#### Environmental Pollution xxx (2017) 1-11



Contents lists available at ScienceDirect

# **Environmental Pollution**



journal homepage: www.elsevier.com/locate/envpol

# Sensitivity of the early life stages of a mayfly to fine sediment and orthophosphate levels $\stackrel{\star}{\sim}$

Nicholas C. Everall <sup>a</sup>, Matthew F. Johnson <sup>b, \*</sup>, Paul Wood <sup>c</sup>, Lauren Mattingley <sup>d</sup>

<sup>a</sup> Aquascience Consultancy Limited, Chesterfield, Derbyshire, S42 7JS, UK

<sup>b</sup> School of Geography, University of Nottingham, NG7 2RD, UK

<sup>c</sup> Department of Geography, Loughborough University, LE11 3TU, UK

<sup>d</sup> Salmon and Trout Conservation, Burgate Manor, Fordingbridge, Hampshire, UK

#### ARTICLE INFO

Article history: Received 15 July 2017 Received in revised form 26 September 2017 Accepted 31 October 2017 Available online xxx

# ABSTRACT

The ecological effects of interacting stressors within lotic ecosystems have been widely acknowledged. In particular, the ecological effects of elevated fine sediment inputs and phosphate have been identified as key factors influencing faunal community structure and composition. However, while knowledge regarding adult and larval life stage responses to environmental stressors has grown, there has been very limited research on their eggs. In this study, the eggs of the mayfly *Serratella ignita* (Ephemerellidae: Ephemeroptera) were collected and incubated in laboratory aquaria to hatching under differing concentrations of inert suspended sediment (SS) and orthophosphate (OP), individually and in combination. Results indicate that SS and OP have greater effects on egg survival than OP in isolation or when OP was added to elevated SS treatments. Egg mortality in control treatments was around 6% compared to 45% in treatments with 25 mg l<sup>-1</sup> SS and 52% in 0.3 mg l<sup>-1</sup> OP treatments. Even relatively modest levels of each stressor (10 mg l<sup>-1</sup> SS; 0.1 mg l<sup>-1</sup> OP), below national legal thresholds, had significant effects on egg survival to hatching. The results support calls for legal levels of SS to be reassessed and suggest that more research is required to assess the impacts of pollution on invertebrate egg development given their different sensitivity and exposure pathways compared to other life stages.

© 2017 Elsevier Ltd. All rights reserved.

## 1. Introduction

Freshwater organisms are currently subjected by multiple, simultaneous and interacting pressures, due to the co-occurrence of effects associated with climate and land-use change (Tockner et al., 2010; Mantyka-Pringle et al., 2014; Jackson et al., 2016; Sebater et al., 2016). A meta-analysis of research from marine ecosystems has shown that the effect of multiple stressors on aquatic organisms are complex and most frequently synergistic or additive, with the effects being greater or equal when stressors are combined (Przesławski et al., 2015). In contrast, a recent metaanalysis of freshwater ecosystems reported that the majority of interactions were antagonistic, with effects lower than expected for individual stressors (Jackson et al., 2016); although there has been

E-mail address: m.johnson@nottingham.ac.uk (M.F. Johnson).

https://doi.org/10.1016/j.envpol.2017.10.131 0269-7491/© 2017 Elsevier Ltd. All rights reserved. less research on lotic systems. The uncertainty surrounding the ecological response to multiple, co-occurring stressors can lead to unexpected ecological responses (Christensen et al., 2006; Lindenmayer et al., 2010; Dehedin et al., 2013). For example, Piggott et al. (2012) identified that the negative impacts of fine sediment on invertebrate and algal diversity were greater when water temperature was increased. Holmstrup et al. (2010) reviewed the impacts of multiple stressors on individual organisms, rather than the entire community, and reported that the majority of studies (including temperature, desiccation and chemicals) resulted in synergistic effects.

Aquatic communities are adapted to hydrological regime variability and associated fluxes of solutes and fine sediment (organic and inorganic) derived from the catchment. Lotic ecosystems require sediment inputs to maintain habitat heterogeneity and facilitate nutrient fluxes, but excessive loadings can have negative effects on river ecosystem functioning (Wood and Armitage, 1997; Jones et al., 2012). The US Environmental Protection Agency identified fine sediment deposition as the number one source of stream

 $<sup>\</sup>star$  This paper has been recommended for acceptance by Dr. Chen Da.

<sup>\*</sup> Corresponding author.

impairment and habitat degradation nationwide (USEPA, 2000; Evans-White et al., 2013). Fine sediment may degrade aquatic faunal communities and directly affect individual organisms due to burial, scour or abrasion of soft tissues, clogging of respiration structures (gills of invertebrates and fish), as well as reducing habitat quality and increased emigration from degraded habitats (e.g. Billota and Brazier, 2008; Béjar et al., 2017). In addition, fine sediments can reduce habitat availability by covering coarser sediments, filling interstices and modifying biogeochemical conditions by reducing dissolved oxygen concentrations whilst leading to elevation of the concentrations of pollutants within the substrate (Kemp et al., 2011; Jones et al., 2012; Descloux et al., 2014; Mathers et al., 2017). The majority of research centred on fine sediment deposition on aquatic organisms has focused on invertebrate larval community composition or adult life stages (Roy et al., 2003; Extence et al., 2013; Bona et al., 2016). For example, the detrimental effects of fine sediment on freshwater mussel population has been examined in detail given that many species are national or internationally endangered and have important functional roles in rivers (Denic and Geist, 2015; Lummer et al., 2016). However, with the exception of salmonid fish (e.g. Grieg et al., 2005; Jensen et al., 2009; Sternecker and Geist, 2010; Chapman et al., 2014), few studies have considered the effects of enhanced fine sediment loading on the egg/embryonic life stages of aquatic fauna.

The effects of elevated phosphorus concentrations on aquatic environments, particularly the proliferation of nuisance phytoplankton and both epiphytic and benthic algae has been widely documented (Mainstone and Parr, 2002; Evans-White et al., 2013; Azevedo et al., 2015) and represents a significant threat to water quality and environmental integrity, internationally (Nijboer and Verdonschot, 2004; Smith and Schindler, 2009; Javie et al., 2015). It is well established that nutrient enrichment (eutrophication) has resulted in the reduction of macroinvertebrate community richness through the extirpation of sensitive taxa, particularly within the insect orders Ephemeroptera, Plecoptera and Trichoptera (Ortiz and Puig, 2007; Friberg et al., 2010; Bini et al., 2014). Orthophosphate (OP) or 'soluble reactive phosphorus' is bioavailable to freshwater organisms and the exceedance of the OP standard has been identified as the single largest cause of water bodies not achieving 'good ecological status' in the UK, under the European Union Water Framework Directive (WFD) (Environment Agency, 2012). Phosphorous concentrations have increased in many regions, often linked to human and animal waste; for example, concentrations of Total Dissolved Phosphorous increased by 2000% between 1970 and 2000 in northern Chinese Rivers (Strokal et al., 2016). Phosphorous can be particularly problematic because ecological recovery does not necessarily follow a reduction of concentrations in the environment due to lag times in ecological responses, complex indirect impacts of elevated phosphorous on aquatic communities, and the effects of associated stressors (Javie et al., 2013). In addition, phosphorous can be bound to sediment and remobilised at a later date when phosphorous inputs into the system may be negligible (Meng et al., 2014; Wood et al., 2015; Emelko et al., 2016). Internationally, elevated nutrient and sediment loads are a management priority and are acknowledged to be the primary contributing factor to over 40% of US waters being in poor biological condition (Evans-White et al., 2013).

Some pollutants may have potentially greater effects on early life stages of aquatic biota as they are typically the least mobile and therefore the most vulnerable to disturbance events (Clements and Newman, 2002; Przesławski et al., 2015). Despite a substantial literature on fish eggs and sedimentation (e.g. see Kemp et al., 2011), relationships between aquatic invertebrates (e.g. Denic and Geist, 2015) and especially their egg survival and environmental stressors are almost completely lacking (but see Gleason et al., 2003; Kefford et al., 2010). Therefore, this study focuses on the effects of increasing suspended sediment (SS) and OP concentrations individually and in combination on the survival and hatching success of the eggs of a widespread and ecologically important aquatic insect larvae, *Seratella ignita* (Ephemerellidae: Ephmeroptera) under experimental conditions. This was achieved by investigating whether:

- 1. Elevated SS concentration impaired egg survival and hatching.
- 2. Elevated OP concentration impaired egg survival and hatching.
- 3. Higher concentrations of SS and/or OP had greater effects on egg survival and hatching than lower concentrations.
- 4. SS and OP in combination effect hatching/survival to a greater degree than in isolation.

