Fine Sediment Effects in Trout – New Insights from Laboratory and Field Studies

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Summary

Worldwide native salmonid populations are reported to be in decline. Habitat degradation, and increased anthropogenic fine sediment input in aquatic ecosystems is an important contributing factor. Suspended fine sediment can directly impair the health of free swimming juvenile salmonid fish, either by causing direct physical damage and/or via turbidity. Deposited fine sediment can hinder oxygen supply to salmonid embryos and hence impair their survival. Numerous studies have been published so far. Despite this, we still lack an integrated understanding of how fine sediment affects salmonid fish at different stages of their life-cycle. My thesis contributes to such an integrated understanding in juvenile salmonid fish (**Part 1**), and for salmonid embryo survival (**Part 2**).

In **part 1**, we could demonstrate with an *in vivo* exposure that pulses of suspended mica clay particles affect rainbow trout (*Oncorhynchus mykiss*) mostly *via* turbidity. Moreover, we found that (*i*.) rainbow trout could adapt within days when they cannot avoid sediment plumes, and (*ii*.) that exposure over 24 days can cause cellular changes in inner organs and metabolic stress, even when gill damage is absent and effects on condition are moderate. Altogether, this experiment provides the first systemic investigation of suspended mineral particle pulse effects in a salmonid fish. In an *in vitro* exposure with the epithelial gill cell-line RTgill-W1 we could, to my knowledge, demonstrate for the first time (*i*.) that natural mineral particles can cause cytotoxic effects in gill epithelial cells, and (*ii*.) that clay particles are more cytotoxic than framework silicates. Further, the clay particles differed in the kind of cytotoxic effects induced, causing either oxidative stress or cell membrane damage. Most interesting, our results are the first empirical evidence that clay particles could induce comparable cytotoxic effects in gill epithelial cells than synthetic nano-particles

In **part 2**, a field experiment provides an integrated perspective on the factors affecting brown trout embryo survival in a channelized river. The introduced steps created a repetitive step-pool-glide morphology (“terraces”). Up to hatch salmonid embryos incubate in distinct gravel nest (“redds”), and during this phase they depend on sufficient oxygen supply. Both fine sediment and a modified river structure can affect hyporheic exchange, and hence embryo survival. For such modified rivers, our experiment is, to my knowledge, the first (*i*.) to characterize the factors affecting fine sediment deposition, water exchange and oxygen concentrations in salmonid redds, and (*ii*.) to explicitly identify important predictors for salmonid embryo survival. We found that river morphology and flow dynamics causing fine

sediment re-suspension, rather than fine sediment transport *per se*, were important for maintaining redd gravel permeability over most of the incubation season. Similarly, water exchange and oxygen concentrations in the redds were mostly controlled by processes driven on the intermediate (i.e. individual terraces) and/or regional scale (i.e. river channel). Gravel permeability and the distance of the redd to the next upstream step, which created constant upwelling of short-residence hyporheic water, were the most important predictors for brown trout embryo survival. Altogether, we demonstrated (*i*.) that artificial steps could benefit salmonid embryo survival in channelized and (*ii*.) that appropriate flow dynamics and river structure are essential for sustaining salmonid incubation success.

My thesis clearly illustrates that it is essential to apply a holistic perspective to understand how fine sediment can affect salmonid fish. My results show that numerous factors can contribute. Fine sediment effects in juvenile salmonid fish could be modulated by exposure concentration and duration, the geochemical composition of the particles, but also adaptive responses of the organism. For fine sediment effects on salmonid embryo survival, the particular river system, with its hydrological and geomorphological setting, as well as the developmental stage of the embryo has to be considered. I believe we ultimately need to integrate fine sediment as one aspect of environmental change, and from there to develop strategies to sustain salmonid populations in the 21st century.

Chapter 1

Introduction

"...no stream fisheries biologist or stream ecologist has been involved in a research project for any length of time without serious sedimentation occurring to upset the research plan"
(Waters, 1995)

Sediments are a natural and ubiquitous component of the aquatic environment, and play an important role in the ecological functioning of rivers and streams (Giller and Malmqvist, 1998). Most natural sediment inputs are therefore regularly incorporated in stream processes without causing substantial harm (Waters, 1995). However, anthropogenic fine sediment (i.e. sediments < 2 mm) input in rivers is also increasing worldwide (Owens et al., 2005; Syvitski et al., 2005). In England and Wales sediment yields in some rivers and lakes increased four-fold over the last 100 years (Foster and Lees, 1999). Sediment input in the alpine Rhine is predicted to increase two-fold by the year 2100, mostly related to altered land-use and climate change (Asselman et al., 2003). Similar trends can be observed in the United States, where fine sediment and turbidity are among the top ten threats for aquatic ecosystems health (US EPA, 2009). Especially in high altitude areas of the Northern Hemisphere, climate change will likely further contribute by increasing the frequency and intensity of heavy rainfall events and hence fine sediment input in rivers (Asselman et al., 2003; Dore, 2005; Jentsch and Beierkuhnlein, 2008). It is these observed and predicted increases of fluvial fine sediment that have raised most concerns about negative effects on aquatic biota, including salmonid fish (Wood and Armitage, 1997; Henley et al., 2000; Wilber and Clarke, 2001; Bilotta and Brazier, 2008; Scheurer et al., 2009; Kemp et al., 2011).

Fine sediments in rivers

Fluvial fine sediment originate from various sources, either located in the catchment or in the river channel (Figure 1; Wood and Armitage, 1997; Owens et al., 2005). Natural sources include sand-bars and exposed stream-banks in the channel, as well as open soil and landslides in the catchment area (Waters, 1995). Humans have impacted all these sources, e.g.

by agriculture, forestry, mining, urban development, or other industrial operations (Waters, 1995). Given the variety of sources, fine sediment in rivers is always a mixture of particles of different size and/or geochemical composition (Figure 1; Irion, 1991; Walling, 1996; Evans et al., 2006).

Once the fine sediment particles are in the river, they move through the system in a dynamic equilibrium between downstream transport and storage in the channel (Figure 1; Slaymaker, 2003). Downstream transport occurs mostly during high flow events in suspension ("suspended sediments"; Figure 2), but also as "bed-load" along the gravel-bed (Montgomery and Buffington, 1998). Suspended fine sediment mostly consist of small-sized particles, defined here as the size-classes of silt (< 63 μm) and clay (< 2 μm; Irion, 1991). Bigger particle sizes can be suspended during strong high-flow events (Waters, 1995; Walling et al., 2000), especially in high gradient streams of higher altitudes (e.g. Lenzi and Marchi, 2000). In larger lowland rivers, suspended fine sediment transport during flood events can persist over weeks (e.g. Asselman, 1999; Pont et al., 2002) Humans can create repeated suspended fine sediment pulses over days to weeks, e.g. during dredging (Harvey and Lisle, 1998) or reservoir flushing (e.g. Crosa et al., 2010). During storage, fine sediment particles deposit on the river-bed or infiltrate in gravel interstices, from where they can be re-suspended (Lisle, 1989; Brunke, 1999; Seydell et al., 2009). Fine sediment infiltration decreases hyporheic exchange, and regular re-suspension of deposited fine sediment by "flushing flows" is crucial for the ecological functioning of rivers (Elosegi et al., 2010). The balance between transport and storage (Figure 1) is determined by fine-sediment properties (i.e. particle-size, cohesiveness), as well as gravel bed composition and discharge dynamics (Schälchli, 1995; Brunke, 1999). The latter two factors affect water-level, hydraulic gradients, and bed-shear stress. The sediment re-suspension cycle starts during the rising limb of flood events, suspended fine-sediment transport persist during the event (Figure 2), and fine sediment deposits and infiltrates during the falling limb. Increased fine sediment infiltration also occurs during base-flow when the suspended load is high (Brunke, 1999). In summary, increased fine sediment transport and deposition can occur due to increased fine-sediment input, but also due to anthropogenic river modifications that change discharge dynamics (e.g. hydropower reservoirs) and/or geomorphology (e.g. channelization, relocation) of the river system.

For my thesis, I concluded that free-swimming salmonid fish are often not confronted with a constant fine sediment concentration, but rather with pulses of small-sized particles (< 63µm). Moreover, their impact most likely depends on both the suspended fine sediment concentration and the duration the fish are exposed to the pulses. For salmonid embryo survival, it is important to recognize that the effect of fine sediment on gravel permeability and hyporheic exchange is temporarily dynamic, and also depends on discharge dynamics and geomorphology of the river system.

Fine sediment effects in salmonid fish

To understand how fine sediment can impact salmonid fish has been on the scientific agenda for over a century (Waters, 1995 gives a historic overview). One of the earliest publications in this regard was probably a comment about the loss of trout spawning habitat in a Colorado river affected by mining (Jordan, 1891 cited in Waters, 1995). By now, numerous case studies have investigated aspects of how fine sediment can affect salmonid fish. To no surprise, several comprehensive reviews have been published (e.g. Waters, 1995; Newcombe and Jensen, 1996; Wilber and Clarke, 2001; Newcombe, 2003; Kemp et al., 2011).

