Does fine sediment source as well as quantity affect salmonid embryo mortality and development?

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Abstract

Fine sediments are known to be an important cause of increased mortality in benthic spawning fish. To date, most of the research has focussed on the relationship between embryo mortality and the quantity of fine sediment accumulated in the egg pocket. However, recent evidence suggests a) that the source of fine sediment might also be important, and b) that fitness of surviving embryos post-hatch might also be impacted by the accumulation of fine sediments. In this paper, we report an experiment designed to simulate the incubation environment of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). During the experiment, the incubating embryos were exposed to different quantities of fine (<63 micron) sediment derived from four different sources; agricultural topsoils, damaged road verges, eroding river channel banks and tertiary level treated sewage. Results showed that mass and source are independently important for determining the mortality and fitness of alevin. Differences between species were observed, such that brown trout are less sensitive to mass and source of accumulated sediment. We demonstrate for the first time that sediment source is an additional control on the impact of fine sediment, and that this is primarily controlled by the organic matter content and oxygen consumption of the catchment source material.

Key words

Sediment Sources, Brown Trout, Atlantic Salmon, Fine sediment, organic matter

Introduction

Excess fine sediment in watercourses (defined in this paper as <63 μm) above natural background levels, is recognised as a pollutant, with important consequences for aquatic ecology and ecosystem function (Jones et al. 2011a & b, 2014; Kemp et al. 2011; Collins et al., 2011). Wilkinson and McElroy, (2007) report that agricultural river basin sediment delivery ratios have increased by 10–20% relative to the pre-agricultural landscape, which raises concerns over the environmental and socioeconomic consequences of sediment transfer from agricultural land to downstream aquatic ecosystems (Evans, 2010), adding to threats to food and water security from projected climate change (European Union, 2009). Similarly, evidence from lake and floodplain sediments support concerns over offsite impacts of human activity on the land surface (Foster et al. 2011; Macklin et al. 2010; Collins et al. 2012a). This is further supported by studies of the provenance of contemporary fine sediment deposits in river beds (Collins et al. 2010a,b; 2012b,c, 2014) that tend to show the importance of catchment surface sources; the latter often including topsoil eroded from agricultural land. There is also a growing concern over the impact that different sources of sediment have on the aquatic ecosystem, driven in part by legislation set up to protect and enhance the aquatic environment (Collins et al. 2009, 2011). As a result, there is a growing recognition that management of sediment at source is the most sustainable option for achieving the targets set by the legislation (Collins and McGonigle 2008; Collins et al. 2009, 2011).

In fisheries science, impacts of fine sediment have tended to focus on its accumulation within the spawning gravels of salmonids and specifically, the links between the level of fine sediment (usually expressed as a percentage by weight below a given size) and egg mortality (Reiser 1998; Sear et al. 2008). Other research has sought to explain the link between the physical impact of fine sediment and the

biological response in embryos; highlighting the reduction in the supply of oxygen (Chapman,1988; Greig et al. 2005a; 2007) or the physical occlusion of micropores on the surface of the egg (Greig et al. 2005b).

Further research has explored the physical characteristics of the fine sediment, seeking to understand which grain size is most closely linked to the mortality of embryos (e.g. summary in Collins et al. 2011). Thus, Levasseur et al. (2006) concluded that, although very fine sediment (<63 µm = 0.063 mm) was highly detrimental to embryo survival, larger sediment (up to 2.0 mm) had no corresponding effect. Support for this was observed by Greig et al. (2007) in field studies that showed good survival in spawning gravels with high levels of sand accumulation, citing the permeability of sand compared to other sites where silt/clay occluded the flow of oxygenated water to the embryo. Lapointe et al. (2005) have shown in laboratory experiments, how the lethal effects of silt-clay sediments occur when combined with sand-sized fractions. The sand traps the finer particles that would otherwise have moved through the larger interstices between the gravel framework and reduces permeability, and thus oxygen supply rate, to incubating progeny.

Organic matter content is an important characteristic of fine sediment accumulation in spawning gravels (Collins et al. 2009, 2013, 2014), with two main effects; first, the presence of biological activity driven by organic matter can generate the formation of biofilms, that block the interstitial pores of gravels (Petticrew & Arocena, 2003) and, secondly, decomposition of the organic matter creates an oxygen demand which competes with the demands made by the incubating embryo (Greig et al. 2005a). For Pacific salmon species, Bjornn and Reiser (1991) hypothesized that organic matter accumulation may have deleterious effects on incubating salmon, whilst Petticrew

and Rex (2006) report an 18% reduction in intergravel DO following organic matter loading from dying spent salmon.

Collectively, these observations suggest that sediments with different physical attributes might be expected to have different impacts on incubating embryos. The science of sediment fingerprinting is based on the principle that sediment derived from different sources will be characterised by differing physical or geochemical characteristics (Collins and Walling, 2004; Collins et al. 2010a), thus there is reason to hypothesize that differing sources of sediment will have differing levels of impact on benthic spawning fish.

Recent research has started to develop an evidence base for sub-lethal effects of sedimentation on subsequent life stages (Roussel 2007; Burke, 2011; Louhi et al. 2011). While studies of incubating salmonids typically estimate survival to emergence, this measure fails to account for the possibility that marginal hyporheic conditions may allow for survival to emergence, but with reduced probability of survival to maturity (Silver et al. 1963; Chapman, 1988). Even at sub-lethal levels of DO, studies have demonstrated smaller and lighter embryos (Youngson et al. 2005; Malcolm et al. 2008), deformity, and delayed hatch and emergence (Alderdice et al. 1958; Silver et al.,1963; Shumway et al. 1964). Against this background of potential complexity, laboratory studies have also demonstrated that embryos can endure short periods (7 days) of very low DO (<2 mg L^{-1}) without noticeable effects, depending on temperature and stage of development (Alderdice et al. 1958; Giest et al. 2006; Ciuhandu et al. 2008).

Despite these emerging lines of evidence, there is still comparatively little evidence for the effects of sediment load on sub-lethality in salmonids. There is no evidence to

date to support the importance of different sediment sources on embryo mortality and fitness. This latter research is required in order to link the growing evidence of source specific sediment loads (e.g. associated with specific risky crops in farming, e.g. maize or winter wheat cropping) to benthic spawning fish (see review by Kemp et al. 2011). Therefore, in this paper, we seek to test for the first time; (1) the effects of different sediment source and/or loading on embryo mortality; (2) the effects of different sediment source and/or loading on the development of surviving embryos, and; (3) the differing response of two economically important, benthic spawning salmonid species – brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). The experimental work was undertaken as a component of a large multi-partner research project examining the impacts of fine sediment on fluvial aquatic ecology.

Methods

Experimental Facility and Design

We conducted experiments at the University of Southampton Chilworth hydraulics laboratory Fish Research Facility from 17th November 2010 – 25th January 2011. The facility is a continuous recirculating system, in which water is fed via two main pipes from a biofiltration system to each of 48 separate tanks (Figure 1). The return water from each tank is collected in a return pipe and passed back into the biofiltration system. The return water is then treated to remove any sediment using fine fabric filters and a sand bed filter, before being passed through a UV and biofiltration system which remove any bacteria or biological material. The water is then recirculated via a chiller unit to control temperature, back through the feeder pipes to each tank. Water is fed into each tank through two inflow pipes, located at the bottom and one close to the top of the tank (Figure 1) with a single outlet pipe located near the surface. The design is similar to that reported by Louhi et al. (2011). Dissolved

material, including nutrients, was not removed by the system but their levels were monitored in the feeder tank prior to distribution through the system. Thus, all 48 tanks received the same amount and quality of water throughout the experiments.