#### 2. Methodology

#### 2.1. Target organism

The Blue-Winged Olive Mayfly (Serratella ignita (Poda, 1761): Ephemeroptera: Ephemerellidae) is one of the most common Ephemeroptera species in the British Isles and is present across most of Europe, including the Mediterranean region. Typically nymphs are found in unpolluted, fast flowing systems, emerging between June and September, with nymphs present in the river from March to September (Elliott & Humpesch, 2010; Macadam and Bennett, 2010), although this varies depending on thermal regime and flow permanence (López-Rodríguez et al., 2009). Their life cycle typically includes a long overwintering period in the egg stage. Females of S. ignita produce a ball of eggs attached to the posterior underside of the abdomen. The animal descends to the water surface, releasing the egg mass which sinks and becomes anchored to the substrate via fibrous attachments (Gaino and Bongiovanni, 1992). S. ignita is ecologically important because of its widespread distribution and high abundance, which makes it significant for supporting fisheries. However, numbers have declined in a number of UK rivers over the past 20 years, particularly chalk streams (Bennett and Gilchrist, 2010). S. ignita larvae are known to be sensitive to fine sediment loading and OP concentration, with investigations linking losses of S. ignita to enhanced fine sediment loading effects in European rivers (Everall, 2010; Larsen et al., 2011; Minutoli et al., 2013).

# 2.2. Experimental set-up and overview

Experiments were undertaken in experimental, laboratory chambers (Fig. 1). A total of 24 chambers were run in parallel for the duration of the experimental period, each representing a different treatment. Each experimental chamber housed 3 glass laboratory slides, which acted as a substrate for *S. ignita* eggs. Each slide contained approximately 30 egg masses which were left to develop on the slides for 8 months under either control or experimental treatment conditions. The experimental chambers consisted of plastic funnels with a slide mount lodged above the outflow. The 3 glass slides were held vertically at 45° and in parallel in the slide mount and remained submerged for the duration of the experiment in the 20 mm diameter circular container.

The water used in experiments was aerated and had predetermined concentrations of OP and SS. It was held in 25 I reservoirs, elevated above the experimental chamber and allowed to flow through the system under gravity from a tap, which limited the flow rate to 0.65 ml min<sup>-1</sup>. Water drained through the experimental chamber and out through a pipe to a drain (Fig. 1).

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11



**Fig. 1.** Schematic of *S. ignita* egg dosing rigs for controls and treatments. A reservoir with experiment solution is held above a funnel, within which 3 slides containing *S. ignita* eggs are held in a slide holder. A perforated ring of tubing on the base of the reservoir ensured complete mixing and aeration of experimental water.

## 2.3. Serratella ignita egg collection and laboratory acclimation

Hundreds of swarming gravid adult *Serratella ignita* were collected from above the water surface of the River Manifold in Staffordshire, UK ( $53^{\circ}09'49.15''$ N;  $001^{\circ}51'35.70''$ W) in August 2015. Adult *S. ignita* were carefully transferred in ventilated plastic aquaria ( $40 \times 25$  cm) fitted with temporary cardboard floors to the laboratory where they were placed on top of white plastic trays ( $38 \times 22 \times 5$  cm). Each tray bottom was lined with 36 sterilised glass slides and covered by 2 cm of aerated water from the sample site. The temporary cardboard floors of the adult mayfly aquaria were removed allowing gravid female *S. ignita* to access the river water surface in the glass slide lined trays to lay their eggs.

Over 24 h, the gravid female *S. ignita* laid their egg masses on to the water surface whereupon they sank and became attached to the glass slides lining the trays. Spent *S. ignita* spinners and body parts (Supplementary Material A) were carefully removed from the egg slides using sterilised steel forceps, paying attention not to disturb the deposited egg masses. The egg mass covered slides were left *in situ* for another 24 h to allow egg mass adhesion to the glass slides after which they were transferred to slide holders in the treatment chambers (Supplementary Material B) using sterilised forceps. Prior to transfer, each slide had the number of egg masses per slide recorded in indelible ink on the slide. All 24 experimental chambers had been running for 20 days with a discharge flow of 0.65 ml min<sup>-1</sup> of carbon-limestone filtered tap water prior to slide introduction.

All of the egg mass slides were left to acclimate in flow through chambers supplied with de-chlorinated, filtered tap water for a further 24 h prior to the commencement of experiments with treatment exposures. The acclimation and treatment bioassays were subject to ambient outdoor air temperatures, humidity and light regime during the experimental period between August 2015 and March 2016.

# 2.4. Bioassay design and egg monitoring

A summary of the bioassay treatments used in the experiment are presented in Table 1. Every month of the bioassay the 3 slides in each treatment were placed under a microscope for a few minutes in a wet mount containing the appropriate bioassay test solution for observation. The slides were examined for any egg mass loss, egg mass emergence, and secondary biological growth e.g. fungal hyphomycae. Egg mass emergence was considered when approximately >90% of the viable eggs in the mass had hatched. Egg mass loss was considered to have occurred if a similar proportion of the individual eggs within the mass had died or if the egg mass had fallen from the slide, identification of which was aided by marks left on the slide by displaced egg masses (Supplementary material C). Secondary fungal hyphomycae growth was clearly identifiable under a microscope. In subsequent analysis, the status of egg masses was aggregated across the 3 slides.

After 3 months the slides in each treatment were carefully checked monthly for individual egg mortalities, within egg masses. It was not possible to count every egg within all egg masses. Instead, an egg mass was randomly selected from each of the 3 slides in each experimental chamber and the state of 200 eggs were counted under a microscope. Eggs were recorded as being either healthy or dead, where dead eggs were readily identified because they turned an opaque white, as reported previously by Yeo and Dechoretz (1976). Any egg mass chosen for egg mortality observation had an indelible dot placed on the reverse side of the slide so that it was not chosen again for observation. In subsequent analysis, egg mortality was aggregated across the 3 slides in each experimental chamber, giving a total sample of 600 individual eggs for each treatment.

#### 2.5. Chemical dosing and testing

The base and experimental control water during the acclimation and bioassay testing period was tap water run at 0.65 ml min<sup>-1</sup> through filters housing a mix ratio of 5:1 6 mm limestone chippings: granular activated carbon to reduce background levels of OP in tap water and remove any trace impurities. Test compounds were made up from the base water with the required dose additions of Sigma Aldrich 1000 mg l<sup>-1</sup> Orthophosphate and 1000 mg l<sup>-1</sup> Total Suspended Solids (inert silica particles, diameter 5–100 µm) calibration standards.

Water temperature in experimental test chambers was recorded daily in the control and once a month in other treatments. Daily water temperature mirrored ambient air temperature

Table 1Experimental designs for bioassays.

Chemical treatment	Non	Nominal chemical concentration mg/l				
Suspended sediment (SS)	0	5	10	15	20	25
Orthophosphate (OP)	0	0.05	0.07	0.1	0.2	0.3
$0.07 \text{ mg } l^{-1} \text{ OP} + \text{SS}$	0	5	10	15	20	25
$10 \text{ mg } l^{-1} \text{ SS} + \text{OP}$	0	0.05	0.07	0.1	0.2	0.3

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11

(Supplementary material D). Dissolved oxygen and pH were also recorded once a month across all the treatments. Water samples were taken from all of the bioassay test chambers once a month across the 8 month experimental period using an overflow valve fitted into the treatment rigs (Fig. 1). All samples for chemical analysis were sent to the UKAS Accredited National Laboratory Service for monthly analyses of Total Nitrogen, Ammoniacal Nitrogen, Nitrite, Alkalinity (to pH 4.5 as CaCO3), Orthophosphate, pH, Suspended Solids (at 105 °C), Boron, Calcium, Iron, Lithium, Magnesium, Manganese, Sodium, Water hardness (Total as CaCO3), Arsenic, Selenium, Cadmium, Copper, Lead, Mercury, Nickel and Zinc (Supplementary material E). The test treatments were also tested monthly for respective actual dosed SS and OP levels.