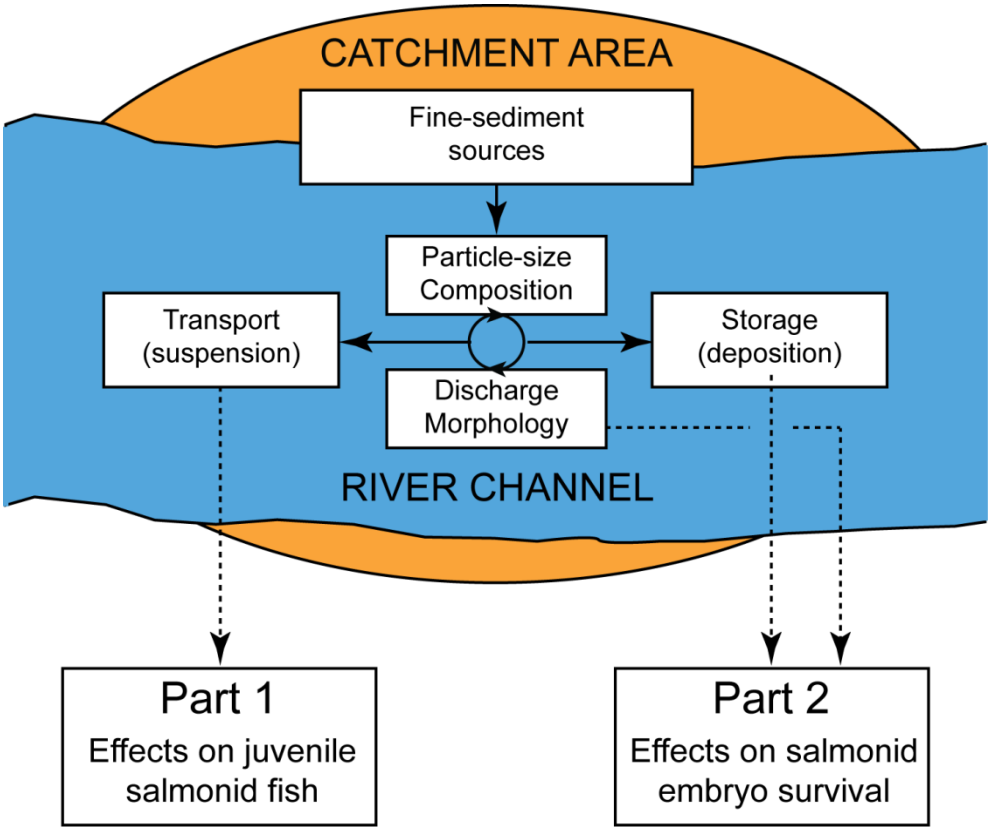

Figure 1 – Schematic illustration of the factors affecting fine sediment input, transport and deposition in a river system and how they relate to aspects of fine sediment effects in salmonid fish investigated in my thesis.

Anthropogenic fine sediment input contributed to the decline of native salmonid populations in North America (Hicks et al., 1991; Nehlsen et al., 1991; Yoshiyama et al., 1998; Katz et al., 2012) and the United Kingdom (Gilvear et al., 2002). In Switzerland brown trout catches decreased by more than 50% since the early 1980s (Burkhardt-Holm et al., 2005). To investigate the underlying causes the five-year interdisciplinary research project FISCHNETZ was initiated (Fischnetz, 2004; Burkhardt-Holm and Scheurer, 2007). Its results indicate that the catch decline was related to reduced fishing activities, a change in stocking but also a decrease of brown trout population sizes (Fischnetz, 2004). Increased fine sediment, and its negative effect on gravel permeability, was one factor that contributed to population declines (Borsuk et al., 2006; Schager et al., 2007). My PhD thesis originated as a follow-up project of FISCHNETZ (Scheurer et al., 2009). Its aim was to further investigate the impact of fine sediments on health and reproduction of salmonid fish.

How fine sediment can affect salmonid fish is closely linked to the salmonid life-cycle. Here I briefly describe the general life-cycle pattern for the brown trout (Jonsson and Jonsson, 2011). Brown trout spawn, fertilize and bury their eggs in distinct gravel nests ("redds") in late autumn to early winter. Embryos incubate in redds for several months ("intra-gravel stage"). During this intra-gravel stage the embryos depend on sufficient interstitial oxygen and water exchange to ensure oxygen supply and to remove metabolic waste products (Greig et al., 2007; Malcolm et al., 2008). Hatched embryos stay in the redd gravel until yolk-sac depletion, and then emerge to the water-column. Emergence usually takes place in late spring to early summer. After emergence, the fish feed and grow until late autumn, and then overwinter. After two to three years they reach maturity and close the reproductive cycle by contributing their own progeny to the population. For details and the diversity of life history strategies, also in the genus *Oncorhynchus*, see for example Crozier et al. (2008) and Jonsson and Jonsson (2011). Fine sediment can affect salmonid fish at any stage, either as suspended sediments or deposited in the redd gravel (Figure 1; Newcombe and Jensen, 1996). My thesis contributes new insights on the effect of fine sediment on juvenile salmonid fish (**Part 1**, Figure 1) and intragravel embryo survival (**Part 2**, Figure 1).

Part 1 – Effects on juvenile salmonid fish

Background

It is clear by now that exposure concentration and duration are important for suspended fine sediment effects in free swimming salmonid fish (Newcombe and Jensen, 1996). Individual studies indicate that specific particle properties, such as particle-size and angularity, influence responses (Servizi and Martens, 1987; Lake and Hinch, 1999). It has been also suggested that the geochemical composition of the particles affects responses, but this has not been studied prior to my thesis (Waters, 1995; Bilotta and Brazier, 2008). For juvenile sockeye salmon, a 96h LC_{50} of 17560 mg L^{-1} was reported for natural particles ≤ 74 µm, which decreased to 1800 mg L^{-1} with particle sizes of 180–740 µm (Servizi and Martens, 1987). In rainbow trout, 10000 mg L^{-1} kaolin particles ≤ 30 µm caused 28% mortality (Goldes et al., 1988). Altogether, sub-lethal responses pre-dominate under many environmental conditions, except maybe for very long suspension events and/or bigger particle sizes (Alabaster and Lloyd, 1982; Waters, 1995; Newcombe and Jensen, 1996). Indirect sub-lethal effects can be related to fine sediment induced changes in primary production but also decreased drift of invertebrate food sources or a reduction in benthic food sources (Wood and Armitage, 1997; Shaw and Richardson, 2001; Suttle et al., 2004). Direct sub-lethal effects could be related to physical damage and/or turbidity (Waters, 1995).

For direct physical damage, the gill was often considered a primary target organ. Yet, the evidence for gill damage is inconclusive, especially for small-sized (< 63µm) suspended fine sediment particles (Waters, 1995). For this particle size, structural damage has been reported in some studies (270–4887 mg L^{-1}, Herbert and Merkens, 1961; Servizi and Martens, 1987; Goldes et al., 1988), while other studies found no effect with similar concentrations and exposure durations (Redding et al., 1987; Lake and Hinch, 1999; Shrimpton et al., 2007). Based on altered hematocrit and leucocrit values, it has been suggested that the particles impair respiratory function by "irritating" gill epithelia (Redding et al., 1987; Lake and Hinch, 1999). For me this conclusion based solely on changes in hematocrit and leucocrit is problematic. Changes in these primary hematological end-points could "simply" reflect acute physiological stress (Houston, 1997) induced by turbid water as an unusual perceived stressor (Barton, 2002; Boonstra, 2013). It is also known that many of the gill lesions reported in the above cited studies (e.g. epithelial lifting, lamellar fusion, hyperplasia, and thickened gill epithelia) can be triggered via the stress axis (Mallatt, 1985). In summary, gill damage would most likely trigger a physiological stress response. However, histological gill damage as well

as primary and secondary responses of the stress axis alone are no proof for an impaired gill function. To document an impaired gill function, histological analyses should be complemented with biochemical measurements in the gill as well as systemic end-points of blood composition (Evans, 1987; Houston, 1997).

Turbidity can induce behavioral responses in salmonid fish (Waters, 1995). Well documented are avoidance of sediment plumes, surface swimming and alarm reactions/spurt swimming (Bisson and Bilby, 1982; Sigler et al., 1984; McLeay et al., 1987; Newcombe and Jensen, 1996). In rainbow trout avoidance can be a response to any new object ("neophobia", Sneddon et al., 2003). These behavioral responses could be therefore related to structural gill damage causing stress, or “simply” to turbidity as a new perceived stressor (Barton, 2002). Turbidity can also impair foraging success and feeding in salmonids, which has been reported to decrease growth and weight (Berg and Northcote, 1985; Shaw and Richardson, 2001; Sweka and Hartman, 2001b; De Robertis et al., 2003). Other authors related growth and weight effects to increased energy demands caused by increased activity, physiological stress and respiratory impairment (McLeay et al., 1987; Sweka and Hartman, 2001a; Shrimpton et al., 2007). Increased swimming might also indicate an adaptive response, since for example brook trout switched from drift feeding to active searching to compensate for turbidity effects (Sweka and Hartman, 2001a). Altogether, reduced feeding and growth is considered a significant sub-lethal effect of suspended fine sediment (Waters, 1995), but there is still controversy about how this effect manifests. Possible physiological adaptations of salmonids to turbidity induced food deprivation and metabolic stress (e.g. Simpkins et al., 2003; Harmon et al., 2011) have been not investigated so far.

Mineral particle uptake in the gill and inner organs could contribute to cytotoxic effects of mineral particles in salmonid fish (Goldes et al., 1986; Martens and Servizi, 1993; Newcombe and Jensen, 1996). In sockeye salmon, on average 14 mineral particles per 100 gill lamellae were taken up, and particles were also found in the spleen (Martens and Servizi, 1993). The magnitude of mineral particle uptake in inner organs was not quantified yet. However, 80000 plastic microspheres (1 µm diameter) accumulated in juvenile rainbow trout (1.5–2.9 g total weight) within 24 h water-borne exposure (Moore et al., 1998). After 24 days around 5000 microspheres were found in spleen and kidney. In mammalian cells, mineral particles can cause inflammatory responses, oxidative stress, and also affect membrane stability (Donaldson and Borm, 2007). I see no reason, why this should not be also the case in fish

cells. In fish, phagocytosis of particles by macrophages is one possible defense mechanism (Ellis et al., 1976). If and to what extent mineral particles cause cellular damage at their site of uptake has not been investigated so far.

My laboratory experiments

Chapter 2 describes an *in vivo* exposure that provides a systemic investigation of the effects of suspended mineral particle pulses in a salmonid fish, the rainbow trout (*Oncorhynchus mykiss*). Studies so far focused on individual aspects of suspended fine sediment effects in juvenile salmonid fish. In isolation many of the applied physiological end-points (e.g. cortisol, hematocrit, leucocrit) are not sufficient to demonstrate persistent pathologic effects. Changes in these end-points could be also a response to a novel cue and/or adaptations to survive the perturbation. Therefore, I designed this experiment to investigate physical damage as well as physiological, systemic, and apical responses that could manifest and persist during fine sediment pulse exposure. I explicitly focused on the fish as an organism to understand, firstly, how effects manifest at different levels of the biological organization, and secondly, how these effects relate to each other. I conceived the experiment, and had the lead for the entire experiment from conducting the exposure up to publishing the data.