To determine whether alevin growth and mortality were affected by fine sediment load and (or) sediment source, we applied sediment from four different sources (river bank, damaged Road verge, agricultural topsoils and treated sewage sludge) at five loads (1% (14 g), 3% (41 g), 6% (82 g), 9% (123 g), 15% (205 g) by wet weight) plus an independent zero sediment control for each source treatment. We applied the same treatment (source x load) to each of 10 separate baskets within a single tank (Figure 1).

The four different sediment sources were collected from the catchment of the River Ithon, Wales, UK, and were selected based on previous sediment fingerprinting studies that had identified the main contributors as (1) agricultural surface soils, (2) eroding river bank material (sampled from below the surface soil level), (3) damaged Road verges, and (4) final treatment sewage sludge (Collins et al. 2012d). All catchment source material samples were collected in October 2009, corresponding with the start of the salmonid spawning season. The sampling strategy was spatially representative of the River Ithon catchment and the distribution of the key sediment source types therein (see Greig et al., 2007 for further catchment details). All accessible watercourses and their surrounding fields and roads were visited to search for suitable sediment sampling sites. 30 sites were sampled for each of the individual sediment sources. A sample of final treatment sewage sludge was collected from a Sewage Treatment works within the River Ithon catchment. This material represents the final stage of solids treatment and can be released into the

environment during overflow periods or as a result of accidental release (cf Collins et al. 2010a, b; 2012a,b).

All samples from each sediment source type were passed through a <63 *μm* sieve into buckets. The buckets were then left to stand for 2 days in a dark, temperature controlled environment to allow the sediment to settle. This was to ensure that fine sediment would not be lost during decanting. After this period of settling, excess water was decanted and the remaining slurry was oven dried at 30 degrees for ca. 36 hours (or until ready). Higher temperatures were avoided to avert the risk of destroying the organic content of the samples. This process resulted in a damp cake-like mixture for each of the study catchment sediment sources. Sub-samples of the damp sediment were oven dried to determine differences in water content between source samples. This was used to correct the total wet mass applied to each incubation basket within each experimental tank.

Treatment 2 was defined by the load (mass) of sediment added to the egg zone within each individual incubation basket. The range of quantities of sediment added was based on a national dataset of salmon and trout redd data compiled by the authors. Data from over 83 bulk gravel samples from natural and artificial Atlantic salmon redds were derived from published (Greig et al. 2007; 2005b; Walling et al. 2003, Milan et al. 2000; Crisp & Carling 1989) and unpublished sources. A cumulative frequency curve for the proportion of silt-clay accumulated in the redd gravels was plotted and values were extracted to represent the full range of silt/clay accumulation found in natural and artificial spawning redds across England and Wales.

187 Diploid brown trout eggs were obtained from 10 females fertilized with sperm pooled
188 from five males from the same stock. Wild Atlantic salmon eggs were sourced from 3
189 females fertilised with sperm from 3 males. The unfertilised eggs of both species
190 were transported from the hatchery in ice cooled polystyrene boxes and fertilised at
191 the experimental site. All eggs were water hardened for two hours at 7-9 °C.
192 Twenty-five eggs were deposited evenly on washed gravels (replicating freshly cut
193 redd gravels (Crisp & Carling, 1989)) in an egg basket in a layer 10 cm (Grieg et al.,
194 2007) below the gravel (4–32 mm) surface within 3 hours of fertilization. More
195 washed river gravel was carefully added over the top of the eggs along with a short
196 stainless steel tube for injecting sediment into the egg basket at a later date. Each
197 egg basket consisted of a cylinder open at the surface with 1 mm plastic mesh
198 (diameter 8 cm, height 20 cm). All eggs used in the experiment were of similar initial
199 mass (brown trout mean mass 0.083 ± 0.004 g, n = 25; Atlantic salmon mean mass
200 0.092 ± 0.009 g, n = 25).
201
202 Ten plastic mesh baskets were placed into each replicated tank and washed gravel
203 carefully placed around them until flush with the surface. This was repeated for all 48
204 tanks giving a total of 480 individual baskets (Figure 1). Prior to egg planting,
205 conductiometric standpipe (see Greig et al. 2005c) readings were made in each
206 gravel-filled basket of three tanks to determine the intra-gravel flow velocity (IGFV)
207 through the egg zone and to test for consistency across the baskets and tanks. Using
208 this data, we set the inflow rate at 1.15 L min^{-1} to achieve a clean gravel IGFV of 849
209 cm hr^{-1}, which replicated conditions in good quality spawning habitat measured at UK
210 field sites by Grieg et al. (2007). Consistency between tanks was good, with a
211 variation of +/-71 cm hr^{-1} (8.76%) between equivalent baskets in each tank.
212 Unfortunately, measurement of IGFV after injection of fine sediments was not
213 possible since the technique requires injection of a saline and alcohol solution which

would have affected the survival of the embryos (Greig et al. 2005c). However, measurements of inflow and outflow from each tank after sediment treatment showed no difference between tanks. Thus, any change in IGFV, and hence oxygen supply rate to incubating embryos, was the result of the treatments as planned.

Physical and Chemical Parameters

Water quality was monitored throughout the period of incubation to hatch. Manual sampling of the water entering the tanks was conducted every 3 days; whilst dissolved oxygen (Aandera 4175 Optode, accuracy +/-5%), temperature (Aandera 4175 Optode, accuracy +/- 0.5%), water level (Druck PTX1830 Series, accuracy +/- 0.06%) and turbidity (Analite 9000, accuracy +/- 1%) were sampled every minute within the feeder tank (i.e. after filtration and biological treatment) and the average logged every 10 minutes on a Delta2 logger. Light levels experienced by each tank/basket were kept constant by covering each tank with a black lid.

Eight small baskets containing 50 eggs but no gravels, were placed on the surface of the substrate in the control tanks and monitored every 3 days for embryo development. Records of the number of live, dead and hatched eggs in these baskets were made. These were used as a check on the predicted time of hatching, to determine the end point of the experiment when the sediment filled baskets could be withdrawn.

After 143 degree days, each tank was isolated in turn and the same quantity and source of fine sediment was injected into each egg basket within the tank via the stainless steel tube. The injected material consisted of a pre-weighed mass of sediment that was blended with 250 mL of water drawn from the incubation tanks. Half the solution was injected into the egg zone and the other half injected into the

gravels above the egg zone. This approach was selected to mimic the process of colmation observed in both flume and field conditions (e.g. Sear et al. 2008). Continuous release of sediment into the recirculating water was not feasible as this would have afforded no control over the sediment mass treatment. Injection into each basket reduced the release of fines into the overlying water column; movement of sediment between baskets within each tank would therefore only result from IGFV. Differences between baskets in each tank were quantified at the end of the experiment by measuring the mass of sediment (inorganic and organic) in each of the 480 separate baskets.