Mean physical-chemical properties for each of the bioassay chambers from 8 monthly samples across the study period are presented in Table 2. Individual monthly measurements of physical-chemical conditions are presented in Supplementary material D and clearly indicate that background water quality was stable with low level of trace chemicals in the control and test diluent water during the tests (Supplementary material E). With the exception of the test variables, there were no significant differences found between diluent physical-chemical conditions in the bioassays across the 8 month experiment (p < 0.01; ANOVA). The measured concentrations of SS and OP in water samples also display good spatial and temporal stability with the dosed concentration. In control experiments, OP concentrations were ~0.04 mg l<sup>-1</sup> despite ~96–98% phosphate removal from the baseline diluent tap water, similar to the performance of phosphorous removal of other workers using this type of filter (e.g. Hussain et al., 2011).

## 2.6. Statistical analysis

A regression modelling approach was used to examine the impact of concentration gradients of OP and SS on egg mortality. The number of dead eggs recorded over time, from a sub-sample of 600 eggs, was recorded for different concentrations and combinations of OP or SS. Regression models were developed for the association between the total number of dead eggs and the

## Table 2

Mean physical-chemica	l properties fo	r each bioassay
-----------------------	-----------------	-----------------

concentration of OP and/or SS for different exposure periods. In addition, regression models were developed for the association between the percentage of egg masses that emerged at the end of the experiment and OP and SS concentrations, and combinations to examine additive effects.

## 3. Results

#### 3.1. Egg mortality within bioassays

The number of dead eggs recorded within egg masses increased as the concentration of OP and SS increased above control levels. Egg mortality in control experiments were consistent between treatments and remained low, averaging 5.8% of sampled eggs across all treatments and ranging from 27 to 42 eggs out of 600. As concentration of SS and OP increased, there were substantial increases in egg mortality, representing a 972% increase under the highest OP levels and 1261% increase under the highest SS concentration over control levels. Mortality increased exponentially with SS and OP concentration when dosed individually (Fig. 2a; b), with significant regression models developed between OP or SS concentration and mortality after 71 days of exposure (SS p < 0.01,  $R^2 = 0.88$ ; OP p < 0.01,  $R^2 = 0.99$ ). After 183 days of exposure, exponential relationships between OP or SS concentration remained significant (p < 0.01 in both cases) with high explanatory power (98% of variance in both cases) (Table 3).

When SS and OP were dosed in combination, mortality increased over equivalent concentrations in isolation (Fig. 2c; d). The increase in egg mortality when 0.07 mg l<sup>-1</sup> OP was added was small, but consistent and the relationship remained exponential. In contrast, the addition of 10 mg l<sup>-1</sup> of SS to OP concentrations resulted in a marked increase in mortality and a change in the relationship between egg mortality and OP concentration from exponential to linear (Table 3).

In control runs and low doses of SS and OP (<5 mg  $l^{-1}$  and <0.1 mg  $l^{-1}$ , respectively), egg mortality did not increase over time, but remained around 6% of sampled eggs (Fig. 3a; b). Although mortality increased when SS was elevated to above 10 mg  $l^{-1}$ , egg mortality did not increase substantially over time, only increasing

Parameter	Mean water quality	$t \pm $ s.d. ( $n = 10$ )				
Nominal SS concentration (mg l <sup>-1</sup> )	0	5	10	15	20	25
Actual SS concentration (mg $l^{-1}$ )	<3	$5.2 \pm 0.2$	$10.0 \pm 0.1$	$14.9 \pm 0.2$	$20.0 \pm 0.2$	$24.7\pm0.6$
Water temperature (°C)	13.4 ± 4.9	13.4 ± 4.9	13.5 ± 4.9	$13.4 \pm 5.0$	13.3 ± 5.0	13.3 ± 4.9
Dissolved oxygen (mg l <sup>-1</sup> )	$10.5 \pm 0.4$	$10.3 \pm 0.4$	$10.3 \pm 0.4$	$10.3 \pm 0.4$	10.2 ± 0 0.2	$10.3 \pm 0.4$
рН	$8.0 \pm 0.1$	$8.0 \pm 0.1$	$8.0 \pm 0.1$	7.9 ± 0.3	7.9 ± 0.1	$7.9 \pm 0.1$
Nominal OP concentration (mg l <sup>-1</sup> )	0	0.05	0.07	0.1	0.2	0.3
Actual OP concentration (mg l <sup>-1</sup> )	$0.04 \pm 0.01$	$0.05 \pm 0.01$	$0.07 \pm 0.02$	0.11 ± 0.01	$0.20 \pm 0.01$	$0.30 \pm 0.01$
Water temperature (°C)	13.4 ± 5.1	13.1 ± 4.9	$13.0 \pm 5.0$	13.0 ± 4.9	12.9 ± 5.0	$12.8 \pm 5.0$
Dissolved oxygen (mg $l^{-1}$ )	$10.5 \pm 0.4$	$10.2 \pm 0.3$	$10.2 \pm 0.3$	$10.3 \pm 0.3$	$10.2 \pm 0.3$	$10.3 \pm 0.4$
рН	$7.9 \pm 0.1$	7.9 ± 0.1	7.9 ± 0.1	7.8 ± 0.3	7.8 ± 0.1	$7.8 \pm 0.1$
Nominal SS concentration (mg l <sup>-1</sup> )	0	5	10	15	20	25
Actual SS concentration (mg $l^{-1}$ )	<3	$5.2 \pm 0.4$	$10.0 \pm 0.1$	$15.0 \pm 0.1$	$20.1 \pm 0.2$	$25.0\pm0.2$
Nominal OP concentration (mg l <sup>-1</sup> )	0.07	0.07	0.07	0.07	0.07	0.07
Actual OP concentration (mg l <sup>-1</sup> )	$0.04 \pm 0.01$	$0.08 \pm 0.01$	$0.07 \pm 0.01$	$0.07 \pm 0.004$	0.07 ± 0 0.003	$0.07 \pm 0.01$
Water temperature (°C)	13.7 ± 5.2	13.6 ± 4.7	13.6 ± 4.5	13.3 ± 4.9	13.0 ± 4.7	$12.9 \pm 4.8$
Dissolved oxygen (mg l <sup>-1</sup> )	$10.4 \pm 0.4$	$10.0 \pm 0.5$	$10.2 \pm 0.3$	$10.2 \pm 0.3$	$10.2 \pm 0.4$	$10.0 \pm 0.3$
рН	$8.0 \pm 0.1$	$8.1 \pm 0.1$	$8.0 \pm 0.1$	$8.0 \pm 0.3$	$8.0 \pm 0.1$	$8.2 \pm 0.2$
Nominal OP concentration (mg l <sup>-1</sup> )	0	0.05	0.07	0.1	0.2	0.3
Actual OP concentration (mg l <sup>-1</sup> )	$0.04 \pm 0.01$	$0.05 \pm 0.004$	$0.07 \pm 0.004$	$0.10 \pm 0.01$	$0.21 \pm 0.01$	$0.31 \pm 0.01$
Nominal SS concentration (mg l <sup>-1</sup> )	10	10	10	10	10	10
Actual SS concentration (mg $l^{-1}$ )	<3	$9.9 \pm 0.5$	$10.0 \pm 0.2$	$10.2 \pm 0.3$	9.9 ± 0.3	$10.0 \pm 0.1$
Water temperature (°C)	13.3 ± 4.6	13.3 ± 4.7	13.4 ± 5.2	13.4 ± 4.7	13.1 ± 5.0	$13.0 \pm 4.9$
Dissolved oxygen (mg $l^{-1}$ )	$10.2 \pm 0.4$	$10.7 \pm 0.4$	$11.0 \pm 0.6$	$10.3 \pm 0.5$	$10.1 \pm 0.4$	$10.1 \pm 0.3$
рН	$8.0\pm0.1$	8.1 ± 0.1	8.0 ± 0.2	7.9 ± 0.3	$8.3\pm0.2$	8.3 ± 0.3

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11



**Fig. 2.** Regressions of the mortality of *S. ignita* eggs after 72 days exposure (open circles) and 183 days exposure (filled circles) against (a) SS, (b) OP), (c) SS in addition to 0.07 mg  $l^{-1}$  OP, and (d) OP in addition to 10 mg  $l^{-1}$  SS.