Chapter 3 describes an *in vitro* experiment with rainbow trout gill epithelial cells**.** In this experiment we tested the hypothesis that mineral particles of different geochemical composition differ in their cytotoxic potential in salmonid gill epithelial cells. This hypothesis was based on two notions: Firstly, similar particle concentrations differ in their ability to cause histological damage in salmonid gills (see above), and, secondly, in my *in vivo* exposure mica particles caused no histological damage, but lipid peroxidation in the gill after 24 days exposure (**Chapter 2**). To test this, we developed an *in vitro* approach with the epithelial gill cell line RTgill-W1 (Bols et al., 1994). We applied this procedure to test four different mineral particles common in European and North American watersheds. The data in this chapter was generated by me and Simon Herzog, who conducted his MSc thesis as part of my project (Herzog, 2012). Further, I analyzed the data and had the lead for writing the manuscript.

Part 2 – Effects on salmonid embryo survival

Background

Increased fine sediment deposition in salmonid redds can decrease embryo survival during the intra-gravel stage (e.g. Jensen et al., 2009 and studies cited therein). This effect is mostly related to a decreased gravel permeability, which can hinder water flow through the egg pocket, and hence oxygen supply to the embryos (Greig et al., 2005; Malcolm et al., 2008). Moreover, the water source is important since upwelling of oxygen depleted groundwater can cause critical conditions even with sufficient water exchange (Malcolm et al., 2008).

The water-flow through salmonid redds can be described as function of gravel permeability (i.e. hydraulic conductivity) and hydraulic gradients (Darcy`s law; Lapointe et al., 2004). In rivers hydraulic gradients depend on discharge dynamics as well as the geomorphology of the river system over multiple scales: On the catchment scale, the river location relative to the aquifer modulates the relative contribution of surfacewater and groundwater to hyporheic exchange (Baxter and Hauer, 2000). Groundwater can have a profound impact on salmonid embryo survival, since it is often oxygen depleted (Malcolm et al., 2008). On the reach scale, bed-form and river structure, such as for example gravel bars, steps or log jams, create hydraulic gradients that drive local hyporheic exchange (e.g. Buffington and Tonina, 2009). Finally, the redd morphology itself can create hydraulic gradients driving pumping flows through the egg-pocket (Tonina and Buffington, 2009). Temporarily, water exchange varies with river discharge (Malcolm et al., 2004; Malcolm et al., 2006). High-flows contribute to temporal dynamics since they, firstly, re-suspend fine-sediments from the gravel bed, and, secondly, change bed-form and river structure (Schälchli, 1995; Montgomery and Buffington, 1998). All these factors jointly affect hyporheic exchange and oxygen concentrations, and hence oxygen supply to salmonid embryos during intragravel incubation (Greig et al., 2007). Thus, to understand how fine sediment affects salmonid embryo survival in a particular river system these spatial and temporal dynamics need to be incorporated (Gibbins et al., 2008; Malcolm et al., 2008; Newson et al., 2012).

The geomorphology of many Swiss streams is heavily modified (Woolsey et al., 2005), which agrees with many salmonid streams worldwide (Brookes, 1988; Gilvear et al., 2002; Wohl, 2006). Common modifications include channel relocation, straightening and lateral stabilization (“channelization”), as well as artificial structures for flow regulation and bed-stabilization. Common artificial in-stream structures are dams, weirs, and artificial steps.

Figure 2 – Our study river at base-flow (**A**) and during a high-flow event (**B**). Open triangles give flow direction, and dashed lines mark artificial steps. **Picture A:** During base-flow the water is clear, and almost no suspended material is transported. Marked are artificial redds at the position of the egg-capsules (asterisks), and two suspended sediment samplers (4). Inset **A1** shows an egg-capsule with brown trout eggs, scale bar = 1cm. Inset **A2** shows one artificial redd with egg capsules (asterisk, red lines, cf. **A1**) as well as the piezometer pipes in pit and tail (1), one bed-load sampler (2), and one accumulation basket (3), scale bar = 1m. For details of the redd structure see Figure 1A in Chapter 5 (page 84). For sampling devices see Figure 2 in Chapter 4 (page 67). **Picture B:** During high-flow the water became distinctly turbid. Grey pipes on river bank contain cables for continuous oxygen and temperature measurements.

All these modifications could impact salmonid embryos, because they change discharge dynamics, geomorphology, and hence hyporheic exchange and fine sediment accumulation (Allan, 2004; Elosegi et al., 2010). Channelization and decreased geomorphological diversity can limit hyporheic exchange in salmonid streams (Malcolm et al., 2008). Despite this, we still lack empirical studies that investigated how anthropogenic river modifications and fine sediment jointly affect salmonid incubation success in modified river environments.

Field experiment

Our field experiment (**Chapters 4–6**) was designed to contribute to an integrated process based understanding of the factors affecting brown trout embryo survival in heavily modified rivers. For this we selected a heavily modified river of the Swiss Plateau with a viable brown trout population, but not affected by hydropower or waste-water treatment plants.

The field experiment was conceived, set up and conducted by me and my PhD fellow Yael Schindler Wildhaber (Institute of Environmental Geosciences, University of Basel). I had the lead of all experimental procedures regarding the incubation of the brown trout embryos (biotic part). For the abiotic part I was involved in method development, and conducted the field work and sample collection together with Yael Schindler Wildhaber. For this experiment we used artificial brown trout redds, which is a common approach in fine sediment research (e.g. Rubin and Glimsäter, 1996; Malcolm et al., 2003; Greig et al., 2005; Levasseur et al., 2006). An example of an artificial brown trout redd used in our study is shown in Figure 2.

Chapter 4 describes the methods developed to measure fine sediment transport and deposition. It further describes and compares the dynamics of fine sediment transport and deposition in our study river, among our three study sites, and how they relate to the discharge dynamics of the river. For this chapter I was involved in sample collection and contributed to the writing of the manuscript.

Chapter 5 describes how fine sediment deposition, discharge dynamics, and river morphology affected water exchange and oxygen concentrations in the artificial redds. The results are discussed in the context of scale-dependent exchange processes (cf. Huber et al., in press). For this chapter I was involved in sample and data collection, contributed in discussions to the data analyses, and was involved in the writing of the manuscript.

In **Chapter 6** the most important predictor variables for brown trout embryo survival in our study river were identified using multivariate statistical modeling. Further, I synthesized the

process based understanding of the factors affecting brown trout embryo survival that we obtained during our project (Chapters 4–6 and Huber et al., in press). For this chapter I developed, coordinated and applied all procedures to assess embryo survival. I analyzed the embryo survival data, developed and applied the multivariate statistical data analysis, and wrote the manuscript.

References

Alabaster JS, Lloyd L. Water quality criteria for freshwater fish. Vol 716 London: Butterworths, 1982.

Allan JD. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology Evolution and Systematics 2004; 35: 257-284.

Asselman NEM. Suspended sediment dynamics in a large drainage basin: the River Rhine. Hydrological Processes 1999; 13: 1437-1450.

Asselman NEM, Middelkoop H, van Dijk PM. The impact of changes in climate and land use on soil erosion, transport and deposition of suspended sediment in the River Rhine. Hydrological Processes 2003; 17: 3225-3244.

Barton BA. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 2002; 42: 517-525.

Baxter CV, Hauer FR. Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 2000; 57: 1470-1481.

Berg L, Northcote TG. Changes in territorial, gill flaring, and feeding behavior in juvenile coho salmon (*Oncorhynchus kisutch*) following short term pulses of suspended sediment. Canadian Journal of Fisheries and Aquatic Sciences 1985; 42: 1410-1417.

Bilotta GS, Brazier RE. Understanding the influence of suspended solids on water quality and aquatic biota. Water Research 2008; 42: 2849-2861.

Bisson PA, Bilby RE. Avoidance of suspended sediment by juvenile coho salmon. North American Journal of Fisheries Management 1982; 2: 371-374.

Bols NC, Barlian A, Chirinotrejo M, Caldwell SJ, Goegan P, Lee LEJ. Development of a cell-line from primary cultures of rainbow trout, *Oncorhynchus mykiss* (Walbaum), gills. Journal of Fish Diseases 1994; 17: 601-611.

Boonstra R. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. Functional Ecology 2013; 27: 11-23.

Borsuk ME, Reichert P, Peter A, Schager E, Burkhardt-Holm P. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecological Modelling 2006; 192: 224-244.

Brookes A. Channelized Rivers: Perspectives for Environmental Management. Chichester: Wiley, 1988.

Brunke M. Colmation and depth filtration within streambeds: Retention of particles in hyporheic interstices. International Review of Hydrobiology 1999; 84: 99-117.

Buffington JM, Tonina D. Hyporheic Exchange in Mountain Rivers II: Effects of Channel Morphology on Mechanics, Scales, and Rates of Exchange. Geography Compass 2009; 3: 1038-1062.

Burkhardt-Holm P, Giger W, Guttinger H, Ochsenbein U, Peter A, Scheurer K, et al. Where have all the fish gone? Environmental Science & Technology 2005; 39: 441a-447a.

Burkhardt-Holm P, Scheurer K. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquatic Sciences 2007; 69: 51-70.

Crosa G, Castelli E, Gentili G, Espa P. Effects of suspended sediments from reservoir flushing on fish and macroinvertebrates in an alpine stream. Aquatic Sciences 2010; 72: 85-95.

Crozier LG, Hendry AP, Lawson PW, Quinn TP, Mantua NJ, Battin J, et al. Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications 2008; 1: 252-270.