When 50% hatch was reached, each tank was isolated in turn and all ten baskets removed. This occurred after 456 (Brown trout) and 513 (Atlantic salmon) degree days. The sediment from each basket was tipped into counting trays and all live and dead eggs and alevin were identified. A sample of fifteen alevin were taken from baskets 2, 3 and 5 in each tank and where insufficient were available, additionally from baskets 1 and 10. Alevin were preserved in a solution of 4% formaldehyde. The total wet mass and wet yolk sack mass were weighed using a Mettler Toledo AB204-5 balance accurate to 0.0001 g. Each alevin was also measured for length using a Nikon E100 microscope at 50x magnification. Errors in length measurement were checked by repeat measurement and found to be <0.1 mm.

After removal of the eggs and alevin, the sediment from each basket was wet sieved through a 63 µm sieve and dried to constant mass. The mass of fine sediment <63 µm and > 63 µm was recorded for each basket. Organic matter content of the <63 µm fraction was determined through loss on ignition (LOI). Samples for LOI were wet sieved to less than 63 µm and oven dried. Crucibles and samples were weighed before and after heating in a carbolite furnace for 2 hours at 550°C. To determine

absolute particle size distributions, a single sample of sediment from each tank was sieved at 63 µm using tap water. The <63 µm fraction was retained and dispersed in a 0.05% sodium hexametaphosphate solution. Samples were subsequently ultrasonicated in order to ensure that particles were in suspension. The sediment samples were vigorously shaken and a 30 mL aliquot was used for the grain size measurement. The aliquot was then agitated for 1 hour prior to measurement on a shaker bed. Measurements were made in triplicate, using a Malvern Mastersizer 2000.

Statistical Analyses

Although treatments were applied to each basket independently and data from each basket handled separately in the statistical analysis, each set of 10 baskets was nested within a single tank making it potentially difficult to separate any effect of the tank from that of the treatment. This design was chosen as there was a significant concern that we would not be able to apply different levels of sediment treatment to individual baskets randomly within tanks without the treatment applied to one basket potentially affecting neighbouring baskets in some way (particularly where large amounts of organic sediments were added), which would tend to homogenize the treatments. Therefore, we opted for a less statistically robust design (i.e. all baskets within a tank received the same treatment) which gave us more confidence that the baskets would experience the desired treatment. To determine if the tanks had any effect, eight control tanks, to which no sediment was added, were included in the range of treatments tested (see above). These were located at the start and end of each line of tanks to capture any variation based on distance along the line of replicated tanks (Figure 1).

General Linear Models (GLM) were used to perform ANCOVAs to test for the effects of sediment source and quantity, and interactions between these effects on specific response variables of the two fish species using SAS 9.1. Sediment source (d.f. 3) and fish species (d.f. 1) were included as fixed main factors, whereas mass of sediment added (d.f. 1) and mass of sediment recovered (d.f. 1) were included as continuous variables (d.f. 1). The ANCOVA model was species|source|mass. If effects were significant, pairwise comparisons were performed for the class effects species and source using post hoc tests (Tukey's HSD). Significance was set at 0.05 in all tests. An initial test was undertaken using both the mass of sediment and mass of organic matter recovered from the baskets as response variables (model = species|source|mass added), to verify that the experimental addition of sediment had been successful. Where sub-lethal measures of alevin performance were used, individuals were nested within the baskets they were incubated in, and basket (d.f. 9) and individual treated as random variables (model = species|source|mass basket individual(basket)). Type III (orthogonal) sums of squares used throughout as these are more appropriate for unbalanced designs and for the assessment of interactions among variables. All data were either arcsine (e.g. % survival) or log transformed to ensure homoscedasticity when necessary.

It should be noted that in our experimental design, to avoid homogenization of treatments, all the replicates of each sediment source x mass treatment were contained within an individual tank. Hence, any potential effect of the tanks was confounded with treatment. To test for any effect of tank, for each response variable a separate GLM analysis was conducted on the control tanks (n = 4 for each species) to which no sediment was added. Here, the effect of the tanks was compared to the effects of the baskets and, for sub-lethal effects, individuals. In these analyses tank and species were fixed main effects, and basket a random effect nested within tank x

species (model = species|tank basket(tank)). Where sub-lethal effects were considered, a further level of hierarchical nesting was included, with individual alevins a random effect nested within baskets (model = species|tank basket(tank) individual(basket)). Where these analyses indicated no significant effect of tank it was assumed that tank had no influence and the replicates of each treatment were assumed independent of tank.

Where an effect of sediment source on the fish was detected, a further test was undertaken using mass of organic matter recovered (as a continuous variable, d.f. 1), to determine if any effect was attributable to differences in the organic content of sampled material collected from the different catchment sediment sources. In this case the model was as above, but with organic mass recovered from each basket used rather than the mass of sediment added.

Results

Characterising Sediment Sources

In this analysis, the characteristics of the source material pertinent to the incubation experiment included absolute particle size, organic matter content and for the first time, sediment oxygen demand (SOD both 5 day (labile) and 20 day (refractory)). SOD has been highlighted by Greig et al. (2005b) as influencing the oxygen supply rate to incubating embryo. Physical differences between the study catchment sediment source materials are shown in Table 2. Sewage Treatment Work (STW) sediment had a significantly higher organic matter and Organic carbon content than the other sources ($p = 0.0192$). In terms of absolute particle size, damaged Road verge had the highest clay content (2%), River Bank had no detectable clay content and Agricultural topsoil had the second highest clay content and was the finest sediment source material overall. STW and Road verge had the highest SOD for

both 5 day and 20 day tests. Agricultural topsoils had the lowest SOD of all sources tested in the experiment.

Physical conditions during incubation and hatch

The physical conditions within the experimental spawning gravels were constant over time. Monitoring of nitrite, nitrate and ammonia showed a sharp and short (<24 hours) increase post sediment injection (Table 1), but levels remained below those reported as critical for incubating salmonids (Westin 1974; Kincheloe et al. 1979; Sonderberg et al. 1983; Timmons et al. 2002;). A decision was taken, one week after injection, to isolate and end the sewage treatment work sediment experiments with > 3% (41 g) by mass of sediment introduced, since these were suspected as a potential cause of deterioration in water quality. All eggs recovered from these tanks were found to be dead. Water quality in the recirculation facility continued to remain below critical levels across all replicated tanks for both species.

A short (<12 hour) increase in turbidity occurred in tanks when sediment was being injected, replicating the pulse of sediment delivery that occurs during natural flood events in river catchments. During sediment injection all fine sediment was contained within the tank being treated, ensuring that baskets in each tank received the same treatment, but no between-tank physical effects of sediment injection were incurred. Water temperature varied with diurnal fluctuations in air temperature, but within a range that was below critical for salmonids (Crisp 1990).