#### Table 3

Regression equations and significance values for OP and/or SS concentration against egg mortality. Note relationships are all exponential with the exception of OP + SS, where the strongest relationship was linear. All regressions are significant (p < 0.01).

Treatment	Time (days)	Equation	R <sup>2</sup>
OP	72	25.495e <sup>5.265x</sup>	0.88
	121	29.162e <sup>7.225x</sup>	0.98
	183	29.324e <sup>8.189x</sup>	0.98
SS	72	22.817e <sup>0.099x</sup>	0.99
	121	25.832e <sup>0.115x</sup>	0.97
	183	27.70e <sup>0.120x</sup>	0.98
OP + SS	72 121 183	$\begin{array}{r} 781.39x + 5.8258 \\ 921.45x + 35.319 \\ 1155.2x + 51.769 \end{array}$	0.98 0.92 0.93
SS + OP	72	22.446e <sup>0.095x</sup>	0.96
	121	27.646e <sup>0.113x</sup>	0.98
	183	36.937e <sup>0.111x</sup>	0.98

by about 10% of the sampled eggs over the duration of experiments (10 mg  $l^{-1}$  10%–20%; 15 mg  $l^{-1}$  19%–29%). When SS was above 20 mg  $l^{-1}$ , mortality increased through time linearly, from 45% to 80% of sampled eggs in the case of the highest dose (Fig. 3a). For OP, mortality consistently increased through time for all treatments except the control; however this was limited to less than 6% for all treatments, except the highest two concentrations (Fig. 3b). Similar patterns were observed when OP and SS were dosed in combination, with egg mortality increasing through time with the rate of mortality increasing as dosage increased. When SS was added to OP treatments, egg mortality increased faster and to a higher percentage of sampled eggs in comparison to when OP was added to SS treatments (Fig. 3c and d).

#### 3.2. Egg mass emergence in bioassays

The number of egg masses that emerged decreased exponentially as concentration of OP or SS increased (OP R<sup>2</sup> = 0.998; SS R<sup>2</sup> = 0.963; p < 0.01 in both cases). OP effects were discernible from control treatments at 0.1 mg l<sup>-1</sup> and from SS controls at 10 mg l<sup>-1</sup> (Fig. 4a and b). The emergence of egg masses exposed to OP declined substantially when 10 mg l<sup>-1</sup> of suspended sediment was added to treatments, supporting the findings of individual egg counts (Fig. 4a). An exponential relationship between egg emergence and OP persisted with the addition of SS (p < 0.01; R<sup>2</sup> = 0.998) but with greater egg mass emergence at concentrations of OP 0.1 mg l<sup>-1</sup> and above. In contrast, when 0.07 mg l<sup>-1</sup> of OP was added to SS treatments, there was no clear difference between egg mass emergence with and without OP (Fig. 4b).

The 3 separate slides within each treatment indicated very high consistency in results observed. The largest difference in egg mass emergence between the 3 slides within each treatment was 27% for those subjected to 0.3 mg l<sup>-1</sup> OP plus 10 mg l<sup>-1</sup> SS (Fig. 5).

# 4. Discussion

#### 4.1. Effects of multiple stressors

When low levels of SS were added to OP treatments, the mortality rate of eggs increased markedly, indicating that SS and OP had a greater impact on *S. ignita* when combined than when present individually. However, when low levels of OP were added to SS treatments, there was no discernible effect on mortality rates above SS in isolation, suggesting SS had a greater effect on egg and egg mass survival than OP.

Studies focusing on multiple stressors have consistently reported fine sediment to be a more pervasive stressor to the abundance of individual invertebrate species (Wagenhoff *et. al.*, 2012)

# **ARTICLE IN PRESS**

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11



Fig. 3. Mortality of S. ignita eggs through time under differing concentrations of (a) SS; (b) OP; (c) SS plus 0.07 mg  $l^{-1}$  OP, and; (d) OP plus 10 mg  $l^{-1}$  SS.

and invertebrate communities (Piggott et al. 2015; Elbrecht et al., 2016) than enhanced nutrient concentrations, indicating that priority should be given to minimising fine sediment over nutrient inputs. However, contrasting results have been found in some cases where chemical composition of fine sediment was more important than sediment quantity in controlling invertebrate community composition (von Bertrub et al., 2013). For example, Anderson et al. (2006) found uncontaminated sediments had no effect on the survival of several invertebrate species (Hyalella Azteca [amphipoda; Hyalellidae]; Procloeon sp. [Ephemeroptera; Baetidae]; Chironomus dilutes [Diptera; Chironomidae]). In the current experiments, suspended sediment was inert silica particles, clearly demonstrating that it is the deposition of sediment, rather than associated chemicals, that effected S. ignita egg development. A potential explanation for the difference between studies is that the species examined by Anderson et al. (2006) were characteristic of slow flowing lowland streams or marginal habitats dominated by macrophytes, where fine sediment concentrations and accumulations were naturally high. This contrasts with S. ignita, which is typical of moderate flowing streams with coarse substrates and where SS concentration is likely to be much lower than lowland reaches. As such, S. ignita is adapted to environments with naturally lower concentrations of fines and as a result fine sediment potentially acts as a stressor at lower concentrations than for many species adapted to slow flowing habitats (Elliott and Humpesch, 2010). Consequently, it is likely that the relative significance of SS and associated contaminates will depend on the receptor species and their association with specific habitats.

Each female *S. ignita* produces many eggs and as a result the effect of the elevated egg mortality on the viability of populations is difficult to assess. For example, it is possible that if hatching success was high in rivers, density dependent processes may result in many early instar larvae perishing, reducing the population level effects of egg mortality due to anthropogenic stressors. Therefore the results here do not necessary imply population level impacts. In

addition, *S. ignita* is a common species in the UK and Europe and often occurs in high abundance. However, *S. ignita* larvae have been shown to be highly sensitive to sedimentation and their presence is used in national biological metrics to indicate reduced fine sediment pressure (Extence et al., 2013). In addition, the proportion of individuals commonly surviving through to reproduction is not known and there is both anecdotal and documented evidence that the abundance of *S. ignita* has declined over the past 20 years in some English rivers (Bennett and Gilchrist, 2010).

#### 4.2. The effect of suspended sediment on eggs

The results of this study indicate that the egg stage of *Serratella ignita* is susceptible to sustained high levels of SS concentration during their 8 month developmental period. Concentration dependent mortality of *S. ignita* eggs was evident at annual mean equivalent concentrations of 10–25 mg l<sup>-1</sup> but levels of fine sediment <10 mg l<sup>-1</sup> displayed no markedly higher egg mortality than the control treatments. The cause of egg mortality is hypothesised to be reduced oxygen transfer due to sediment coating egg surfaces. Additionally, the build-up of fine sediment over time caused some of the egg masses to be dislodged from the slides after 6 months of exposure in the 20 and 25 mg l<sup>-1</sup> treatments. These egg masses were eroded and lost within the dosing rig sumps and, therefore, it is not clear if the individual eggs were still viable; however, in watercourses dislodgement exposing egg masses to scour damage, burial and predation would be highly disadvantageous.

Other experiments examining the effect of fine sediment covering on invertebrate eggs have reported reduced survival and hatching for *Chironomus cloacalis* (Diptera; Chironomidae), *Physa acuta* (Gastropod; Physidae) and *Gyraulus tasmanica* (Gastropod; Planorbidae) (Kefford et al. (2010). In control treatments without fine sediment, 100% of viable eggs of all three species hatched, but this was reduced when buried with clay (kaolin) or sand; although, the direct effects of suspended sediment were limited. Similarly,

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11



**Fig. 4.** Percentage of *S. ignita* egg masses surviving to emergence under different concentrations of (a) OP in isolation (open circles) and in combination with 10 mg  $l^{-1}$  of SS (closed circles) and (b) SS in isolation (open circles) and in combination with 0.07 mg  $l^{-1}$  of OP. Note, at 0.3 mg  $l^{-1}$  OP plus 10 mg  $l^{-1}$  SS, fungal growth prevented the majority of egg masses from emerging and prevented an accurate count of egg mass emergence.