De Robertis A, Ryer CH, Veloza A, Brodeur RD. Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. Canadian Journal of Fisheries and Aquatic Sciences 2003; 60: 1517-1526.
Donaldson K, Borm P. Particle Toxicology. Boca Raton, FL CRC Press, 2007.
Dore MHI. Climate change and changes in global precipitation patterns: What do we know? Environment International 2005; 31: 1167-1181.
Ellis AE, Munroe ALS, Roberts RJ. Defence mechanisms in fish 1: study of phagocytic system and fate of intraperitoneally injected particulate material in plaice (*Pleuronectes platessa* L). Journal of Fish Biology 1976; 8: 67-&.
Elosegi A, Diez J, Mutz M. Effects of hydromorphological integrity on biodiversity and functioning of river ecosystems. Hydrobiologia 2010; 657: 199-215.
Evans DH. The fish gill - site of action and model for toxic effects of environmental pollutants. Environmental Health Perspectives 1987; 71: 47-58.
Evans DJ, Gibson CE, Rossell RS. Sediment loads and sources in heavily modified Irish catchments: A move towards informed management strategies. Geomorphology 2006; 79: 93-113.
Fischnetz. Dem Fischrückgang auf der Spur. Schlussbericht des Projektes Netzwerk Fischrückgang Schweiz "Fischnetz": Eawag, BUWAL, Dübendorf, Bern, 2004.
Foster IDL, Lees JA. Changes in the physical and geochemical properties of suspended sediment delivered to the headwaters of LOIS river basins over the last 100 years: a preliminary analysis of lake and reservoir bottom sediments. Hydrological Processes 1999; 13: 1067-1086.
Gibbins C, Shellberg J, Moir H, Soulsby C. Hydrological influences on adult salmonid migration, spawning and embryo survival. In: Sear D, DeVries P, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. 65. American Fisheries Society, Bethesda, USA, 2008, pp. 195-224.
Giller PS, Malmqvist B. The Biology of Streams and Rivers. New York: Oxford University Press, 1998.
Gilvear DJ, Heal KV, Stephen A. Hydrology and the ecological quality of Scottish river ecosystems. Science of the Total Environment 2002; 294: 131-159.
Goldes SA, Ferguson HW, Daoust PY, Moccia RD. Phagocytosis of the inert suspended clay kaolin by the gills of rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 1986; 9: 147-151.
Goldes SA, Ferguson HW, Moccia RD, Daoust PY. Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 1988; 11: 23-33.
Greig SM, Sear DA, Carling PA. The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management. Science of the Total Environment 2005; 344: 241-258.
Greig SM, Sear DA, Carling PA. A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. Hydrological Processes 2007; 21: 323-334.
Harmon KJ, Bolinger MT, Rodnick KJ. Carbohydrate energy reserves and effects of food deprivation in male and female rainbow trout. Comparative Biochemistry and Physiology A - Molecular & Integrative Physiology 2011; 158: 423-431.
Harvey BC, Lisle TE. Effects of suction dredging on streams: A review and an evaluation strategy. Fisheries 1998; 23: 8-17.
Henley WF, Patterson MA, Neves RJ, Lemly AD. Effects of sedimentation and turbidity on lotic food webs: a concise review for natural resource managers. Reviews in Fisheries Science 2000; 8: 125-139.

Herbert DWM, Merkens JC. The effect of suspended mineral solids on the survival of trout. International Journal of Air and Water Pollution 1961; 5: 46-55.
Herzog S. Cytotoxic effects of mineral particles on fish gill cells *in vitro*. Environmental Sciences. MSc. University of Basel, Basel, 2012, pp. 29.
Hicks BJ, Hall JD, Bisson PA, Sedell JR. Responses of salmonids to habitat changes. In: Meehan WR, editor. Influences of forest and rangeland management on salmonid fishes and their habitats. 19, 1991, pp. 483-518.
Houston AH. Review: Are the classical hematological variables acceptable indicators of fish health? Transactions of the American Fisheries Society 1997; 126: 879-894.
Huber E, Huggenberger P, Epting J, Schindler Wildhaber Y. Zeitliche und räumliche Skalen der Fluss-Grundwasser-Interaktion: Ein multidimensionaler hydrogeologischer Untersuchungsansatz. Grundwasser in press: DOI 10.1007/s00767-013-0220-x.
Irion G. Minerals in rivers. In: Degens ET, Kempe S, Richey JE, editors. Biogeochemistry of majorworld rivers. John Willey & Sons, New York, 1991, pp. 265-283.
Jensen DW, Steel EA, Fullerton AH, Pess GR. Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. Reviews in Fisheries Science 2009; 17: 348-359.
Jentsch A, Beierkuhnlein C. Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. Comptes Rendus Geoscience 2008; 340: 621-628.
Jonsson B, Jonsson N. Ecology of Atlantic salmon and brown trout: Habitat as a template for life histories. Vol 33. New York.: Springer, 2011.
Jordan DS. Report of explorations in Colorado and Utah during the summer of 1889, with an account of the fishes found in each of the river basins examined. U.S. Fish Commission Bulletin 1891; 9: 1-40.
Katz J, Moyle P, Quiñones R, Israel J, Purdy S. Impending extinction of salmon, steelhead, and trout (Salmonidae) in California. Environmental Biology of Fishes 2012: 1-18.
Kemp P, Sear D, Collins A, Naden P, Jones I. The impacts of fine sediment on riverine fish. Hydrological Processes 2011; 25: 1800-1821.
Lake RG, Hinch SG. Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 1999; 56: 862-867.
Lapointe M, Bergeron N, Berube F, Pouliot M, Johnston P. Interactive effects of substrate sand and silt contents, redd-scale hydraulic gradients, and interstitial velocities on egg-to-emergence survival of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 2004; 61: 2271-2277.
Lenzi MA, Marchi L. Suspended sediment load during floods in a small stream of the Dolomites (northeastern Italy). Catena 2000; 39: 267-282.
Levasseur M, Bergeron N, Lapointe M, Bérubé F. Effects of silt and very fine sand dynamics in Atlantic salmon (*Salmo salar*) redds on embryo hatching success. Canadian Journal of Fisheries and Aquatic Sciences 2006; 63: 1450-1459.
Lisle TE. Sediment transport and resulting deposition in spawning gravels, North Coastal California. Water Resources Research 1989; 25: 1303-1319.
Malcolm IA, Greig SM, Youngson AF, Soulsby C. Hyporheic influences on salmon embryo survival and performance. In: Sear D, DeVries P, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. 65. American Fisheries Society, Bethesda, USA, 2008, pp. 225-248.
Malcolm IA, Soulsby C, Youngson AF. High-frequency logging technologies reveal state-dependent hyporheic process dynamics: implications for hydroecological studies. Hydrological Processes 2006; 20: 615-622.

Malcolm IA, Soulsby C, Youngson AF, Hannah DM, McLaren IS, Thorne A. Hydrological influences on hyporheic water quality: implications for salmon egg survival. Hydrological Processes 2004; 18: 1543-1560.

Malcolm IA, Youngson AF, Soulsby C. Survival of salmonid eggs in a degraded gravel-bed stream: Effects of groundwater-surfacewater interactions. River Research and Applications 2003; 19: 303-316.

Mallatt J. Fish gill structural changes induced by toxicants and other irritants - a statistical review. Canadian Journal of Fisheries and Aquatic Sciences 1985; 42: 630-648.

Martens DW, Servizi JA. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Fisheries and Aquatic Sciences 1993; 50: 586-590.

McLeay DJ, Birtwell IK, Hartman GF, Ennis GL. Responses of Arctic grayling (*Thymallus arcticus*) to acute and prolonged exposure to Yukon placer mining sediment. Canadian Journal of Fisheries and Aquatic Sciences 1987; 44: 658-673.

Montgomery DR, Buffington JM. Channel processes, classification, and response. In: Naiman R, Bilby R, editors. River ecology and management: lessons from the Pacific coastal ecoregion. . Springer-Verlag, New York, 1998, pp. 13-42.

Moore JD, Ototake M, Nakanishi T. Particulate antigen uptake during immersion immunisation of fish: The effectiveness of prolonged exposure and the roles of skin and gill. Fish & Shellfish Immunology 1998; 8: 393-407.

Nehlsen W, Williams JE, Lichatowich JA. Pacific salmon at the crossroads - stocks at risk from California, Oregon, Idaho, and Washington. Fisheries 1991; 16: 4-21.

Newcombe CP. Impact assessment model for clear water fishes exposed to excessively cloudy water. Journal of the American Water Resources Association 2003; 39: 529-544.

Newcombe CP, Jensen JOT. Channel suspended sediment and fisheries: A synthesis for quantitative assessment of risk and impact. North American Journal of Fisheries Management 1996; 16: 693-727.

Newson M, Sear D, Soulsby C. Incorporating hydromorphology in strategic approaches to managing flows for salmonids. Fisheries Management and Ecology 2012; 19: 490-499.

Owens PN, Batalla RJ, Collins AJ, Gomez B, Hicks DM, Horowitz AJ, et al. Fine-grained sediment in river systems: Environmental significance and management issues. River Research and Applications 2005; 21: 693-717.

Pont D, Simonnet JP, Walter AV. Medium-term changes in suspended sediment delivery to the ocean: Consequences of catchment heterogeneity and river management (Rhone River, France). Estuarine Coastal and Shelf Science 2002; 54: 1-18.

Redding JM, Schreck CB, Everest FH. Physiological effects on coho salmon and steelhead of exposure to suspended solids. Transactions of the American Fisheries Society 1987; 116: 737-744.

Rubin JF, Glimsäter C. Egg-to-fry survival of the sea trout in some streams of Gotland. Journal of Fish Biology 1996; 48: 585-606.

Schager E, Peter A, Burkhardt-Holm P. Status of young-of-the-year brown trout (*Salmo trutta* fario) in Swiss streams: factors influencing YOY trout recruitment. Aquatic Sciences 2007; 69: 41-50.

Schälchli U. Basic equations for siltation of riverbeds. J. Hydraul. Eng.-Asce 1995; 121: 274-287.

Scheurer K, Alewell C, Banninger D, Burkhardt-Holm P. Climate and land-use changes affecting river sediment and brown trout in alpine countries - a review. Environmental Science and Pollution Research 2009; 16: 232-242.

Servizi JA, Martens DW. Some effects of suspended Fraser River sediments on sockeye salmon, *Oncorhynchus nerka*. In: Smith HD, Margolis L, Wood CC, editors. Sockeye salmon, *Oncorhynchus nerka*, population biology and future management. 96. Canadian Special Publications in Fisheries and Aquatic Sciences, 1987, pp. 254–264.

Seydell I, Ibisch RB, Zanke UCE. Intrusion of suspended sediments into gravel riverbeds: influence of bed topography studied by means of field and laboratory experiments. Advanc.Limnol. 2009; 61: 67-85.

Shaw EA, Richardson JS. Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. Canadian Journal of Fisheries and Aquatic Sciences 2001; 58: 2213-2221.

Shrimpton JM, Zydlewski JD, Heath JW. Effect of daily oscillation in temperature and increased suspended sediment on growth and smolting in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Aquaculture 2007; 273: 269-276.