GLM tests indicated that the sediment injection procedure was successful in producing the target treatment levels within the egg baskets (Table 3, Figure 2). The mass of sediment recovered from the egg baskets did not differ significantly among treatments with different fish species or sediment sources, but did differ in a highly

significant manner with the mass added ($p < 0.0001$). The interaction between sediment source and mass added was not significant at the 5% level. The mass of organic matter recovered from the egg baskets did not differ significantly among treatments with different fish species, but again did differ significantly with the mass of sediment added ($p < 0.0001$). In contrast to the total mass of sediment recovered from the egg baskets, there were highly significant differences in the mass of organic matter recovered among the sediment sources, and with the interaction of sources and mass added (Table 3), reflecting differences in the characteristics of the sediment added (see Table 2). Thus, we are confident that the individual baskets in a tank were replicated (i.e. no significant difference in the mass of organic matter or total mass of sediment between baskets in a given tank) but there was a significant difference between tanks (treatments).

Sediment, Mortality and Survival

A GLM test using data from the control tanks indicated a significant difference in survival of the two fish species, but no effect of the tanks or individual egg baskets within the tanks (Table 4A, Figure 3a). Mean mortality of brown trout in the egg baskets in the absence of any additional fine sediment was 9.9% whereas for Atlantic salmon it was 74%. The cause of the increased mortality in salmon resulted from the process of transfer from the hatchery to the Chilworth hydraulics laboratory since all physical variables within the facility were well within published tolerances of the particular species, and in previous experiments, survival had been good (>89%) and control batches at the hatchery showed 10.2% mortality for Atlantic salmon and 2.1% for the brown trout. This difference in survival between species was controlled for in subsequent GLM modelling by including species as a main factor. The results thus highlight where there is a difference between the species. However, where there is a

400 significant interaction with other factors, the inclusion of species in the model
401 indicates that the species are reacting differently to the other factors.
402
403 In addition to the difference in mortality between Atlantic salmon and brown trout, the
404 GLM analysis of the experimental addition of fine sediment indicated significant
405 effects of different sediment sources and of the mass added, together with
406 interactions between mass added and species, mass added and sediment source,
407 and mass added, species and sediment source (Table 4B, Figure 3a and 3b). Figure
408 3a shows how the response of trout differs from Atlantic salmon; while both species
409 show an increase in mortality with increasing fine sediment load, trout show a rapid
410 increase in mortality between 1% and 6% wet mass. Average mortality of salmon
411 eggs increases almost linearly between 1% and 9% wet mass added but,
412 unaccountably, mortality decreases after 9%.
413
414 Tukey's test indicated that mortality was significantly higher with STW sediment
415 compared to all other sources. Furthermore, STW sediment caused an increase in
416 mortality at lower added mass than other sources, whilst damaged road verge
417 material caused the next highest mortality for Atlantic salmon. Complete mortality of
418 both species occurred in the tanks containing >3% by mass STW loadings, which
419 were isolated and closed down earlier in the experiment than the remaining
420 treatments. In addition, there was a significant difference in the response of the two
421 fish species to the mass added of different sources (species*source*mass); a lower
422 mass of STW and damaged road verge sediment was required to cause an increase
423 in mortality for Atlantic salmon than for brown trout (Figure 3b).
424
425 When the mass of organic matter recovered was included as a covariable in the GLM
426 analysis (rather than mass added), the effects of species, source and their interaction

on mortality remained significant (Table 4C). There was also a highly significant effect of organic matter and a significant interaction between organic matter and species. However, when the mass of organic matter recovered was included with source (i.e. Organic*Source and Organic*Species*Source), the interactions were not significant. In other words, although there were differences in mortality with different sources, the mass of organic matter recovered was sufficient to explain the differences in mortality between the different sediment sources.

Sub-lethal affects on Alevin

The data from the control tanks again indicated that there was no effect of the tanks or individual egg baskets within the tanks on the three indicators of alevin fitness used, namely; wet mass, length and wet yolk sack mass (Table 5A). For all measures of alevin fitness the differences between the egg baskets and between individuals within egg baskets were not statistically significant.

The GLM analysis of the experimental addition of fine sediment mass indicated significant differences between the two fish species (Table 5B), with brown trout overall lighter (0.0922±0.0144 g *cf* 0.0949±0.0102 g) and shorter (16.01±0.05 mm *cf* 16.97±0.04 mm) and with more yolk sac (0596±0.0006g *cf* 0.0568±0.0004g) than Atlantic salmon for the same relative incubation time (defined by degree days to 50% hatch in the surface egg baskets). Accounting for the inter-species difference in alevin mass, there were significant differences in the mass of alevin exposed to different sources and masses of injected sediment (Table 5B, Figure 4a and 4d); the more sediment added, the smaller the mass of alevin. The interactions between species and mass of sediment added, and species and source were not significant (Table 5B), indicating that alevin mass of both species reacted similarly to the mass of sediment added (Figure 4a) and the different sources (Figure 4d).

The interaction between mass of sediment added and source was significant, with a more pronounced reduction in alevin mass with increasing mass of STW sediment added compared to the other sources. A similar response was seen in the mass of yolk sac, with the exception that the interaction between mass of sediment added and source was not significant (Table 5B).

There were significant differences in alevin length associated with species (as expected trout alevin are shorter), source, mass of sediment added, and the interactions between species and source, mass and species, and mass and source (Table 5B, Figure 4b, 4e). The length of alevin decreased with an increasing mass of sediment added.

When the mass of organic matter recovered from the egg baskets was included in the GLM model rather than the mass of sediment added, the differences between sources of sediment were not significant for alevin length, not significant for yolk sac mass, and significant for alevin mass (Table 5C). A significant effect of mass of organic matter recovered was apparent for all three measures of alevin fitness, with all three measures declining with increasing mass of organic matter. However, the interaction between the mass of organic matter recovered from the baskets and sources was not significant (Table 5C), indicating that the mass of organic matter recovered was sufficient to explain the differences among the sediment sources.

Discussion

The results provide preliminary evidence for both lethal and sub-lethal effects of silt/clay-sized (<63 µm) fine sediment on pre-emergent salmonid embryos (Lapointe

et al. 2005; Sear et al. 2008; Louhi et al. 2011). Increasing the mass of fine sediment resulted in higher mortality in both salmonid species. However, we were unable to find a significant linear relationship between specific size fraction (silt or clay) and mortality. In this respect our findings are similar to those of Louhi et al. (2011) who reported that percentage survival was not related to any specific inorganic absolute grain size. Unlike Louhi et al., (2011), we did find a significant effect of sediment mass on mortality. The absence of an absolute particle size (specifically clay) based effect is counter to the findings of Grieg et al. (2005) and Lapointe et al., (2011) who identified a physically-based rationale for the additional effectiveness of clay via blockage of the micropores on the surface of salmon eggs. The mass of clay reported for all these experiments are similar, but the experimental conditions differ; Greig et al. (2005) measured oxygen uptake in a small container with only 3 eggs directly exposed to clay, whilst Lapointe et al. (2011) and more recently Franssen et al. (2012) demonstrate the importance of a coarser sand sized component that amplifies the effects of silt/clay sized particles by reducing pore sizes and leading to enhanced blocking by fines. It is possible that within the egg baskets used by Louhi et al. (2011) and in this experiment, local concentrations of clay were much lower, resulting in a lower probability of encountering an egg, or a micropore on the egg surface. We demonstrate that in the absence of sand sized particles, concentrations of silt/clay of only 3% by mass result in deleterious effects on both egg mortality and alevin fitness, and that the effect is non-linear in both salmonid fish species.

