, & Youngson, 2002[m]; O'Connor &

720 Andrew, 1998[n]).

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
Salmo salar	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]		
Oncorhynchus kisutch	15[e]	35[e]			5.4–35[i]	7.5–21[h]		30–36[h]	
Oncorhynchus keta	15[e]	35[e]			9.6–62[i]			27[h]	
Oncorhynchus tshawytscha	15[e]	50[e]	30–950[g]	25–225[g]	10.8–69[i]		11[c]		15–40[h]
Oncorhynchus gorbuscha	15[e]	35[e]			6.5–11[i]				

Species									
Oncorhynchus nerka	10[e]	25[e]		10–15[j]	14.5–48[i]				33[h]
Salmo trutta	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]		
Salvelinus fontinalis	5[e]	15[e]	30–70[d]		7.2–10.7[i]		10–22[f]		
Oncorhynchus mykiss	10[e]	25[e]			10.5–46.3[i]	12[h]	16[c]	7.7–24[h]	30-40[h]
Salvelinus confluentus	10[e]	20[e]					11[c]		20–30[b]
Oncorhynchus clarkii	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]

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

Wentworth (1922) class	Grain size (mm)	Treatment				
		Control no sand (n=6) %	10 % sand (n=6) %	20 % sand (n=6) %	30 % sand (n=6) %	40 % sand (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 %

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728 Table 3. Initial number of *B. barbus* 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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731 Table 4. Results of LM testing for the effect of treatment on the time required to 50 %

732 emergence. Mean differences are from estimated least-square means, significant at * $P <$

733 0.05 and ** $P < 0.01$.

Model:		

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

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

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745 Table 5 Results of GLMMs testing: 1) differences in egg to emergence survival between

746 treatments, 2) differences in cumulative proportion of daily emerged larvae to the surface

747 water between treatments. Mean differences are from estimated least-square means,

748 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 (± SE)
Control, day 12 – 10 % sand, day 12	- 1.48	- 0.46 ± 0.31, $P = 0.38$
Control, day 12 – 20 % sand, day 12	- 4.58	- 1.40 ± 0.31, $P < 0.01$**
Control, day 12 – 30 % sand, day 12	- 8.02	- 2.44 ± 0.30, $P < 0.01$**
Control, day 12 – 40 % sand, day 12	- 9.46	- 2.88 ± 0.30, $P < 0.01$**
Control, day 13 – 10 % sand, day 13	- 2.55	- 0.77 ± 0.30, $P < 0.05$*
Control, day 13 – 20 % sand, day 13	- 6.93	- 2.08 ± 0.30, $P < 0.01$**
Control, day 13 – 30 % sand, day 13	- 10.74	- 3.24 ± 0.30, $P < 0.01$**
Control, day 13 – 40 % sand, day 13	- 11.41	- 3.45 ± 0.30, $P < 0.01$**
Control, day 14 – 10 % sand, day 14	- 3.65	- 1.10 ± 0.30, $P < 0.01$**
Control, day 14 – 20 % sand, day 14	- 7.47	- 2.25 ± 0.30, $P < 0.01$**
Control, day 14 – 30 % sand, day 14	- 12.22	- 3.70 ± 0.30, $P < 0.01$**
Control, day 14– 40 % sand, day 14	- 11.72	- 3.55 ± 0.30, $P < 0.01$**
Control, day 15– 10 % sand, day 15	- 2.79	- 0.82 ± 0.29, $P < 0.05$*
Control, day 15 – 20 % sand, day 15	- 6.20	- 1.82 ± 0.29, $P < 0.01$**
Control, day15 – 30 % sand, day 15	- 9.97	- 2.95 ± 0.30, $P < 0.01$**