Sigler JW, Bjornn TC, Everest FH. Effects of chronic turbidity on density and growth of steelheads and coho salmon. Transactions of the American Fisheries Society 1984; 113: 142-150.

Simpkins DG, Hubert WA, Del Rio CM, Rule DC. Physiological responses of juvenile rainbow trout to fasting and swimming activity: Effects on body composition and condition indices. Transactions of the American Fisheries Society 2003; 132: 576-589.

Slaymaker O. The sediment budget as conceptual framework and management tool. Hydrobiologia 2003; 494: 71-82.

Sneddon LU, Braithwaite VA, Gentle MJ. Novel object test: Examining nociception and fear in the rainbow trout. Journal of Pain 2003; 4: 431-440.

Suttle KB, Power ME, Levine JM, McNeely C. How fine sediment in riverbeds impairs growth and survival of juvenile salmonids. Ecological Applications 2004; 14: 969-974.

Sweka JA, Hartman KJ. Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. Canadian Journal of Fisheries and Aquatic Sciences 2001a; 58: 386-393.

Sweka JA, Hartman KJ. Influence of turbidity on brook trout reactive distance and foraging success. Transactions of the American Fisheries Society 2001b; 130: 138-146.

Syvitski JPM, Vorosmarty CJ, Kettner AJ, Green P. Impact of humans on the flux of terrestrial sediment to the global coastal ocean. Science 2005; 308: 376-380.

Tonina D, Buffington JM. A three-dimensional model for analyzing the effects of salmon redds on hyporheic exchange and egg pocket habitat. Canadian Journal of Fisheries and Aquatic Sciences 2009; 66: 2157-2173.

US EPA. National water quality inventory: report to congress (2004 reporting cycle). United States Environmental Protection Agency, Office of Water, Washington DC, 2009, pp. 42.

Walling DE. Suspended sediment transport by rivers: a geomorphological and hydrological perspective. Arch. Hydrobiol. Spec. Issues Advanc. Limnology 1996; 47: 1-27.

Walling DE, Owens PN, Waterfall BD, Leeks GJL, Wass PD. The particle size characteristics of fluvial suspended sediment in the Humber and Tweed catchments, UK. Science of the Total Environment 2000; 251: 205-222.

Waters TF. Sediment in streams. Sources, biological effects, and control. Bethesda, MD: American Fisheries Society, 1995.

Wilber DH, Clarke DG. Biological effects of suspended sediments: A review of suspended sediment impacts on fish and shellfish with relation to dredging activities in estuaries. North American Journal of Fisheries Management 2001; 21: 855-875.

Wohl E. Human impacts to mountain streams. Geomorphology 2006; 79: 217-248.

Wood PJ, Armitage PD. Biological effects of fine sediment in the lotic environment. Environmental Management 1997; 21: 203-217.

Woolsey S, Weber C, Gonser T, Hoehn E, Hostmann M, Junker B, et al. Handbuch für die Erfolgskontrolle bei Fliessgewässerrevitalisierungen: Rhone-Thur Projektes. Eawag, WSL, LCH-EPFL, VAW-ETHZ, 2005.

Yoshiyama RM, Fisher FW, Moyle PB. Historical abundance and decline of Chinook Salmon in the central valley region of California. North American Journal of Fisheries Management 1998; 18: 487-521.

Part 1

Effects on juvenile salmonid fish

Chapter 2

Suspended sediment pulse effects in rainbow trout (*Oncorhynchus mykiss*) – relating apical and systemic responses

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ARTICLE

Suspended sediment pulse effects in rainbow trout (*Oncorhynchus mykiss*) — relating apical and systemic responses

Christian Michel, Heike Schmidt-Posthaus, and Patricia Burkhardt-Holm

Abstract: To provide an integrated perspective on mineral particle effects in salmonids, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to daily mica particle pulses for 8 and 24 days. On day 8, increased immature erythrocyte proportions indicated a previous stress response. This response was absent on day 24, on which condition factor as well as plasma protein and aspartate aminotransferase activity decreased. The latter two related negatively to the hepato-somatic index, suggesting metabolic adaptations. The hepato-somatic index increased on days 8 and 24, while spleen-somatic index increased on day 24. No histopathological damage occurred in gills, liver, spleen, or kidney. However, splenic melano-macrophages increased on both days, and hyaline degenerations of kidney tubular cells were apparent on day 24. Overall, particle pulses affected rainbow trout more via turbidity rather than by physical damage. We conclude that (*i*) rainbow trout may adapt to sediment pulses as early as 8 days of exposure and (*ii*) particle pulses over 24 days can cause structural and metabolic changes in rainbow trout, even when gill damage is absent and apical effects on condition are moderate.

Résumé : Afin d'établir une compréhension intégrée des effets des particules minérales sur les salmonidés, des truites arc-en-ciel (*Oncorhynchus mykiss*) juvéniles ont été exposées quotidiennement à des doses pulsées de particules de mica pendant des périodes de 8 à 24 jours. Au jour 8, des proportions accrues d'érythrocytes immatures indiquaient une réaction de stress antérieure. Cette réaction était absente au jour 24, jour où des diminutions du facteur d'embonpoint ainsi que des protéines plasmatiques et de l'activité de l'aspartate aminotransférase ont été notées. Le fait que ces deux derniers paramètres étaient négativement reliés à l'indice hépatosomatique laisse croire à des adaptations métaboliques. L'indice hépatosomatique avait augmenté aux jours 8 et 24, alors que l'indice splénosomatique avait augmenté au jour 24. Aucun dommage histopathologique ne s'est produit dans les branchies, le foie, la rate ou les reins. Toutefois, une abondance accrue de mélanomacrophages spléniques a été observée aux jours 8 et 24 et des dégénérescences hyalines des cellules tubulaires rénales étaient présentes au jour 24. Dans l'ensemble, les truites arc-en-ciel étaient plus fortement affectées par la turbidité associée aux doses pulsées que par des dommages physiques en découlant. Nous en concluons que (*i*) la truite arc-en-ciel peut s'adapter à des doses pulsées de sédiments dès le huitième jour après l'exposition et (*ii*) les doses pulsées de particules sur plus de 24 jours peuvent causer des changements structuraux et métaboliques chez la truite arc-en-ciel, même si des dommages aux branchies sont absents et que les effets apicaux sur l'embonpoint sont modérés. [Traduit par la Rédaction]

Introduction

Suspended sediments are common in aquatic ecosystems, but sediment loads are also increasing worldwide, often as a result of anthropogenic activities (Waters 1995; Syvitski et al. 2005; Scheurer et al. 2009). In Europe, sediment yields in the alpine Rhine are predicted to increase more than twofold by the year 2100 (Asselman et al. 2003). In England and Wales, historic data suggest that sediment yields in some lowland rivers increased fourfold during the last century (Foster and Lees 1999). The United States Environmental Protection Agency has identified sediments as among the top ten threats for freshwater and marine ecosystems health (US EPA 2009).

Suspended sediments can have detrimental effects on fish, including salmonids (Bilotta and Brazier 2008; Scheurer et al. 2009; Kemp et al. 2011). The effect of mineral particles on free-swimming salmonid fish decreases with particle size and increases with particle concentration and exposure duration (Servizi and Martens 1987; Newcombe and Jensen 1996). Under many environmental conditions, sublethal effects predominate (Alabaster and Lloyd 1982; Waters 1995), especially with particles in the low micrometre to nanometre range (Newcombe 2003). Particles in this range, referred to here as "small-sized particles", may affect salmonid fish via turbidity but also by direct physical damage (Newcombe and Jensen 1996; Newcombe 2003).

Acute sublethal responses of salmonid fish to suspended mineral particle exposure are well documented (table A1 in Newcombe and Jensen 1996). Salmonids regularly experience physiological stress when challenged with suspended mineral particles, a response often paralleled by decreased leucocrit and increased hematocrit values (Redding and Schreck 1982; Redding et al. 1987; Servizi and Martens 1992; Lake and Hinch 1999). Likewise, gill lesions and particle uptake in gills and spleen have been reported (Goldes et al. 1986; Servizi and Martens 1987; Goldes et al. 1988; Martens and Servizi 1993). Therefore, increased hematocrits could be related to the acute stress response (Pearson and Stevens 1991) but also to a threatened respiratory homeostasis (Gallaugher and Farrell 1998). Behavioral responses include avoidance of sediment plumes and "alarm reactions" (Bisson and Bilby 1982; Sigler et al. 1984; Berg and Northcote 1985). Finally, reduced growth and mass of salmonid fish exposed to suspended mineral particles beyond

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4 days has been attributed to increased energy demands (Sweka and Hartman 2001*a*; Shrimpton et al. 2007) but also to reduced feeding in turbid waters (Shaw and Richardson 2001). In summary, numerous studies have investigated specific aspects of suspended mineral particle effects in salmonids, but no study has investigated the effects on the different body systems of a salmonid fish simultaneously. Furthermore, little is known about longer-term systemic responses, which could include adaptations to maintain the metabolic and hematologic homeostasis (cf. Houston 1997; Beyers et al. 1999). This knowledge would provide a more integrated perspective and therefore contribute to a better risk assessment of suspended mineral particle exposure in salmonid fish.

Our study is the first systemic investigation of small-sized suspended mineral particle effects in juvenile rainbow trout (*Oncorhynchus mykiss*). Its aim was to investigate physical damage as well as physiological, systemic, and apical responses that could manifest and persist during sediment exposure. Our analyses cover different levels of biological organization (i.e., cells, organs, and whole animal) and key areas of physiology (i.e., osmoregulation, hematology, and metabolism). This body systems approach (cf. Federici et al. 2007) allowed us to relate effects in target organs (i.e., gills, liver, spleen, and kidney) to systemic and apical responses. Thereby, we investigated (*i*) whether particles caused physical damage and (or) biochemical effects in the gills, (*ii*) whether particles caused histopathological, biochemical, or cellular effects in target organs (i.e., gills, liver, spleen, and trunk kidney), and (*iii*) whether systemic and apical responses related to target organ effects, but also to food deprivation or a stress response. All effects were investigated after 8 and 24 days to assess differences related to exposure duration and also possible adaptive responses.