Higher sediment load was shown to affect alevin fitness in both brown trout and Atlantic salmon. As sediment mass increased, salmon and trout alevin were lighter, shorter and, in salmon, had a smaller yolk sack mass, whilst in trout, after 6% wet mass of sediment was added, the reduction in yolk sac mass was smaller. Whilst this partly agrees with previous studies of salmonid species, our observation of reduced

egg yolk mass runs counter to previous research. Harmor and Garside (1977), Argent and Flebbe (1999) and Youngson et al. (2005), found smaller, lighter alevin with larger residual yolk sacs in conditions of low dissolved oxygen saturation, whilst Louhi et al. (2011) found that yolk sacs in alevin exposed to sedimentation were larger compared to non-sediment controls. Roussel (2007) explained this in terms of a delay in yolk sac absorption under hypoxic conditions – reduced oxygen leads to reduced growth and hence less demand on yolk. Our observations for brown trout and Atlantic salmon differ from these and might be explained by a higher metabolic rate as the alevin attempt to move into more oxygen rich water (Kamler 2002). Thus, whilst growth is reduced due to longer development time, increased metabolism increases the rate of yolk depletion. Alternatively, with a decrease in oxygen supply, metabolic processes can be partly shifted towards less efficient anaerobic processes, less efficient use of resources and therefore greater use of the yolk sac (Kamler 2008). At this stage, we do not know the reason for the observed differences in existing experimental outcomes. Differences in body size and amount of yolk at emergence are reported to have fitness consequences (Miller et al. 1988; Andesen 1988; Skogland et al. 2011). However, two strategies exist: one which maximises mobility whereby the fry are larger with a small yolk mass and are more effective at predator avoidance, and a second in which smaller fry emerge with a larger yolk sack, and are able to avoid risk of starvation (Skoglund et al. 2011). The effects of fine sediment on brown trout and Atlantic salmon in this experiment are counter to either of these strategies, and their fitness is therefore sub-optimal compared to those incubated in the control treatments.

The results permit for the first time, comparison between the response of two common salmonid species. The results show that response to sediment load and sediment source are broadly similar between species but with some species

specificity; brown trout show a change in response to fine sediment mass at around 6% per sediment wet weight. After 6%, rates of mortality, alevin and yolk sac mass loss all decrease, whilst rate of shortening decreases. For Atlantic salmon, such trends are less obvious, but at 9% by wet mass of fines in spawning gravels, rate of mortality decreases and loss of alevin mass increases, whilst rates of change in length and yolk sac mass remain constant. The results show that Atlantic salmon are more sensitive to catchment sediment sources with higher organic matter content than brown trout. The physiological reason for this remains uncertain but may relate to the larger mass of Salmon eggs relative to trout that has been shown to influence oxygen consumption (Einum et al., 2002) and therefore the demand for oxygen from the surrounding spawning habitat.

For the first time, we report that the source of the fine sediment is a control on embryo mortality and the development of pre-emergence alevin. Of the sediment sources used, STW final treated solids and damaged road verge sediments showed the strongest effects on survival and measures of alevin fitness. The organic matter content of both of these sediment sources sampled in the River Ithon study catchment are high and the resulting oxygen demands (SOD 5 day) exerted by the decomposition of the organics are also the highest of all the sediment sources. We found that the difference in embryo survival and Alevin characteristics between catchment sediment sources was explained by the mass of organic matter recovered. Grieg et al (2005a) highlight how the sediment oxygen demand competes with the egg oxygen demand to lower the oxygen supply rate to embryo, whilst Louhi et al. (2011) found that survival of brown trout was correlated to the mass of fine organic matter. Since organic matter content has been shown in these experiments to have a significant effect on alevin fitness, we hypothesize that this is the main mechanism controlling the effects observed for both species of salmonids incubated

562 in STW and damaged road verge sediment. Here, using a preliminary experiment,
563 we have demonstrated an effect of STW sediment at levels as low as 1% by mass of
564 spawning gravels. Thus, highly organic matter from STWs will be deleterious to
565 benthic spawning salmonids, even at low levels of accumulation in spawning gravels,
566 though less so for brown trout. The implications are that organic matter type (since
567 organic matter is found in all sediment sources) as well as quantity will be an
568 important control on the SOD of infiltrated sediments within salmon redds or the
569 spawning substrate used by other lithophilous species. Indeed, Collins et al. (2013,
570 2014) have recently reported the presence of sewage derived organic matter sources
571 in salmon spawning redds within some rural catchments. The same work has also
572 traced the contributions of sediment-associated organic matter ingressing salmonid
573 redds from other important catchment sources including farm yards or steadings and
574 domestic septic tanks.
575
576 Lapointe et al. (2005) and Levasseur et al. (2006) have highlighted the importance of
577 sand in trapping silt and clay within the egg zone. The experiments reported in this
578 paper lend support to this observation since without the presence of sand, over
579 84.0%±6.8 of injected silt/clay (based on the difference between injected mass and
580 recovered mass) was transported out of the egg zone by interstitial flow and into the
581 gravels at the bottom of the experimental incubation tanks. This would have
582 increased mortality and reduced alevin fitness due to the higher mass of silt/clay
583 organic matter retained in the egg zone. Thus, catchments producing both sand and
584 silt/clay sized fractions, potentially from different sources (e.g. coarser sands are
585 derived from river banks in the River Ithon study catchment (Burke 2011)), are likely
586 to have a higher risk of deleterious effects on salmonids. Field experiments by Greig
587 et al. (2007) support this hypothesis, observing that the highest accumulation rates of
588 sand supported high rates of egg survival in the absence of silt/clay sized particles in

589 the wash load. Thus, management of different sediment sources may be necessary
590 in order to reduce cumulative impacts of different sediment sizes and organic matter
591 content on salmonid spawning habitats.
592
593 **Conclusion**
594
595 The principal findings of the present study may be summarized as follows. (1) The
596 effect of fine sediment load is different between sediment sources; final treatment
597 sewage and damaged road verge sediments were found to be significantly more
598 deleterious to mortality and alevin fitness than other sources relative to fine sediment
599 free controls. (2) Organic matter is highlighted as a major characteristic controlling
600 the effectiveness of spawning habitat, principally through its effect on oxygen
601 concentration via SOD (5day), and possibly through its effectiveness in blocking
602 pores. (3) The effect of fine sediment load is different between species, although the
603 overall effect is increased mortality and reduced alevin fitness. (4) Fine sediment
604 (<63 µm) has been shown to effect the mortality and fitness of both brown trout and
605 Atlantic salmon embryos. (5) The experiment confirmed the deleterious effects of
606 increasing fine sediment load on both brown trout and Atlantic salmon. This effect is
607 apparent in surviving alevin via reductions in mass, length and yolk sack mass
608 relative to experimental controls.
609
610 The research has two key implications; first, experiments (both laboratory and field)
611 as well as spawning gravel characterisation, should quantify more carefully the
612 physical characteristics of the sediment treatments used; these should include
613 organic matter content, SOD, grainsize and mass. Secondly, further research is
614 needed to better understand the processes by which organic matter influences the
615 supply of oxygen in spawning gravels. Recent organic sediment fingerprinting and

apportionment techniques have shown site specificity with different organic matter sources dominating in different catchments (Collins et al. 2013, 2014) reflecting the mix of land use and farming types present.

