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

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

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

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

Most of the work on fines accrual and implications for egg development and larval survival and emergence has focused on salmonid fishes. However, it is important to develop understanding of fines impacts on non-salmonid egg incubation for three reasons. First, within 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, Lomnický, & 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

hypothesis tested was that variable subsurface sand content will influence egg-to-emergence survival rates and timing of larval emergence of *B. barbus*. In this study, ‘larval emergence’ refers to both emergence from the subsurface sediment layer to the water column and emergence to the surface sediment layer, with specific references made to each of these throughout the manuscript.

2 MATERIALS AND METHODS

2.1 Experimental setup

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

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

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

Following initial set-up, the system was allowed to run for 7 days before adding fertilized *B. barbus* eggs. Flow velocity was measured three times in each box before sediment was added,

just above the inflow outlet using a side-facing Nortek Vectrino, sampling at 100Hz for 60 seconds. This was to ensure flow conditions were consistent between the different treatments and control. Importantly, no significant differences in the vertical component of velocity were detected (one-way ANOVA; $F_{(4,25)} = 1.07$, $P > 0.05$). Additionally, water velocity within each of the incubator boxes was measured after the addition of sediment. Measurements were made just above the sediment surface (10 - 20 mm) at three locations per incubator box, using the equipment and procedure described above. Mean values of the vertical component of velocity from each trough were used as a proxy for interstitial water velocity at the start of the experiment. Mean velocity was $0.01 \pm 0.002 \text{ ms}^{-1}$, well above the minimal interstitial flow velocity associated with high salmon embryo survival ($4.17 \times 10^{-5} \text{ ms}^{-1}$; Franssen et al., 2012; Greig, Sear, & Carling, 2007). Therefore, it was assumed that initial conditions within incubation boxes were suitable for egg incubation.

2.2 Collection and seeding of *Barbus barbus* eggs

Fertilized *B. barbus* eggs were provided by the Environment Agency of England, a government-funded organisation responsible for fisheries management and regulation. Eggs and milt were extracted from one female (fork length: 690 mm; mass: 4.5 kg) and 2 males (fork lengths: 490 and 530 mm) under anaesthetic, following two rounds of hormone injections (carp pituitary extract; 0.1 ml/kg) over a 24-hour period. A single female was used to ensure consistent levels of fertilisation between experimental replicates. Following fertilization in the hatchery (Figure S1), eggs were immediately transferred to the experimental recirculating system located on site (Figure 1; Figure S1). Approximately 300 eggs were deposited inside each spawning box (Table 3) at a depth of 100 mm and covered with a 50 mm layer of additional sediment. The exact number of eggs per box was determined using image analysis

in Image J (Schneider, Rasband, & Eliceiri, 2012; Figure S1) of photographs of the eggs prior to their deposition in the incubator boxes. Eggs were not counted manually due to time constraints and high sensitivity of *B. barbus* eggs to handling (personal observation by Bašić).

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

described in literature and mimicked natural conditions in UK lowland rivers around the time of spawning.

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

Upon emergence to the water surface, larvae were captured daily from treatment and control incubation boxes, counted and transferred to separate holding cages (0.17 x 0.13 x 0.13 m). Daily enumeration and removal of emergent larvae continued through the emergence period

and after 3 consecutive days of no emergence from any treatment/control, the experiment concluded. Upon experiment completion fish were stocked into a nursery pond but no subsequent measurements of physiology or fate were made.

2.3 Data analysis

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

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

GLMMs were fitted by maximum likelihood using a Laplace approximation (family-binomial; link-logit; Zuur, Ieno, Walker, Saveliev, & Smith, 2009) in R (R Development Core Team, 2011) package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Model assumptions were checked using standard graphical validation for GLMM and LM in R (Zurr et al., 2009). The significance of fixed effect/s for each model was assessed using an *F* test (LM) or Wald test

(GLMMs) in the R car package (Fox & Weisberg 2011). Following a significant effect of treatment on egg to emergence survival, time to 50 % emergence or proportion of emerged larvae, comparisons of covariate adjusted means were conducted via least-squares means with Dunnett adjustments for P values for multiple independent comparisons using multcomp package in R (Hothorn, Bretz, & Westfall, 2008).

3 RESULTS

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

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

The interaction of treatment and experimental time had a significant effect (Wald $\chi^2_{(49)} = 113921$; $P < 0.01$) on the proportion of emerged larvae to the surface water column between treatments (Table 5). Dunnett's pairwise comparison revealed significant differences between proportions of larvae emerged to the surface water in the control and 10% sand treatment from the thirteenth day of the experiment ($z = 2.55$, $P = 0.04$; Table 5) until the nineteenth day of the experiment ($z = 1.33$, $P = 0.47$; Table 5). The daily proportions of emerged larvae to the surface water varied significantly between the control and 20% sand treatment from day 12 (z

= 4.58, $P = 0.01$; Table 5) to day 18 of the experiment ($z = 2.42$, $P = 0.05$; Table 4), but the overall rate of emergence equalized thereafter (Table 5; Figure 4).