Contrast	z	Mean difference (± SE)
Control, day 15 – 40 % sand, day 15	- 9.36	- 2.77 ± 0.30, $P < 0.01$**
Control, day 16 – 10 % sand, day 16	- 3.01	- 0.87 ± 0.29, $P < 0.01$**
Control, day 16 – 20 % sand, day 16	- 4.28	- 1.24 ± 0.29, $P < 0.01$**
Control, day 16 – 30 % sand, day 16	- 7.31	- 2.14 ± 0.29, $P < 0.01$**
Control, day 16 – 40 % sand, day 16	- 6.67	- 1.95 ± 0.29, $P < 0.01$**
Control, day 17 – 10 % sand, day 17	- 2.72	- 0.79 ± 0.29, $P < 0.05$*
Control, day 17 – 20 % sand, day 17	- 3.32	- 0.96 ± 0.29, $P < 0.01$**
Control, day 17 – 30 % sand, day 17	- 5.38	- 1.57 ± 0.29, $P < 0.01$**
Control, day 17 – 40 % sand, day 17	- 4.71	- 1.38 ± 0.29, $P < 0.01$**
Control, day 18– 10 % sand, day 18	- 2.72	- 0.79 ± 0.29, $P < 0.05$*
Control, day 18 – 20 % sand, day 18	- 2.42	- 0.70 ± 0.29, $P = 0.05$
Control, day 18 – 30 % sand, day 18	- 4.08	- 1.19 ± 0.29, $P < 0.01$**
Control, day 18 – 40 % sand, day 18	- 3.59	- 1.05 ± 0.29, $P < 0.05$*
Control, day 19 – 10 % sand, day 19	- 1.33	- 0.38 ± 0.29, $P = 0.47$
Control, day 19 – 20 % sand, day 19	- 1.01	- 0.29 ± 0.29, $P = 0.68$
Control, day 19 – 30 % sand, day 19	- 2.12	- 0.62 ± 0.29, $P = 0.11$
Control, day 19 – 40 % sand, day 19	- 1.62	- 0.48 ± 0.29, $P = 0.30$
Control, day 20 – 10 % sand, day 20	- 0.70	- 0.20 ± 0.29, $P = 0.85$
Control, day 20 – 20 % sand, day 20	- 0.47	- 0.14 ± 0.29, $P = 0.94$
Control, day 20 – 30 % sand, day 20	- 0.81	- 0.24 ± 0.29, $P = 0.79$
Control, day 20 – 40 % sand, day 20	0.32	- 0.09 ± 0.29, P 4 0.98
Control, day 21 – 10 % sand, day 21	- 1.26	- 0.37 ± 0.29, $P > 0.05$
Control, day 21 – 20 % sand, day 21	- 0.79	- 0.23 ± 0.29, $P > 0.05$
Control, day 21– 30 % sand, day 21	- 0.57	- 0.17 ± 0.29, $P > 0.05$
Control, day 21 – 40 % sand, day 21	- 0.11	- 0.03 ± 0.29, $P > 0.05$

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750 **Figure captions**

751 Figure 1 The set-up of the experimental design, showing the input of water from the

752 borehole, its flow through the UV steriliser and heater and its pumping through the

753 system. The inset image shows detailed view of the experimental set-up inside each

754 trough.

755

756 Figure 2 Average marginal effects and 95 % confidence intervals estimated from the

757 generalized linear mixed-effects model testing the impact of Treatment on egg-to-emergence

758 survival of *B. barbus*. Letters above bars indicate statistically homogeneous groups.

759

760 Figure 3 Average marginal effects and 95 % confidence intervals estimated from the

761 generalized linear mixed-effects model testing the impact of Treatment on 50 % emergence

762 time of *B. barbus*. Letters above bars indicate statistically homogeneous groups.

763

764 Figure 4 Line plots showing the marginal effects of variables included in interaction terms

765 (Treatment and Time). X - axis is the explanatory variable value, representing cumulative

766 daily emergence of *B. barbus* larvae to the surface water column. Dots represent mean values

767 of daily larval emergence (± 95 % confidence intervals), where (filled circles) control, (filled

768 squares) 10 % sand, (blank triangles) 20 % sand, (blank squares) 30 % sand and (blank circles)

769 40 % sand. Statistically significant differences between control and each of the treatment in

770 time are presented in Table 5.

771   **Supplementary material**

772   Figure S1 Experimental setup and some of the procedures utilised in the experiment where a)

773   Recirculating system at Calverton fish farm; b) Female *B. barbus* stripping; and c) An example

774   of the photo used in ImageJ for determining the number of eggs per box and treatment at the

775   start of the experiment.