The identification of multiple effects of fine sediment also highlights the inadequacy of current metrics and sediment targets which are based on quantity of sediment of a given grain size, or total daily maximum loads (cf. Collins and Anthony, 2008; Collins et al. 2009, 2011). These are based on the assumption that all fine sediments are of equal impact on aquatic ecology. Our research points to specific sediment and species effects. High sediment inorganic sediment loads with low SOD, are likely to be less damaging to trout and salmon, and less damaging than materials derived from high SOD organic sources, although impacts will still occur (e.g. entombing of alevin – Greig et al., 2005a). Resource managers now have evidence to support the development of sediment screening techniques that would enable them to target particular sediment source control strategies in the landscape. Critically, these strategies must not focus solely on the proportion of different sources of fine sediment, but also on the characteristics of the mobilised sediment delivered to rivers from individual sources.

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652
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Tables & Figures

Table 1: Water quality summary for the experimental period.

Table 2: Summary of sediment source characteristics used in the experiments. Note the high levels of organic matter and 5-day Sediment Oxygen Demand associated with the STW and Road verge sources.

Table 3: Statistical results of General Linear Model of the effect of sediment addition on the total mass and mass of organic matter recovered from the baskets.

Table 4: Statistical results of General Linear Model of effects of sediment addition on mortality. A) Comparison among the control tanks (0 g sediment added) to determine the influence of tanks and basket (nested within tanks). B) Comparison among experimental treatments to determine the influence of species (i.e. trout or salmon), source of sediment added (i.e. Road verge, agricultural, river bank or sewage works), mass of sediment added and basket. Basket was regarded as a random factor and mass of sediment added as a continuous variable. C) Comparison among experimental treatments to determine the influence of species (i.e. trout or salmon), source of sediment added (i.e. Road verge, agricultural, river bank or sewage works), and mass of organic sediment recovered from the basket. Mass of organic sediment recovered was regarded as a continuous variable.

Table 5: Statistical results of General Linear Model of effects of sediment addition on the mass, length and mass of yolk sac of surviving alevins. A) Comparison among the control tanks (0 g sediment added) to determine the influence of tanks, basket (nested within tanks), and individual fish (nested within baskets). B) Comparison

among experimental treatments to determine the influence of species (i.e. trout or salmon), source of sediment added (i.e. Road verge, agricultural, river bank or sewage works), mass of sediment added, basket, and individual fish (nested within baskets). Both basket and individual fish were regarded as random factors and mass of sediment added as a continuous variable. C) Comparison among experimental treatments to determine the influence of species (i.e. trout or salmon), source of sediment added (i.e. Road verge, agricultural, river bank or sewage works), and mass of organic sediment recovered from the basket. Mass of organic sediment recovered was regarded as a continuous variable.

Figure 1: Chilworth Experimental Spawning facility showing the recirculation system and water quality controls. Diagram also shows details of the individual tanks used to incubate Atlantic salmon and Brown trout eggs.

Figure 2: Sediment mass treatment showing the mean (bars) and standard deviation of the mean (error bars) of sediment mass injected from the egg baskets after hatch. Missing values refer to STW tanks that were isolated and stopped early (see text for details). Missing bank data (tank 41) occurred due to laboratory error.

Figure 3: Variation in mean mortality (±SE) of brown trout and Atlantic salmon with a) mass of sediment added to the egg baskets and b) source of sediment added to the egg baskets. Letters above means indicate significant differences between sources, upper case for both species, lower case within species.

Figure 4: Variation in mean (±SE) alevin mass (a, d), alevin length (b,e) and yolk sac mass (c, f) of brown trout and Atlantic salmon with a, b, c) variation in mass of sediment added to the egg baskets and d, e, f) variation in the source of sediment

971 added to the egg baskets. Letters above means indicate significant differences

972 between sources, upper case for both species, lower case within species.

973 Table 1

Parameter	Mean	Standard deviation	Range
Temperature (°C)	7.40	0.60	5.43 - 9.37
Dissolved Oxygen (mg L^{-1})	10.02	0.23	9.45-11.01
Water Level in reservoir (cm)	37.27	1.72	34.88 - 62.97
pH	7.98	0.17	7.6 - 8.2
NH_4^+ (mg L-1)	0.27	0.19	0.0 - 0.5
NO_3^- (mg L-1)	14.17	13.11	0.0 - 40.0
NO_2^- (mg L-1)	0.23	0.31	0.0 - 1.0

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975

Table 2

Source	% Organic Content (LOI)	% Organic Carbon	5day Sediment Oxygen Demand $mgO_2/g/day$	20day Sediment Oxygen Demand $mgO_2/g/day$	% Silt	% Clay	D_{10} (µm)	D_{50} (µm)	D_{90} (µm)
Sewage Treatment Works (Tertiary Treated Waste)	56.54 (6.62)	60.0 (5.0)	12.97 (2.39)	7.40 (1.92)	99.85	0.15	8.36	24.19	50.05
Road verge	14.53 (0.94)	9.0 (8.0)	10.69 (0.49)	1.34 (0.84)	97.93	2.07	3.53	13.19	39.67
River bank	7.66 (0.69)	3.0 (3.0)	6.83 (2.10)	0.97 (0.39)	100.00	0.00	37.87	49.59	63.49
Agriculture (Field)	14.05 (1.01)	6.0 (7.0)	3.91 (1.18)	0.88 (0.56)	98.08	1.92	3.43	11.92	37.52

Figures in brackets are 1 standard deviation of mean. For % Organic Carbon figures in brackets are CV. LOI is Loss on Ignition at 550^{o}C

Table 3.

	Species		Source		Mass added		Source* Mass added	
	$F_{1,216}$	p	$F_{3,216}$	p	$F_{1,216}$	p	$F_{3,216}$	p
Mass recovered	2.19	0.140	0.81	0.488	2685	<0.0001	2.22	0.0861
Organic mass recovered	1.97	0.161	1093	<0.0001	2820	<0.0001	889	<0.0001

Table 4.

A)

	Species		Tank		Basket	
	$F_{1,39}$	p	$F_{3,39}$	p	$F_{36,39}$	p
Mortality	368.7	<0.0001	0.64	0.595	0.87	0.667

B)

	Species		Source		Species*Source		Mass		Mass*Species		Mass*Source		Mass*Species* Source		Basket	
	$F_{1,451}$	p	$F_{3,451}$	p	$F_{3,451}$	p	$F_{1,451}$	p	$F_{1,451}$	p	$F_{3,451}$	p	$F_{3,451}$	p	$F_{9,451}$	p
Mortality	645.9	<0.0001	14.28	<0.0001	2.57	0.054	115.5	<0.0001	13.91	0.0002	99.27	<0.0001	28.12	<0.0001	0.69	0.722

C)

	Species		Source		Species*Source		Organic		Organic*Species		Organic*Source		Organic*Species*Source	
	$F_{1,211}$	p	$F_{3,211}$	p	$F_{3,211}$	p	$F_{1,211}$	p	$F_{1,211}$	p	$F_{3,211}$	p	$F_{3,211}$	p
Mortality	250.1	<0.0001	138.3	<0.0001	7.28	0.0001	288.06	<0.0001	50.83	<0.0001	0.56	0.647	0.51	0.668

Table 5.