Gleason et al. (2003) found that burial to 0.5 cm caused a 99.7% reduction in the emergence of invertebrate eggs from wetlands. The impact of SS on invertebrates is complex because of associated contaminates; for example, the source of sediment has been shown to be important for salmonid embryo development, primarily because the organic matter content of sediment consumes oxygen as it degrades, potentially reducing oxygen availability to developing embryos (Sear et al., 2016). In addition, the influence of other stressors confound ecological response; for example, Doretto et al. (2017) found that high availability of coarse particulate organic matter mitigated the negative effects of fine sediment, which clogged interstitial spaces in artificial substrates in the Po River, Italy.

Fish eggs are negatively effected by fine sediment (Kemp et al., 2011). Much of the research on fish embryo development and fine sediment has focused on the clogging of interstitial spaces in salmonid fish redds and associated reduction of interstitial flow volume and velocity (Jensen et al., 2009; Chapman et al., 2014). However, deposition of clay particles directly onto salmon (*Salma salar*) eggs has been shown to reduce oxygen exchange across the egg membrane and increase mortality (Grieg et al., 2005). This mechanism is also hypothesised to be responsible for mayfly egg mortality in these experiments. Research has also demonstrate that salmonid fish egg development can be effected by sedimentation by the prevention of the expulsion of metabolic wastes from the egg chorion (Chapman, 1988; Bennett et al., 2003). Concentrations of nitrates and ammonia may significantly affect salmonid egg



**Fig. 5.** Percentage of *S. ignita* egg masses surviving to emergence under different concentrations of (a) OP (b) SS for each of the 3 slides in each treatment indicated separately.

development (e.g. Sternecker et al. 2013) and Reynolds and Guillaume (1998) found phosphate concentrations of 0.5 mg  $l^{-1}$  resulted in earlier emergence of European Bitterling (*Rhodeus sericeus*) embryos from eggs deposited within the gills of freshwater mussels. However, little research has investigated the link between elevated phosphorous concentration and fish embryo development.

## 4.3. Phosphorous effects on egg development

Mortality of S. ignita eggs was evident at annual mean equivalent concentrations of 0.1–0.3 mg l<sup>-1</sup> OP but levels of biologically available phosphorous  $< 0.1 \text{ mg l}^{-1}$  resulted in no higher egg mortality than in control treatments. The cause of egg mortality in the highest dose of 0.3 mg  $l^{-1}$  appeared to be related to the growth of aquatic fungal filaments smothering the egg masses after 1 month of exposure. The adhesive, mucous coating of mayfly eggs has been postulated to protect the egg from bacterial and fungal attack (Gaino et al., 2009), although any protection appeared to have been lost as a result of elevated OP stimulating microbial growth, resulting from the high availability of phosphorous. From light microscopy examination of fungal smothered eggs both undetermined aquatic hyphomycete species and Fusarium aquaeductum were identified coating the egg surfaces. Aquatic hyphomycetes growing on fish eggs have been found to be pathogenic (Wedekend et al., 2010) and Fusarium species have been documented

7

parasitizing eggs of the Penaeid prawn *Marsupenaeus japonicus* (Momoyama, 1987).

Egg mortality in treatments with lower OP levels, where there was no evidence of fungal growth, suggested other direct impacts of elevated biologically available phosphorous levels. Chronic exposure to sub-lethal concentrations of phosphates have been reported to have negative effects on early stages of aquatic fauna; for example, abnormal embryonic development in sea urchin (*Lytechinus variegatus*) (Bottger and McClintock, 2002). In addition, the cells of more complex organisms have also shown impaired gene expression (Rutherford et al., 2006) and cell membrane scrambling (Voelkl et al., 2014) with increasing extracellular phosphate concentrations. Therefore, it is hypothesised that egg mortality in the range of continuous OP exposures of 0.1–0.2 mg l<sup>-1</sup> may have been due to direct physiological and genotoxic impacts.

## 4.4. Concentrations of OP and SS in rivers

The ability of an organism to survive exposure to a stressor is dependent upon the concentration of the parameter and duration of exposure (Tabak and Gibbs, 1991; Zhao and Newman, 2006; Cope et al., 2008). The total duration of exposure to a concentration of suspended sediment is acknowledged to be a key variable determining its effect on aquatic biota (Billota and Brazier, 2008). For example, Maturana et al. (2014) found that continuous, chronic exposure of sediment had a greater detrimental impact on salmonid embryos than instantaneous pulses of sediment. The exposure conditions here were not directly comparable to natural conditions within a river, where pulsed and intermittent exposure of organisms to sediments and nutrients are common (Alabaster and Lloyd, 1980; Davies and Bothwell, 2012; Outram et al., 2014). However, SS and OP levels used in these experiments represent relatively modest concentrations for many English rivers, typically below WFD specified thresholds.

The Environment Agency (EA), the statutory environmental regulator in England, recorded 32549 spot measurements of OP across England in 2015 and 22% of those were above 0.3 mg l<sup>-1</sup>, the highest concentration used in these experiments. Monthly spot measures were made at 1812 locations across England in 2015, where at least 9 measurements were made throughout the year. Of these sites, 22% had annual average values higher than 0.3 mg l<sup>-1</sup> and over half had average concentrations above 0.1 mg l<sup>-1</sup>, the lowest concentration used in these experiments with a statistical effect on egg survival. 26% of sites had OP levels above 0.1 mg l<sup>-1</sup> in every measurement made throughout the year (Table 4). These results are consistent with the work of Worrell et al. (2016) who calculated that annual average OP concentrations have declined from 0.19 to 0.1 mg l<sup>-1</sup> between 1974 and 2012 in England, Wales

and Scotland, based on routine monitoring data. Therefore, whilst concentrations of OP are declining across Europe (Bouraoui and Grizzetti, 2011), in many streams concentrations remain above those found to exert an effect in the experiments reported in this study. Fewer sites were sampled for suspended sediment but of the 129 sites with 9 or more measurements in 2015, 9% had average values higher than 25 mg  $l^{-1}$  and 39% had values over 10 mg  $l^{-1}$ , found to effect egg hatching in these experiments (Table 4). OP legal levels are dependent on site specific characteristics and physiochemical conditions but it is clear that low-to- moderately elevated levels can have direct effects on insect egg development, which may be accelerated at higher concentrations by fungal growth. In addition, elevated OP in rivers alters primary production leading to important indirect implications for dissolved oxygen concentrations and water temperature because excessive plant and algae growth can shade the water column, which may also impact insect egg development (Humpesch, 1980; Elliot, 1987; Pritchard et al., 1996; Bennett, 2007; Rotvit and Jacobsen, 2013).

The current experimental findings support the growing concern that the annual mean SS guideline standard of 25 mg l<sup>-1</sup> in the UK is not sufficient (WWF-UK, 2007). This is supported by other studies that have identified effects of fine sediment on invertebrate survival at levels  $>8 \text{ mg l}^{-1}$  in Canadian freshwaters (Rosenberg and Wiens, 1978; Quinn et. al., 1992). In these experiments, egg masses were lost because of sediment coverage and the weight of deposited sediment dislodging them, although it is not clear whether dislodgement occurred during or after egg health had deteriorated, potentially reducing their adhesive properties. In rivers, this would probably result in the burial and/or damage of eggs. The coating of eggs with sediment has implications for oxygen transfer which will be partly controlled by the extent of sediment coverage on the egg surface, as well as the particle size and shape. These parameters will be at least partially dependent on the flow velocity and sediment properties and are likely to be less well correlated to suspended sediment concentrations. Therefore, the results support the assertion of Billota and Brazier (2008) and Kefford et al. (2010) that standards should move away from turbidity or suspended sediment concentrations to focus on settlement rates and sediment properties. Similarly, the source of sediment could have different effects on egg mortality because of its ability to harbour other pollutants, including phosphorous.