Materials and methods

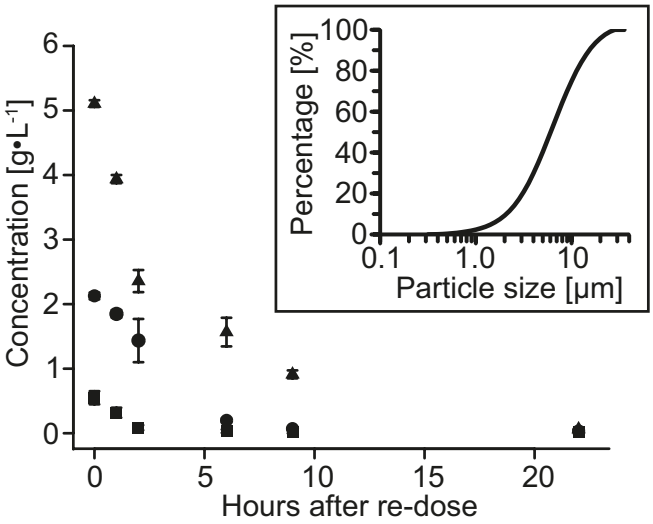
Experimental procedures

Juvenile rainbow trout were obtained from a trout hatchery (Pisciculture de Vionnaz Hess SA, Switzerland). Fish were acclimatized for 2 weeks before random distribution to 12 experimental tanks (60 L volume; $n = 12$ fish per tank). During distribution, total mass and standard length of each fish were recorded. Initial total mass and standard length of fish was 44.30 ± 0.37 g and 14.45 ± 0.04 cm (mean ± SE, $n = 144$) respectively. No initial differences for either parameter or condition factor were detected between experimental tanks. Following transfer the fish were rested for 4 days before the onset of particle exposure. During the experiment fish were fed commercial trout pellets (HOKOVIT Silvercup bio, H.U. Hoffmann AG, Switzerland) at a rate of 1% of body mass per day. Water flow in tanks was adjusted to approximately 300 mL·min^{-1}.

Fish were exposed to daily pulses of small-sized mica particles (Fig. 1; Aspanger Bergbau und Mineralwerke GmbH & Co KG, Austria). These particles are extracted from a natural deposit via wet processing and hence can be expected to be comparable to particles also transported in natural rivers (e.g., Martens and Servizi 1993; Atteia et al. 1998). Treatment levels were as follows: 0 mg·L^{-1} (control) and 300 (low), 1300 (medium), and 5000 mg·L^{-1} (high) nominal initial particle concentrations. Three replicate tanks per treatment level were applied. Treatment levels were chosen to cover a range of particle concentrations that salmonid fish could experience in the field, either naturally (Tramblay et al. 2010; Mitchell 2012; Kröger et al. 2013) or related to human activities (Crosa et al. 2010). The maximum concentration was set below the level that kaolin, another phyllosilicate particle, caused mortality in rainbow trout (Goldes et al. 1988).

For re-dosing, mica particles were introduced into 10 L Duran glass jars (Schott Duran Group GmbH, Germany) located on a rack above the experimental tanks. Particles were suspended by vigorous stirring with a magnetic stirrer bar (500 r·min^{-1}, ≥10 min) and complete suspension was checked visually before re-dosing. Then approximately 20 L water was siphoned from the tank, and the particle suspension was carefully infused using a hose and gravity-driven water flow. Control tanks were treated similarly except that they were infused with clear water. Re-dosing was repeated every 24 h. Fish were fed at least 30 min prior to the next sediment pulse, and pulses were applied when feeding activity had ceased.

Fig. 1. Suspended mineral particle concentration in exposure tanks during the 24 h between re-dosing. Symbols denote mean ± SE concentration in treatment levels (squares = low, circles = medium, triangles = high). The inset on the upper right shows the cumulative size distribution of the mica particle mixture used in the experiment.



Initial particle concentrations in tanks were inferred from 500 mL water samples every 4 days collected in the center of each tank immediately after re-dosing. The time course of the suspended particle concentration was determined from samples taken 1, 3, 6, 9, and 22 h after re-dosing collected once weekly in the center of one tank per treatment level. Samples were dried to constant mass in a heating cabinet and weighed (±0.1 mg). Particle concentrations (*w*/*v*) were calculated from the amount of suspended matter in the tank minus the mean mass of suspended matter in control tanks. Every 3 days temperature, pH (WTW 3210 pH meter), conductivity (WTW 330i conductivity meter), and dissolved oxygen concentrations (oxygen dipping probe; PreSens GmbH, Germany) were monitored.

Sampling

Rainbow trout were sampled on days 8 and 24 of the exposure ($n = 5$ fish per tank per sampling day). Feeding was stopped the day before sampling, and fish were sampled 24 h after the last sediment pulse. No sediment pulses were applied on the sampling days. On each sampling day, five fish were collected per tank and killed with buffered tricaine methanesulfonate (MS-222, Sigma Aldrich GmbH, Switzerland). For hematological analyses, blood was collected from two of these fish ($n = 6$ fish per treatment level per sampling day) with heparinized syringes from the caudal vein. For biochemical analyses, organ samples (second left gill arch, liver, spleen, and trunk kidney) from the same fish were collected, snap-frozen in liquid nitrogen, and stored at −80 °C. For histological analyses, the same organs were sampled from the three remaining fish per tank ($n = 9$ fish per treatment level per sampling day). Tissues were carefully excised, fixed in 10% phosphate-buffered formalin (Carl Roth GmbH, Switzerland), and kept at 4 °C in the dark until processing. Total body mass (±0.1 g) and standard length (±0.1 cm) as well as spleen and liver mass (±0.1 mg) were recorded for all fish. Spleen-somatic index (SSI), hepato-somatic index (HSI), and mean specific growth rate per tank were calcu-

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lated as described in Schubert et al. (2008). Fulton's condition factor K was calculated as $K = 100 \times (\text{mass} \times \text{length}^{-3})$, using total mass and standard length. Regardless of sampling day, no effect of sampling order on spleen mass and spleen-somatic index was observed.

Hematology and plasma analysis

Hematological analyses ($n = 6$ fish per treatment level per sampling day) were performed according to standard methods (e.g., Handy and Depledge 1999). Duplicate erythrocyte counts and hematocrit and leucocrit measurements were conducted immediately after blood collection. In parallel, blood smears were prepared and stained with a modified Wright–Giemsa stain (Sigma-Aldrich, Switzerland). Hemoglobin levels were measured in triplicate with the cyanmethemoglobin method. Blood samples were then centrifuged (5000*g*, 4 °C, 5 min), and plasma was stored at −80 °C. Plasma electrolytes (Na^+, K^+, Cl^-, Ca^{2+}), marker enzymes (aspartate aminotransferase (ASAT), alanine aminotransferase), and blood glucose were measured with an automated clinical analyzer (Beckman Coulter UniCel DXC600). Plasma total protein was determined with the semimicro modification of the Lowry method described in Handy and Depledge (1999).

Erythron composition and mature erythrocyte morphology were investigated by image analysis of blood smears (Houston and Murad 1992). Pictures were taken under oil immersion (1000× magnification) as described below for histological analyses. Immature, mature, degrading, and dividing erythrocytes were counted on 20 images per blood smear from each of two smears per fish ($n = 40$ pictures per fish analyzed; mean = 1846, minimum = 1247, maximum = 2336 cells per fish counted). Erythrocyte maturation stages were identified following Lehmann and Stürenberg (1974). Lengths and widths of five mature erythrocytes were measured on six randomly selected pictures per fish ($n = 30$ cells per fish). From these, the erythrocyte area ($0.25 \times \pi \times \text{length} \times \text{width}$) and shape factor (width/length) were calculated. Erythron analyses were conducted with sample names blinded and replaced by random numbers to avoid subjective bias.

Biochemical analysis

Biochemical analyses were conducted on the second left gill arch, liver, spleen, and trunk kidney from all fish included in the hematological analysis ($n = 6$ fish per treatment level per sampling day). For enzyme assays, tissues were homogenized in 19 volumes of ice-cold SEID buffer (McCormick 1993) with an Ultra-Turrax (IKA Werke, Germany). Homogenates were centrifuged (5000*g*, 4°C, 1 min), and enzyme activities were measured in supernatants. Na^+K^+-ATPase activity was measured following McCormick (1993). Lactate dehydrogenase (LDH) activity was measured following Bergmeyer (1974), modified for 96-well plates. Enzyme activities were determined from the NADH-related absorption decrease over 10 min in a plate reader (340 nm wavelength, 21 °C; Infinite M200, Tecan Group Ltd., Switzerland). Samples were measured in duplicate. To investigate particle exposure-related oxidative cell damage, thiobarbituric acid-reactive substances (TBARS) were determined. Tissues were homogenized in 15 volumes of ice-cold phosphate-buffered saline with an Ultra-Turrax (IKA Werke, Germany). This assay was conducted in triplicate following Holt et al. (1986) and Rau et al. (2004), modified for 96-well plates. Protein content in homogenates was determined with the semimicro modification of the Lowry method described in Handy and Depledge (1999). Enzyme activities and TBARS content were normalized to tissue protein. All chemicals for biochemical analyses were purchased from Sigma-Aldrich (Switzerland).

Histological analysis

Histological assessments were conducted on the second left gill arch, liver, spleen, and trunk kidney ($n = 9$ fish per treatment level per sampling day). Tissues were automatically processed (TP1020 tissue processor, Leica Microsystems AG, Switzerland), and eight sections per fish (3–5 μm thickness) were mounted on microscope slides. All sections were stained with haematoxylin and eosin. Additionally, spleen and kidney sections were stained with Prussian blue to detect changes in tissue iron content related to erythrocyte turnover. For histo-pathological examination (Nikon Eclipse 400 microscope), sections were first screened (100–200× magnification) and then examined in detail (400× magnification). The quantitative analyses described below were conducted on fish from the control and the high particle treatment level (each $n = 9$ fish per sampling day). Digital images were taken with a Nikon DXM 1200 F digital camera and Nikons ACT-1 software (version 2.63).