A)

	Tank		Basket		Tank*Basket		Individual	
	$F_{3,89}$	p	$F_{9,89}$	p	$F_{12,89}$	p	$F_{35,89}$	p
Mass	1.60	0.195	1.39	0.202	0.75	0.628	0.99	0.502
Length	0.68	0.564	1.13	0.350	0.38	0.911	0.78	0.799
Yolk Sac	1.34	0.267	1.08	0.387	1.66	0.129	0.88	0.651

B)

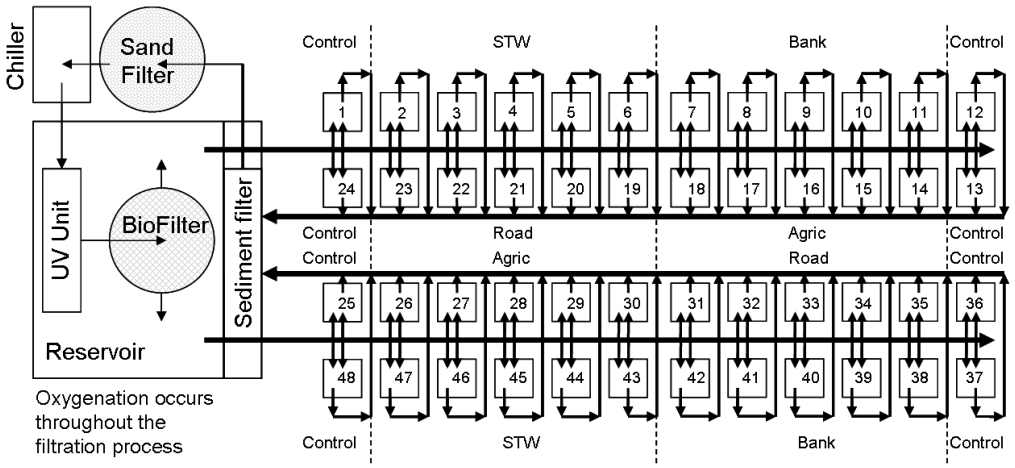
	Species		Source		Species* Source		Mass		Mass*Species		Mass*Source		Basket		Individual	
	$F_{1,588}$	p	$F_{3,588}$	p	$F_{3,588}$	p	$F_{1,588}$	p	$F_{1,588}$	p	$F_{3,588}$	p	$F_{9,588}$	p	$F_{35,588}$	p
Mass	7.89	0.005	3.04	0.029	0.47	0.702	15.33	<0.0001	2.38	0.123	2.47	0.043	1.36	0.204	0.96	0.536
Length	120.0	<0.0001	2.82	0.038	16.73	<0.0001	12.1	0.0005	2.38	0.035	3.35	0.019	1.43	0.172	0.29	1.000
Yolk Sac	10.73	0.001	4.44	0.004	1.56	0.199	6.58	0.0105	0.00	0.9998	1.51	0.211	1.52	0.135	1.29	0.128

C)

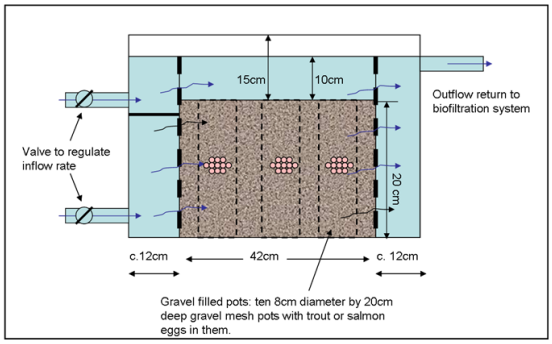
	Species		Source		Species*Source		Organic		Organic*Species		Organic*Source		Basket		Individual	
	$F_{1,536}$	p	$F_{3,536}$	p	$F_{3,536}$	p	$F_{1,536}$	p	$F_{1,536}$	p	$F_{3,536}$	p	$F_{9,536}$	p	$F_{33,536}$	p
Mass	8.25	0.004	2.65	0.048	1.16	0.325	14.19	0.0002	2.74	0.099	0.22	0.883	1.25	0.262	0.58	0.972
Length	84.91	<0.0001	2.13	0.948	17.14	<0.0001	11.09	0.0009	2.71	0.100	1.15	0.328	1.50	0.144	0.47	0.996
Yolk sac	8.00	0048	2.21	0.086	0.96	0.412	5.52	0.019	0.01	0.937	0.22	0.882	0.49	0.882	1.29	0.130