## 4.5. Management implications

Previous research on the larval and adult stage of invertebrates indicates that elevated SS and OP are pervasive issues in river management (Friberg et al., 2010; Jones et al., 2012; Bini et al., 2014; Mathers et al., 2017). Internationally, OP concentrations remain

Table 4

Analysis of national routine spot measures of OP and SS made by the Environment Agency in 2015 for WFD compliance across England.

		Count	Average (mg l <sup>-1</sup> )	Percentage of sites where the average is				
				>0.3 mg l <sup>-1</sup>	$>0.1 \text{ mg } l^{-1}$	$>25 \text{ mg } l^{-1}$	$>10 \text{ mg } l^{-1}$	
All sites All sites	OP SS	32549 2029	0.27 16.6	21.8	51.6	9.2	31.6	
		Count	ount Average	Percentage of sites where every measurement is				
			$(mg l^{-1})$	>0.3 mg l <sup>-1</sup>	$>0.1 \text{ mg } l^{-1}$	$< 0.1 \text{ mg } l^{-1}$	$< 0.3 \text{ mg } l^{-1}$	
Sites > 9 samples	OP	1812	0.30	22.2	52.9	21.8	52.7	
		Count	Average (mg l <sup>-1</sup> )	Percentage of sites where every measurement is				
				>25 mg l <sup>-1</sup>	>10 mg l <sup>-1</sup>	<10 mg l <sup>-1</sup>	$<25 \text{ mg } l^{-1}$	
Sites > 9 samples	ss	129	12.7	9.4	39.0	49.6	10.9	

high and rising in many river systems, in particular due to agricultural intensification and population increases coupled with the direct discharge of untreated human waste (Tysmans et al., 2013; Strokal et al., 2016; Yan et al., 2016). Despite reductions in both SS and OP concentrations in river systems across Europe and North America, many rivers still show clear signs of negative impact (Javie et al., 2015; Blaas and Kroeze, 2016). This is likely to be partly related to the indirect impacts of OP and SS, their interaction with other stressors, lags in ecological response, and remobilisation of OP bound to sediments long after inputs into the river system have been reduced (Javie et al., 2012). However, the results presented here suggest that relatively low levels of both SS and OP can negatively affect invertebrate egg development. Therefore, it is possible that by focusing on the larval and adult stage of invertebrate development, important information is being missed about the tolerance of species during what is potentially their most vulnerable developmental stage. More information is needed on the effect of stressors on egg development as impaired hatching could have significant implications for invertebrate populations at lower pollutant concentrations than those observed to effect larval and adult stages of the same species.

#### 5. Conclusions

The effects of environmental pollutants on the eggs of aquatic invertebrates are not well understood despite the fact that eggs are potentially the most vulnerable life stage of many invertebrates. Relatively modest levels of SS and OP have highly significant detrimental effects on the mortality of S. ignita eggs, with potentially significant implications for populations of mayfly. Fine sediment was the more pervasive stressor, increasing mortality of eggs exposed to OP enrichment, whereas elevated OP levels did not significantly increase mortality in comparison to those exposed only to fine sediment. The direct mechanism for the detrimental effects on eggs is likely to be complex but suspended sediment settled onto eggs, coating them and under high dosage  $(>0.2 \text{ mg } l^{-1})$  resulting in dislodgement. High OP levels  $(>0.2 \text{ mg l}^{-1})$  fuelled the growth of hyphomycete, which negatively affected eggs. The mechanism by which lower levels of OP  $(0.1-0.2 \text{ mg } l^{-1})$  negatively impacted eggs, in the absence of hyphomycete growth, is not known. Current legal limits of SS and OP in the European Union are above those found to have an effect in the experiments reported in the study and suggests management needs to focus on elevated SS levels. Although levels are dropping across Europe – substantially in the case of OP – the results of these experiments support growing concern about current guidelines relating to SS and associated organic contaminants and the need for more stringent regulation.

#### Acknowledgements

We would like to thank Salmon and Trout Conservation UK for funding the chemical analysis and Ben Heynes for assistance with field work.

# Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.10.131.

#### References

Alabaster, J.S., Lloyd, R., 1980. Water Quality Criteria for Freshwater Fish. Food and Agriculture Organization of the United Nations, Butterworths, London. Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., Tjeerdema, R.S., 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): relative effects of pesticides and suspended particles. Environ. Pollut. 141, 402–408.

- Azevedo, L.B., van Zelm, R., Leuven, R.S.E.W., Hendriks, A.J., Huijbregts, M.A.J., 2015. Combined ecological risks of nitrogen and phosphorus in European freshwaters. Environ. Pollut. 200, 85–92.
- Béjar, M., Gibbins, C.N., Vericat, D., Batalla, R.J., 2017. Effects of suspended sediment transport on invertebrate drift. River Res. Appl. https://doi.org/10.1002/rra.3146.
- Bennett, C.J., 2007. The ecology of a seven year study of the life cycle of the mayfly *Ephemera danica*. Freshw. Forum 27, 3–14.
- Bennett, C., Gilchrist, W., 2010. Chapter 22. Riverflies. In: Maclean, N. (Ed.), Silent Summer. The State of Wildlife in Britain and Ireland. Cambridge University Press, Cambridge, UK.
- Bennett, D.H., Connor, W.P., Eaton, C.A., 2003. Substrate composition and emergence success of fall chinook salmon in the snake river. Northwest Sci. 77, 93–99.
- Billota, G.S., Brazier, R.E., 2008. Understanding the influence of suspended solids on water quality and aquatic biota. Water Res. 42, 2849–2861.
- Bini, L.M., Landeiro, V.L., Padial, A.A., Siqueira, T., Heino, J., 2014. Nutrient enrichment is related to two facets of beta diversity for stream invertebrates across the United States. Ecology 95, 1569–1578.
- Blaas, H., Kroeze, C., 2016. Excessive nitrogen and phosphorus in European rivers: 2000 – 2050. Ecol. Indic. 67, 328–337.
- Bona, F., Doretto, A., Falasco, E., La Morgia, V., Piano, E., Ajassa, R., Fengoglio, S., 2016. Increased sediment loads in alpine streams: an integrated field study. River Res. Appl. 32, 1316–1326.
- Bottger, S.A., McClintock, J.B., 2002. The effects of organic and inorganic phosphates on fertilization and early development in the sea urchin *Lytechinus variegatus* (Echinodermata: echinoidea). J. Exp. Zool. 292, 660–671.
- Bouraoui, F., Grizzetti, B., 2011. Long term change of nutrient concentrations of rivers discharging in European seas. Sci. Total Environ. 409, 4899–4916.
- Chapman, D.W., 1988. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc. 117, 1–21.
- Chapman, J.M., Proulx, C.L., Veilleux, M.A.N., Levert, C., Bliss, S., André, M.-È., Lapointe, N.W.R., Cooke, S.J., 2014. Clear as mud: a meta-analysis on the effects of sedimentation on freshwater fish and the effectiveness of sediment-control measures. Water Res. 56, 190–202.
- Christensen, M.R., Graham, M.D., Vinebrooke, R.D., Findlay, D.L., Paterson, M.J., Turner, M.A., 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Glob. Change Biol. 12, 2316–2322.
- Clements, W.H., Newman, M.C., 2002. In: Community Ecotoxicology. Hierarchical Ecotoxicology Series. John Wiley and Sons Ltd, Hoboken, USA.
- Cope, W.G., Bringolf, R.B., Buchwalter, D.B., Newton, T.J., Ingersoll, C.G., Wang, N., Augspurger, T., Dwyer, F.J., Barnhart, M.C., Neves, R.J., Hammer, E., 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. J. North Am. Benthol. Soc. 27, 451–462.
- Davies, J.M., Bothwell, M.L., 2012. Responses of lotic periphyton to pulses of phosphorus: P-flux controlled growth rate. Freshw. Biol. 57, 2602–2612.
- Dehedin, A., Maazouzi, C., Puijalon, S., Marmonier, P., Piscart, C., 2013. The combined effects of water level reduction and an increase in ammonia concentrations on organic matter processing by key freshwater shredders in alluvial wetlands. Glob. Change Biol. 19, 763–774.
- Denic, M., Geist, J., 2015. Linking stream sediment deposition and aquatic habitat quality in Pearl Mussel streams: implications for conservation. River Res. Appl. 31, 943–952.
- Descloux, S., Datry, T., Marmonier, P., 2014. Trait-based structure of invertebrates along a gradient of sediment colmation: benthos versus hyporheos responses. Sci. Total Environ. 466, 265–276.
- Doretto, A., Bona, F., Piano, E., Zanin, I., Eandi, A.C., Fenoglio, S., 2017. Trophic availability buffers the detrimental effects of clogging in an alpine stream. Sci. Total Environ. 592, 503–511.
- Elbrecht, V., Beerman, A.J., Goessler, G., Neumann, J., Tollrian, R., Wagner, R., Wlecklik, A., Piggott, J.J., Matthaei, C.D., Leese, F., 2016. Multiple-stressor effects on stream invertebrates: a mesocosm experiment manipulating nutrients, fine sediment and flow velocity. Freshwaer Biol. 61, 362–375.
- Elliott, J.M., Humpesch, U.H., 2010. Mayfly larvae (*Ephemeroptera*) of Britain and Ireland: keys and a review of their biology, 66. Scientific Publications of the Freshwater Biological association.
- Elliot, J.M., 1987. Temperature-induced changes in the lifecycle of Leuctra nigra (Plecoptera: Leuctridae) from a lake district stream. Freshw. Biol. 18, 177–184.
- Emelko, M.B., Stone, M., Silins, E., Allin, D., Collins, A.L., Williams, C.H.S., Martens, A.M., Bladon, K.D., 2016. Sediment-phosporus dynamics can shift aquatic ecology and cause downstream legacy effects after wildfire in large river systems. Glob. Change Biol. 22, 1168–1184.
- Environment Agency, 2012. Freshwater eutrophication: a nationally significant water management issue. Briefing note for 10.12.2012 workshop. Accessible at. http://www.rivermease.co.uk/wp-content/uploads/2015/07/Freshwater\_Eutrophication\_Briefing\_Note\_December\_20121.pdf.
- Evans-White, M.A., Haggard, B.E., Scott, J.T., 2013. A review of stream nutrient criteria development in the United States. J. Environ. Qual. 42, 1002–1014.
- Everall, N.C., 2010. The Aquatic Ecological Status of the Rivers of the Upper Dove Catchment in 2009. Natural England Commissioned Report NECR046. Natural England, Sheffield.
- Extence, C.A., Chadd, R.P., England, J., Dunbar, M.J., Wood, P.J., Taylor, E.D., 2013. The assessment of fine sediment accumulation in rivers using macro-invertebrate