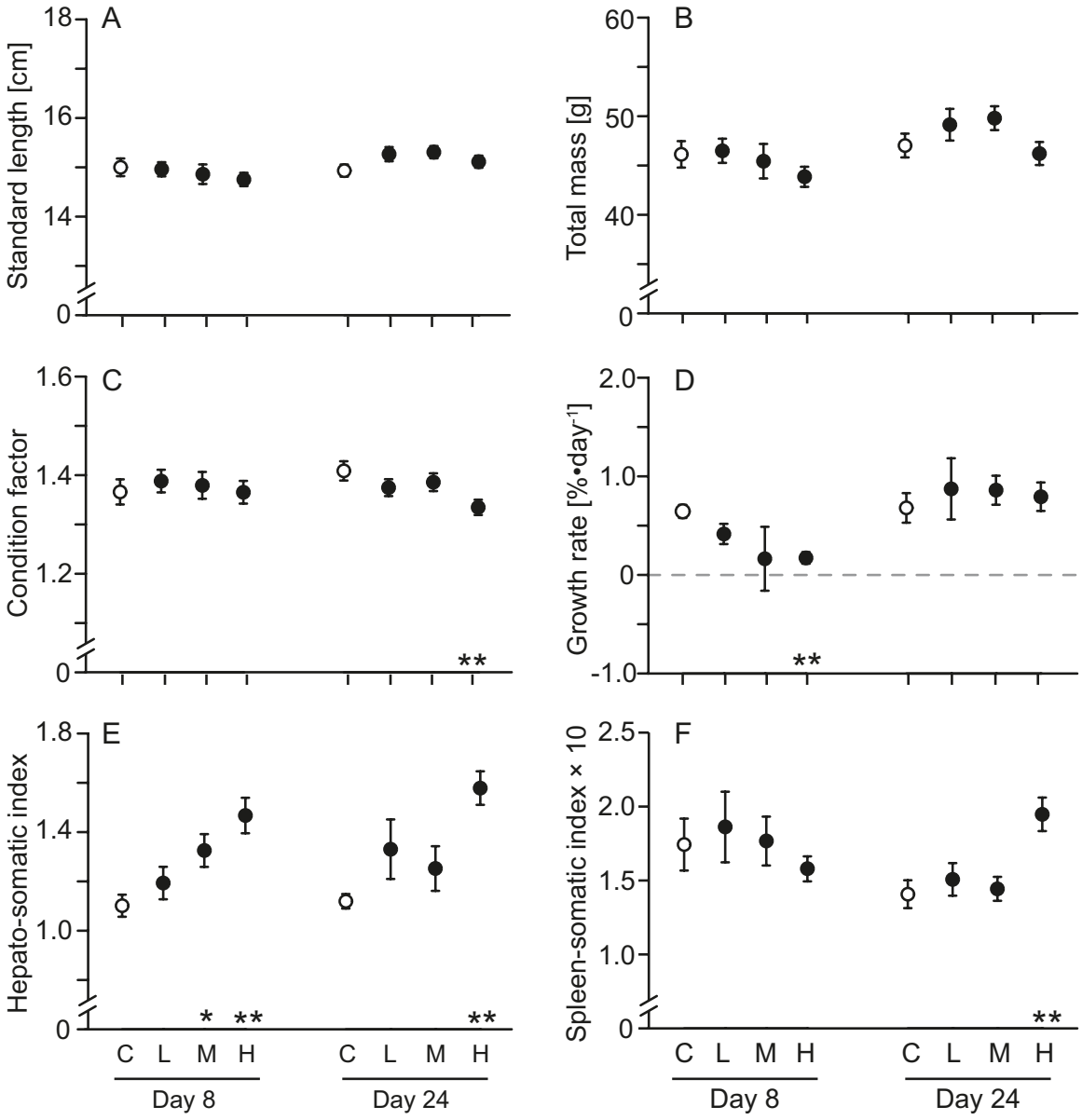
In spleen and kidney, the number and area fraction of melano-macrophage aggregates (MAs) was quantified (Schwindt et al. 2006), with four digital images analyzed for each of two sections ($n = 8$ images per fish). In the red pulp of the spleen, accumulations of individual macrophages containing distinct dark granules were observed (henceforth termed "granular macrophages", abbreviated GM). To quantify these, we defined a granular macrophage accumulation (GMA) as an area with ≥10 GMs present per microscope field (200× magnification). Then two sections were screened for GMAs along three nonoverlapping longitudinal transects covering the entire organ. Granular macrophages were counted in three representative microscope fields per section located along transects (maximum of $n = 6$ fields per fish counted). Counting fields were separated ≥1000 μm to avoid counting the same accumulation twice. In the kidney, hyaline degeneration of tubular cells was observed. To quantify this effect, the number of pictures showing this effect was recorded ($n = 8$ images per fish screened). This number was used as a measure for the degree of degenerated tubules present. All image analyses were conducted with the software ImageJ for microscopy version 1.46f (Collins 2007). All quantitative histological analyses were conducted with sample names blinded and replaced by random numbers to avoid subjective bias. For better visualization, the contrast of the shown pictures (Fig. 3) has been adjusted with the Auto Contrast function of Adobe Photoshop CS3 version 10.0.1.

Statistical analysis

All statistical analyses were conducted in the open-source statistics software R version 2.12.0 (R Development Core Team 2011). Significance was accepted at $p \leq 0.05$. A two-way ANOVA was applied to test for differences in particle concentrations between treatment levels and time points after re-dose. For this analysis, the particle concentration was $\log_{10}$-transformed. One-way ANOVA was used to test for differences in water chemistry parameters and water flow between (*i*) experimental tanks and (*ii*) treatment levels as well as for differences in mass, length, and condition factor between experimental tanks before the onset of exposure. Linear regression analysis was used to test for a relationship between organ indices (HSI, SSI) and hematological and plasma parameters. For all these analyses, linear models were applied (function: lm). Model assumptions were evaluated according to standard procedures (e.g., Venables and Ripley 1994), and no violations were observed.

All other data were analyzed with mixed effects models (Zuur et al. 2009). Tanks were included as random effect to account for repeated sampling from the same tank (cf. chapter 5 in Zuur et al. 2009). Continuous response variables were analyzed with linear mixed effect models (function: lmer, lme4 package) and "treatment level" as a categorical explanatory variable. Significance was tested via likelihood ratio tests (Zuur et al. 2009). Response variables were $\log_{10}$-transformed to adjust them to model assumptions. If outliers were observed, the model was fitted with and without these data points, and significance was only accepted when supported in both analyses. Once a significant main effect was detected, each particle treatment level was compared with the respective control. For this we used the Markov chain Monte

Fig. 2. Apical effects and organ indices in rainbow trout exposed to suspended mica particles for 8 and 24 days. Symbols denote mean ± SE; asterisks near the *x* axis denote significant differences from control on respective days (*, $p < 0.05$; **, $p < 0.01$). Labels on the *x* axis denote control (C) and low (L), medium (M), and high (H) particle treatment levels, grouped according to sampling days.



Carlo (MCMC) resampling approach implemented in the function pvals.fnc (languageR package; Baayen et al. 2008). Proportional data were analyzed with generalized linear mixed effect models (glmm) using a binomial error structure and a logit link function (function: glmmPQL, MASS package; Venables and Ripley 1994). Fixed and random effect terms were included as described above. Significance was tested with Wald χ^2 tests (function: wald.test, aod package). Once a significant main effect was detected, each particle treatment level was compared with the respective control using Wald *t* tests (Bolker et al. 2009). Model fit and assumptions were evaluated according to standard procedures (Zuur et al. 2009), and no violations were observed.

Results

Exposure system

Particle concentrations in exposure tanks showed a characteristic time course (Fig. 1) and differed significantly between treatment levels ($p < 0.01$) and time points after re-dose ($p < 0.01$). Qualitative observations indicate that regardless of treatment level, sediment pulses reduced the visual water clarity to less than approximately 5 cm. After 24 h, visual water clarity in the low particle concentration tanks had mostly recovered to control levels. In the medium and high particle concentration tanks, visual water clarity was still reduced to visual water clarities below approximately 25 and 15 cm, respectively. No differences between treatment levels could be observed for pH (means: 7.88–7.90), temperature (means: 13.50–13.55 °C), and oxygen (means: 9.68–9.75 mg·L^{-1}). Similarly, no differences in water flow were detected between experimental tanks (means: 296.63–296.86 mL·min^{-1}). A slight but significant increase of the conductivity was detected in the high particle concentration tanks ($p < 0.01$; control: 291.00 ± 10, low: 290.75 ± 0.53, medium: 293.76 ± 0.94, and high: 295.00 ± 1.07 μS·cm^{-1}; mean ± SE).

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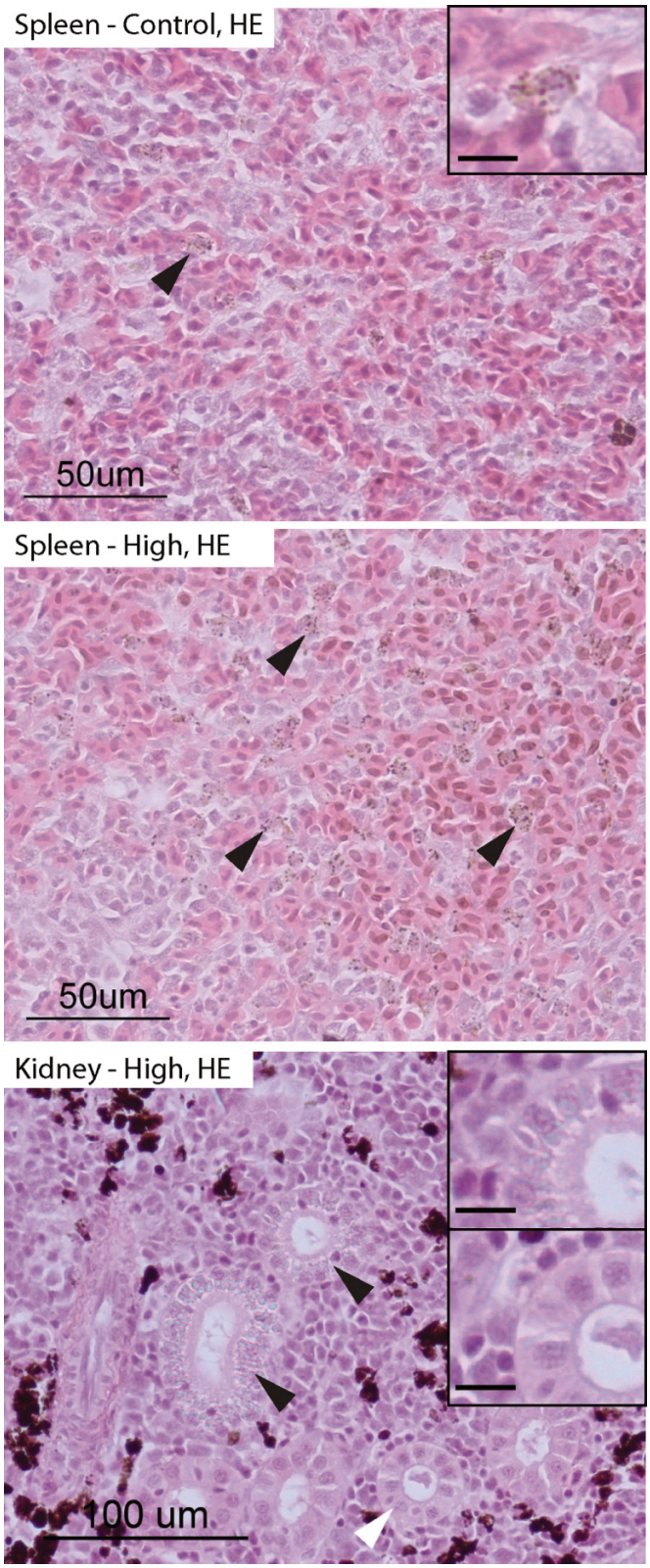