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

4 DISCUSSION

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

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.

Other investigations into emergence of salmonid species found limited impacts of fine sediment on larval survival. For example, no significant differences in survival and total emergence were detected for *Salmo salar* and *Oncorhynchus mykiss* larvae between treatments with variable fine sediment content (Fudge et al., 2008; MacCrimmon & Gots, 1985). However, emergence patterns changed with sediment treatment such that in MacCrimmon and Gots (1985), mean time to initial emergence to the water column and time to median emergence

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

influence on the survival of *B. barbus* eggs or larvae during the experiment. For salmonid species, there is typically an inverse relationship between sand content and recruitment (Lapointe et al., 2004; Sear et al., 2016; Zimmerman & Lapointe, 2005), with mortalities expected for UK salmonids (e.g. *Salmo salar*, *Salmo trutta*) where < 1 mm and < 2mm particles make up over 5.4 - 15 and 10 - 20 % of the bed, respectively (Table 1). Pacific Salmon are also less tolerant of substrates with fines exceeding 7.5 - 21 (< 1 mm) and 11 % (< 2 mm) (Table 1). Longer incubation time of salmonid eggs and larvae (four to six months) in the gravel (Hendry, Hensleigh, & Reisenbichler, 1998; Malcolm, Middlemas, Soulsby, Middlemas, & Youngson, 2010; Murray & McPhail, 1988) can increase risk of entombment (Franssen et al., 2012; Fudge et al., 2008; Sternecker & Geist, 2010) and likelihood of asphyxiation resulting from elevated sedimentation rates during early development (e.g. Bowerman et al., 2014; Levasseur et al., 2006; Sear et al., 2016). In contrast, *B. barbus* spawns during late spring in warmer conditions, thus their incubation time is significantly shorter, often less than two weeks, depending on temperature (Wijmans, 2007; Kemp et al., 2011). Salmonid eggs and larvae are typically buried at comparatively greater depths in the substratum (mean bottom egg pocket depth: 28.6 cm; Table 1) as opposed to shallow spawners such as *B. barbus* (around 5 cm; personal observation by Bašić), which may impose a higher risk of entombment (Lisle, 1989, Montgomery et al., 1996; Wijmans, 2007). However, risks of predation and egg entrainment are presumably higher for shallow-spawning fishes. A shorter incubation time appears to have been advantageous for *B. barbus* in this study, although spring/summer spawning and shallow burial depth could also increase egg and larval predation risk, given elevated metabolic rates and so, food requirements of piscivorous predators around that time. As incubation time and egg burial depth could have significant implications for reproductive success of different fish species, greater understanding of the nature of spawning and its impact

on species resistance to environmental stressors is therefore required to aid conservation efforts.

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

Increased sedimentation can reduce the natural resilience of freshwater ecosystems to present and future perturbations, with growing populations and so, demands for food and agriculture, likely to exacerbate ecological impacts. Furthermore, climate change scenarios project increased air temperatures and alterations of precipitation patterns (UKCP09: Murphy et al., 2009), potentially reducing river flows and increasing sedimentation rates. Anthropogenic activities and their impacts on river hydrology and geomorphology can have major implications for lithophilic species, potentially influencing fish recruitment and viability. With scarce knowledge on early development of non-salmonid lithophilic fishes, there is a requirement to investigate impacts of fine sediment on egg survival and larval emergence of a range of species

under *in-* and *ex-situ* conditions. Coupled with knowledge of spawning habitat conditions in rivers for a variety of species, these data can inform threshold setting based on the quality of fluvial sediment and the biological impact. Such data could be used to inform managers on the efficiency of different sediment mitigation options in relation to predefined biological targets, as a first step in seeking to appropriately manage fish spawning habitats (Bašić et al., 2017).

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

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7 AUTHORS' CONTRIBUTION STATEMENT

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

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

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

Analysed the data: TB, AP, RB;

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

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, 1993ⁱ; Lorenz & Filer, 1989^j; Louhi et al., 2008^k;
719 Magee, McMahon, & Thurow, 1996^l; Moir, Soulsby, & Youngson, 2002^m; O'Connor &
720 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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724 Table 2. Grain size distributions of sand gravel mixtures used in control and treatment
725 incubation boxes, expressed in weight percentages. Number of replicates are represented
726 by n.

Treatment						
Wentworth (1922) class	Grain	Control	10 %	20 %	30 %	40 %
	size	no sand	sand	sand	sand	sand
	(mm)	(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 %
Gravel	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
	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. 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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Table 4. Results of LM testing for the effect of treatment on the time required to 50 % emergence. Mean differences are from estimated least-square means, significant at * $P < 0.05$ 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 ± 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 , $P < 0.01^{**}$

Table 5 Results of GLMMs testing: 1) differences in egg to emergence survival between treatments, 2) differences in cumulative proportion of daily emerged larvae to the surface water between treatments. Mean differences are from estimated least-square 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	<i>z</i>	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, $P > 0.05$

Figure captions

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

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

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

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