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11

community response. River Res. Appl. 29, 17–55.

- Friberg, N., Skriver, J., Larsen, S.E., Pedersen, M.L., Buffagni, A., 2010. Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication. Freshw. Biol. 55, 1405–1419.
- Gaino, E., Sartori, M., Rebora, M., 2009. Chorionic fine structure of eggs from some species of Proboscidoplocia (Ephemeroptera; Ephemeroidea). Ital. J.Zool. 68, 1–8.
- Gaino, E., Bongiovanni, E., 1992. The egg of Pseudocentroptilum pennulatum: a scanning electron microscopy (SEM) study (Ephemeroptera, Baetidae). Bolletino della Soc. Entomoogical Ital. 12, 195–200.
- Gleason, R.A., Euliss Jr., N.H., Hubbard, D.E., Duffy, W.G., 2003. Effects of sediment load on emergence of aquatic invertebrates and plants from wetland soil egg and seed banks. Wetlands 23, 26–34.
- Grieg, S.M., Sear, D.A., Carling, P.A., 2005. The impact of fine sediment accumulation on the survival of incubating salmon progeny: implications for sediment management. Sci. Total Environ. 344, 241–258.
- Holmstrup, M., Bindesbøl, A.-M., Oostingh, G.J., Duschl, A., Scheil, V., Køhler, H.-R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C., Spurgeon, D.J., 2010. Interactions between effects of environmental chemicals and natural stressors: a review. Sci. Total Environ. 408, 3746–3762.
- Humpesch, U.H., 1980. Effect of temperature on the hatching time of eggs of five *Ecdyonurus* spp. (Ephemeroptera) from Austrian streams and English streams, rivers & lakes. J. Animal Ecol. 49, 317–333.
- Hussain, M., Hussain, S.M., Afzal, M., Javid, A., Abdullah, S., Raza, S.A., Iqbal, M., 2011. Effect of low level of mineral phosphorus and medium level of nitrogen on planktonic productivity and increase in fish production. J. Animal Plant Sci. 21, 642–645.
- Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Change Biol. 22, 180–189.
- Javie, H.P., Sharpley, A.N., Flaten, D., Kleinman, P.J.A., Jenkins, A., Simmons, T., 2015. The pivotal role of phosphorus in a resilient water-energy-food security nexus. J. Environ. Qual. 44, 1049–1062.
- Javie, H.P., Sharpley, A.N., Scott, J.T., Haggard, B.E., Bowes, M.J., Lesley, B.M., 2012. Within-river phosphorus retention: accounting for a missing piece in the watershed phosphorus puzzle. Environ. Sci. Technol. 46, 13284–13292.
- Javie, H.P., Sharpley, A.N., Withers, P.J.A., Scott, J.T., Haggard, B.E., Neal, C., 2013. Phosphorus mitigation to control river eutrophication: Murky waters, inconvenient truths, and "postnormal" science. J. Environ. Qual. 42, 295–304.
- Jensen, D.W., Steel, E.A., Fullerton, A.H., Pess, G.R., 2009. Impact of fine sediment on egg-to-fry survival of Pacific Salmon: a meta-analysis of published studies. Rev. Fish. Sci. 17, 348–359.

Jones, J.I., Murphy, J.F., Colins, A.L., Sear, D.A., Nadan, P.S., Armitage, P.D., 2012. The impact of fine sediment on macroinvertebrates. River Res. Appl. 28, 1055–1071.

- Kefford, B.J., Zalizniak, L., Dunlop, J.E., Nugegoda, D., Choy, S.C., 2010. How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? Environ. Pollut. 158, 543–550.
- Kemp, P., Sear, D., Collins, A., Nadan, P., Jones, I., 2011. The impact of fine sediment on riverine fish. Hydrol. Process. 25, 1800–1821.
- Larsen, S., Pace, G., Ormerod, S.J., 2011. Experimental effects of sediment deposition on the structure and function of macroinvertebrate assemblages in temperate streams. River Res. Appl. 27, 257–267.
- Lindenmayer, D.B., Likens, G.E., Krebs, C.J., Hobbs, R.J., 2010. Improved probability of detection of ecological "surprises". Proc. Natl. Acad. Sci. U. S. A. 107, 21957–21962.
- López-Rodríguez, M.J., de Figueroa, J.M.T., Alba-Tercedor, J., 2009. The life history of Serratella ignita (Poda, 1761) (Insecta: Ephemeroptera) in a temporary and permanent Mediterranean stream. Aquat. Sci. Research Across Boundaries 71, 179–188.
- Lummer, E.M., Auerswald, K., Geist, J., 2016. Fine sediment as environmental stressor affecting freshwater mussel behavior and ecosystem services. Sci. Total Environ. 571, 1340–1348.
- Macadam, C.R., Bennett, C.J., 2010. A Pictorial Guide to the British Ephemeroptera. Field Studies Council, Shrewsbury, UK.
- Mainstone, C.P., Parr, W., 2002. Phosphorus in rivers ecology and management. Sci. Total Environ. 282–283, 25–47.
- Maturana, O., Tonina, D., McKean, J.A., Buffington, J.M., Luce, C.H., Caamaño, D., 2014. Modeling the effects of pulsed versus chronic sand inputs on salmonid spawning habitat in a low-gradient gravel-bed river. Earth Surf. Process. Landforms 39, 877–889.
- Mantyka-Pringle, C.S., Martin, T.G., Moffatt, D.B., Linke, S., Rhodes, J.R., 2014. Understanding and predicting the combined effects of climate change and landuse changes on freshwater macroinvertebrates and fish. J. Appl. Ecol. 51, 572–581.
- Mathers, K.L., Rice, S.P., Wood, P.J., 2017. Temporal effects of enhanced fine sediment loading on macroinvertebrate community structure and functional traits. Sci. Total Environ. 599–600, 513–522.
- Meng, J., Yao, P., Yu, Z., Bianchi, T.S., Zhao, B., Pan, H., Li, D., 2014. Speciation, bioavailability and preservation of phosphorus in surface sediments of the Changjiang Estuary and adjacent East China Sea inner shelf. Estuar. Coast. Shelf Sci. 144, 27–38.
- Minutoli, R., Granata, A., Guglielmo, L., 2013. Potential use of ecotoxicological biomarkers in *Serratella ignita* (Ephemeroptera) larvae for Alcantara river (Sicily, Italy) water quality assessment. J. Limnol. 72, 394–399.