999 Fig1

a

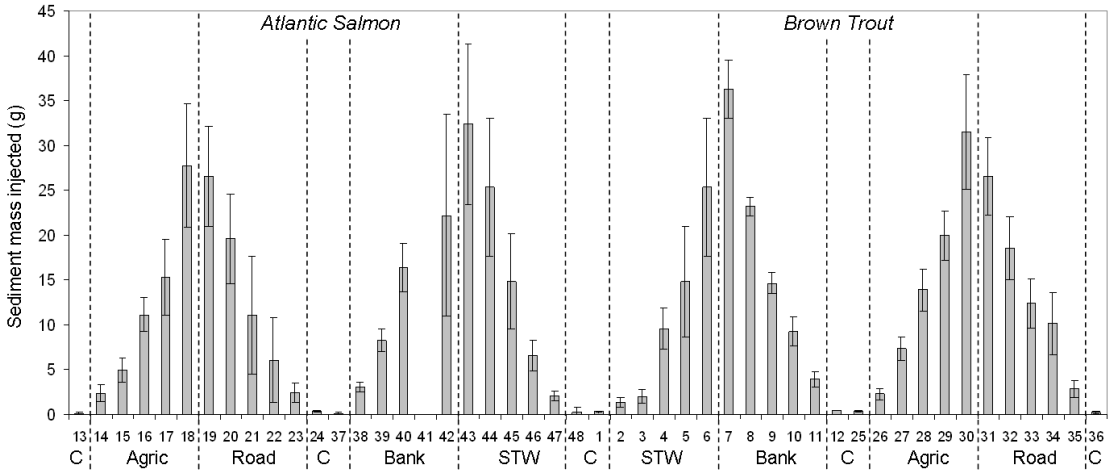


b



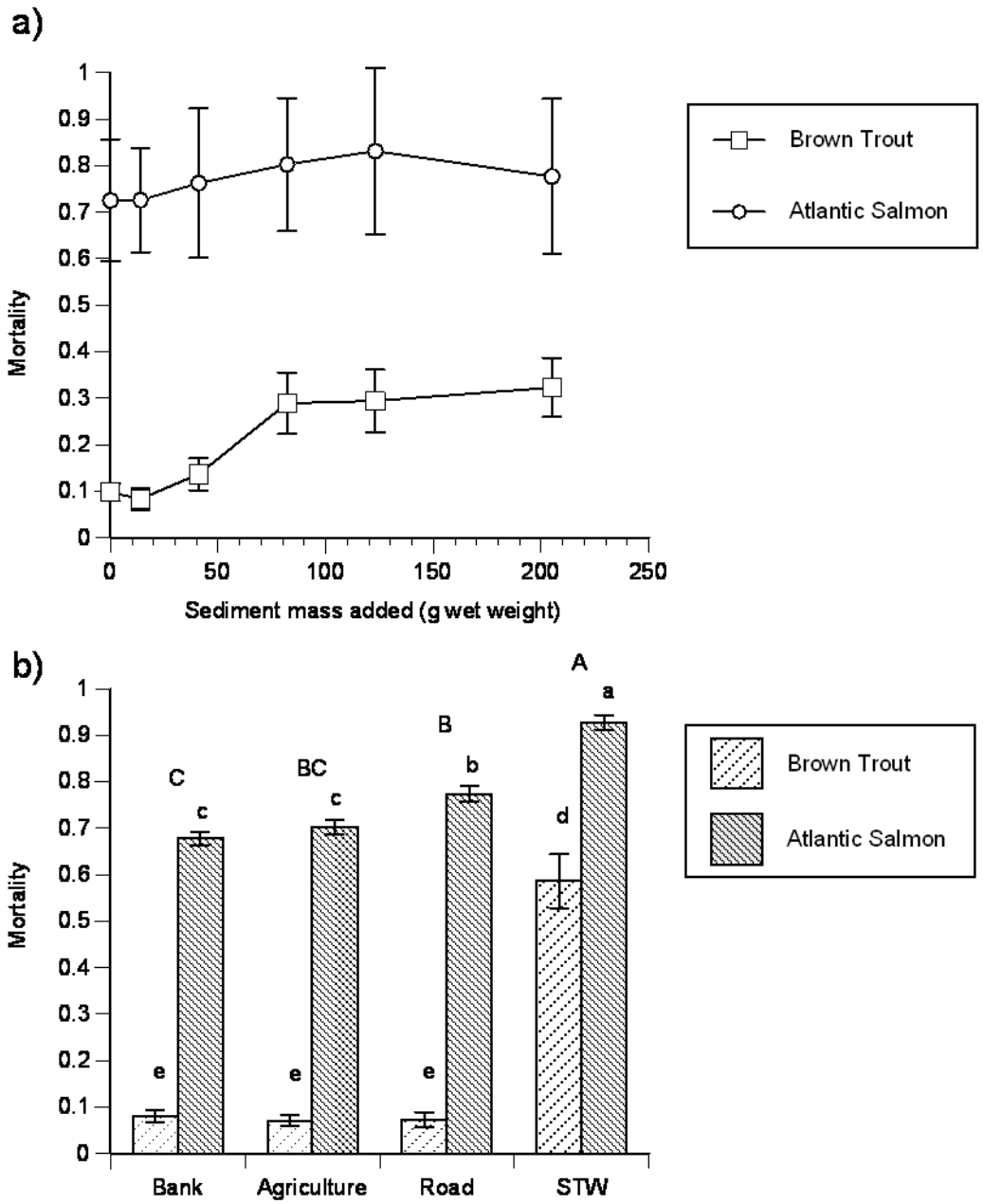
1000

1001 Fig2



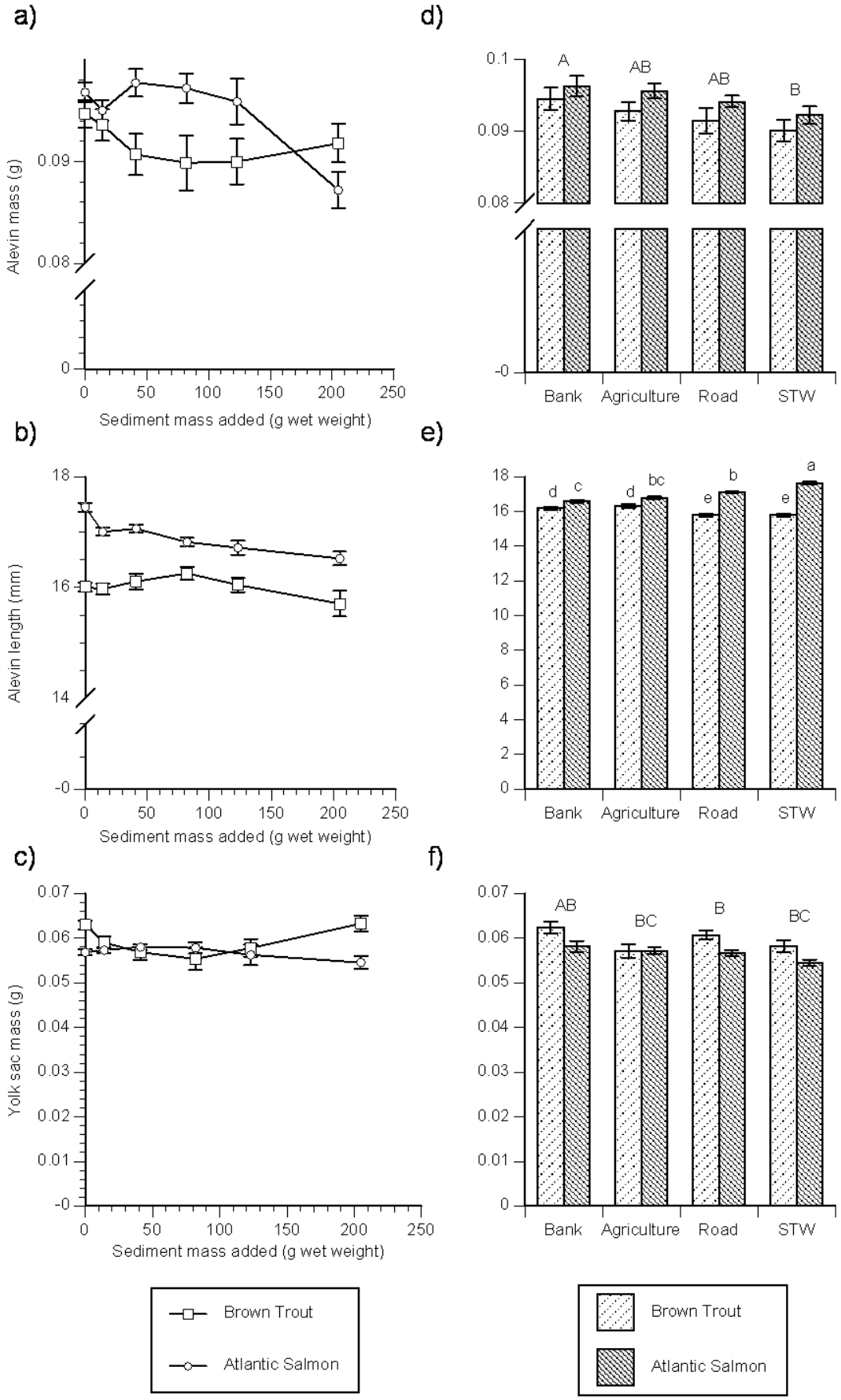
1002

1003 Fig3



1004

1005 Fig4



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