- Momoyama, K., 1987. Distributions of the hyphae in kuruma shrimps. Penaeus japonicas, infected with Fusarium solani. Fish. Pathol. 22, 15–23.
- Nijboer, R.C., Verdonschot, P.F., 2004. Variable selection for modelling effects of eutrophication on stream and river ecosystems. Ecol. Model. 177, 17–39.
- Ortiz, J.D., Puig, M.A., 2007. Point source effects on density, biomass and diversity of benthic macroinvertebrates in a Mediterranean stream. River Res. Appl. 23, 155–170.
- Outram, F.N., Lloyd, C.E.M., Jonczyk, J., Benskin, C.McW.H., Grant, F., Perks, M.T., Deasy, C., Burke, S.P., Collins, A.L., Freer, J., Haygarth, P.M., Hiscock, K.M., Johnes, P.J., Lovett, A.L., 2014. High-frequency monitoring of nitrogen and phosphorus response in three rural catchments to the end of the 2011 – 2012 drought in England. Hydrol. Earth Syst. Sci. 18, 3429–3448.
- Piggott, J.J., Lange, K., Townsend, C.R., Matthaei, C.D., 2012. Multiple stressors in agricultural streams: a mesocosm study of interactions among raised water temperature sediment addition and nutrient enrichments. PLoS One 7, e49873. https://doi.org/10.1371/journal.pone.0049873.
- Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. Glob. Change Biol. 21, 1887–1906.
- Pritchard, G., Harder, L.D., Mutch, R.A., 1996. Development of aquatic insect eggs in relation to temperature and strategies for dealing with different thermal environments. Biol. J. Linn. Soc. 58, 221–244.
- Przesławski, R., Byrne, M., Mellin, C., 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Glob. Change Biol. 21, 2122–2140.
- Quinn, J.M., Davies-Colley, R.J., Hickey, C.W., Vickers, M.L., Ryan, P.A., 1992. Effects of clay discharges on streams. Hydrobiologia 248, 235–247.
- Reynolds, J.D., Guillaume, H.P., 1998. Effects of phosphate on the reproductive symbiosis between bitterling and freshwater mussels: implications for conservation. J. Appl. Ecol. 35, 575–581.
- Rosenberg, D.M., Wiens, A.P., 1978. Effects of sedimentation on microbenthic invertebrates on a northern Canadian river. Water Res. 12, 753–763.
- Rotvit, L., Jacobsen, D., 2013. Egg development of Plecoptera, Ephemeroptera and Odonata along latitudinal gradients. Ecol. Entomol. 39, 177–185.
- Roy, A.H., Rosemond, A.D., Leigh, D.S., Paul, M.J., Wallace, J.B., 2003. Habitat specific responses of stream insects to land cover disturbance: biological consequences and monitoring implications. J. North Am. Benthol. Soc. 22, 292–307.
- Rutherford, R.B., Foster, B.L., Bammler, T., Beyer, R.P., Sato, S., Somerman, M.J., 2006. Extracellular phosphate alters cementoblast gene expression. J. Dent. Res. 85, 505–509.
- Sear, D.A., Jones, J.I., Collins, A.L., Hulin, A., Burke, N., Bateman, S., Pattison, I., Nadan, P.S., 2016. Does fine sediment source as well as quantity affect salmonid embryo mortality and development? Sci. Total Environ. 541, 957–968.
- Sebater, S., Barcelo, D., De Castro-Català, N., Ginebreda, A., Kuzmanovic, M., Petrovic, M., Picó, Y., Ponsatí, L., Tornés, E., Muñoz, I., 2016. Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. Environ. Pollut. 210, 308–314.
- Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24, 201–207.
- Sternecker, K., Geist, J., 2010. The effects of stream substratum composition on the emergence of salmonid fry. Ecol. Freshw. Fish 19, 537–544.
- Strokal, M., Ma, L., Bai, Z., Luan, S., Kroeze, C., Oenema, O., Velthof, G., Zhang, F., 2016. Alarming nutrient pollution of Chinese rivers as a result of agricultural transitions. Environ. Res. Lett. 11, 024014.
- Tabak, L.M., Gibbs, E., 1991. Effects of aluminium, calcium and low pH on egg hatching and nymphal survival of Cloeon traingulifer McDunnough (Ephemeroptera: Baetidae). Hydrobiologia 218, 157–166.
- Tockner, K., Pusch, M., Borchardt, D., Lorang, M.S., 2010. Multiple stressors in coupled river-floodplain ecosystems. Freshw. Biol. 55, 135–151.
- Tysmans, D.J.J., Löhr, A.J., Kroeze, C., Ivens, W.P.M.F., van Wijnen, J., 2013. Spatial and temporal variability of nutrient retention in river basins: a global inventory. Ecol. Indic. 34, 607–615.
- US Environmental Protection Agency, 2000. National Water-Quality Inventory. Office of Water, US Environmental Protection Agency, Washington, DC.
- Voelkl, J., Alzoubi, K., Mamar, A.K., Ahmed, M.S.E., Abed, M., Lang, F., 2014. Stimulation of suicidal erythrocyte death by increased extracellular phosphate concentrations. Kidney Blood Press. Res. 38, 42–51.
- von Bertrub, M.G., Krein, A., Stendera, S., Thielen, F., Hering, D., 2013. Is fine sediment deposition a main driver for the composition of benthic macroinvertebrate assemblages? Ecol. Indic. 24, 589–598.
- Wagenhoff, A., Townsend, C.R., Matthaei, C.D., 2012. Macroinvertebrate responses along broad stressor gradients of deposited fine sediment and dissolved nutrients: a stream mesocosm experiment. J. Appl. Ecol. 49, 892–902.
- Wedekend, C., Gessner, M.O., Vazquez, F., Maerki, M., Steiner, D., 2010. Elevated resource availability sufficient to turn opportunistic into virulent fish pathogens. Ecology 91, 1251–1256.
- Wood, P.J., Armitage, P.D., 1997. Biological effects of fine sediment in the lotic environment. Environ. Manag. 21, 203–217.
- Wood, S.A., Depree, C., Brown, L., McAllister, T., Hawes, I., 2015. Entrapped sediments as a source of phosphorus in epilithic cyanobacterial proliferations in low nutrient rivers. PLoS One 10, e0141063. https://doi.org/10.1371/journal.pone. 0141063.
- Worrell, F., Jarvie, H.P., Howden, N.J.K., Burt, T.P., 2016. The fluvial flux of total reactive and total phosphorus from the UK in the context of a national phosphorus budget: comparing UK river fluxes with phosphorus trade imports and

N.C. Everall et al. / Environmental Pollution xxx (2017) 1-11

- exports. Biogeochemistry 130, 31–51. WWF-UK, 2007. Review of UKTAG Proposed Standard for Suspended Solids. APEM Scientific Report, 20pp. Yan, Z., Han, W., Peñuelas, J., Sardans, J., Elser, J.J., Du, E., Reich, P.B., Frang, J., 2016.
- Phosphorus accumulates faster than nitrogen globally in freshwater ecosystems

- under anthropogenic impacts. Ecol. Lett. 19, 1237–1246. Yeo, R.R., Dechoretz, N., 1976. Diquat and copper-ion residues in salmon-spawning channel. Weed Sci. 405–409.
- Zhao, Y., Newman, M.C., 2006. Effects of exposure duration and recovery time during pulsed exposures. Environ. Toxicol. Chem. 25, 1298–1304.