Fig. 3. Cellular responses in spleen and kidney of juvenile rainbow trout exposed to suspended mica particle for 24 days. Spleen: granular macrophage accumulations (arrowheads mark examples for individual macrophages) in a control (upper panel) and a high treatment level fish (middle panel), both stained with hematoxylin and eosin (HE). Inset shows individual granular macrophage (bar = 10 μm). Kidney: example of tubular hyaline degenerations (black arrowheads) in high treatment level fish from day 24, stained with HE. The white arrowhead marks a tubule with regular appearance. Insets show a detail of the degenerated tubule marked by the upper arrowhead (top inset) and the regular tubule (bottom inset; bars = 20 μm).

General effects

All fish survived over the entire experimental period, and no injuries or phenotypic pathologies could be observed. No particle accumulations in the digestive tract of particle-exposed rainbow trout were observed during sampling.

Effects on growth and condition

On day 8, no significant effects on length, absolute mass, and condition factor were observed (Figs. 2A–2C). Nonetheless, the specific growth rate, a measure of relative mass gain, was more than 75% reduced in the medium and high particle concentration tanks ($p = 0.019$; Fig. 2D). On day 24, growth rates had recovered to control levels (Fig. 2D). At this time point, the condition factor was slightly decreased in the highest particle concentration ($p = 0.026$; Fig. 2C).

Effects in target organs

The gills of rainbow trout exposed to suspended mica particles showed no histopathological damage compared with controls, regardless of exposure concentration and duration. Similarly, no changes in Na^+K^+-ATPase and LDH activity could be observed (Fig. A1). On day 24, lipid peroxidation was significantly increased, but only in the low particle concentration fish ($p = 0.044$; Fig. A1).

In the liver, no large-scale histo-pathological effects were observed. However, on day 8 the hepato-somatic index was increased in the medium and high particle concentration tanks ($p = 0.019$; Fig. 2E). On day 24 this effect was still observable in the highest particle concentration ($p = 0.045$; Fig. 2E).

In the spleen, no large-scale histo-pathological effects were observed. However, the density of MAs was increased almost twofold in the highest particle concentration on day 8 ($p = 0.019$; control = 22.76 ± 2.45, high = 41.53 ± 7.91 MAs·mm^{-2}; mean ± SE). A similar pattern of a more than twofold increase could be observed for the MA area proportion (control = 0.19 (0.14, 0.27), high = 0.46 (0.31, 0.69); mean ± boundary estimates of proportions). The latter was nonsignificant given the bigger variation in the particle-exposed fish. On day 24, increased numbers of individual granular macrophages could be observed in the red pulp of the spleen of fish from the highest particle concentration (Fig. 3; Table 1). Also on this day, the spleen-somatic index was markedly increased in the highest particle concentration ($p = 0.002$; Fig. 2F).

In the kidney, no large-scale histo-pathological effects were observed. However, increased hyaline droplet degeneration of tubular cells could be noted in particle-exposed rainbow trout on day 24 (Fig. 3; Table 1).

Systemic effects

On day 8, all primary hematological parameters were unchanged (Table 2), but the proportion of immature erythrocytes was increased almost twofold in the medium and high particle treatment levels ($p = 0.001$; Fig. 4). Immature erythrocytes in these fish were mostly late pro-erythrocytes, distinguished by a round to oval shape and a basophilic cytoplasm (cf. Lehmann and Stürenberg 1974). In parallel, mature erythrocyte length and area were slightly decreased ($p = 0.039$ and $p = 0.045$, respectively; Table 3). Also on day 8, hematocrit and erythrocyte numbers were negatively related to spleen-somatic index (Fig. 5A). Finally, a trend for a negative relationship between plasma ASAT activity and the hepato-somatic index was observable (Fig. 5B).

On day 24, immature erythrocyte proportions were no longer affected by particle exposure (Fig. 4). At this time point, the total number of circulating erythrocytes was slightly decreased in the highest particle concentration tanks ($p = 0.029$; Table 2). Further,

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Table 1. Cellular alterations in spleen and kidney of rainbow trout exposed to suspended mica particles.

		Spleen (GMAs)				Kidney (tubules)	
Day	Level	n_{fish}	Mean	Min.	Max.	n_{fish}	Pic (%)
8	Control	5 (9)	24	18	30	2 (9)	11
	High	6 (9)	22	15	30	4 (9)	24
24	Control	2 (9)	26	18	34	2 (9)	13
	High	7 (9)	50	25	85	8 (9)	47

Note: Spleen: n_{fish} = number of fish with granular macrophage accumulations, GMAs (number fish examined in parentheses) as well as mean, minimum, and maximum granular macrophage counts per microscope field. Kidney: n_{fish} = number of fish with degenerated tubules (number of fish examined in parentheses); Pic (%) = percentage of pictures with this effect. Effects are shown in Fig. 3.

Table 2. Hematology of rainbow trout exposed to suspended mica particles.

		Suspended sediment level			
Parameter	Day	Control	Low	Medium	High
Hematocrit (%)	8	34.8±6.0	32.7±1.4	34.0±1.6	37.1±1.9
	24	35.4±1.3	34.3±1.8	35.9±0.6	33.1±1.6
Leucocrit (%)	8	1.16±0.1	1.54±0.1	1.31±0.2	1.64±0.1
	24	1.75±0.2	1.90±0.1	1.48±0.1	1.64±0.2
Erythrocytes	8	2.04±0.1	1.98±0.3	1.92±0.1	2.08±0.2
(10^{12} cells·L^{-1})	24	2.25±0.1	2.08±0.1	2.51±0.1	1.90±0.1**
Hemoglobin (g·L^{-1})	8	110.1±11.1	101.0±5.1	99.0±5.2	121.6±13.6
	24	97.8±3.0	97.6±10.3	95.5±3.8	97.7±7.1

Note: Data are mean ± SE; asterisks (**, $p < 0.01$) denote significant difference from control on respective day.

Table 3. Erythrocyte morphology parameters in rainbow trout exposed to suspended mica particles.

		Suspended sediment level			
Parameter	Day	Control	Low	Medium	High
Cell width	8	9.71±0.12	9.33±0.09	9.36±0.17	9.36±0.08
(μm)	24	9.62±0.29	9.39±0.18	9.28±0.14	9.88±0.25
Cell length	8	14.91±0.17	14.38±0.16	14.23±0.26*	14.19±0.13*
(μm)	24	15.04±0.67	15.04±0.67	13.92±0.13	15.07±0.65
Cell area	8	114.00±2.71	105.49±0.92	105.01±3.48*	104.54±1.29*
(μm^2)	24	114.56±8.88	106.18±2.60	101.75±2.39	117.62±8.24
Shape factor	8	0.65±0.00	0.65±0.01	0.66±0.01	0.66±0.01
	24	0.64±0.01	0.66±0.01	0.67±0.01	0.66±0.01

Note: Data are mean ± SE; asterisks (*, $p < 0.05$) denote significant difference from control on respective day.

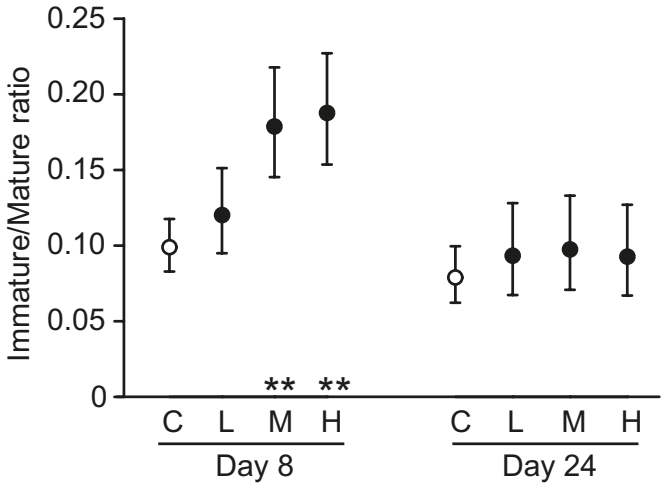
on day 24, plasma ASAT activity was reduced almost twofold in the highest particle concentration ($p = 0.049$; Table 4). The plasma protein content showed a similar pattern of decrease (Table 4). Moreover, plasma protein content and ASAT activity were negatively related to the hepato-somatic index (Fig. 5B).

A schematic summary of all effects of the particle exposure on the different body systems of rainbow trout is shown in Fig. 6.

Discussion

Studies over the past few decades have focused on individual aspects of suspended mineral particle effects in salmonid fish (reviewed in Waters 1995; Newcombe and Jensen 1996). However, our knowledge of how suspended sediments affect individual salmonid fish at different levels of their biological organization remains limited. This knowledge would clearly improve our understanding of how salmonid fish react to and cope with suspended sediment exposure. Therefore, we have provided the first systemic investigation of the effects of small-sized suspended mineral particles on a salmonid fish, the rainbow trout.

Fig. 4. Immature erythrocyte proportions in the blood of rainbow trout exposed to suspended mica particles. Symbols denote mean ± upper and lower boundary estimated from the glmmPQL model fit. Asterisks (**, $p < 0.01$) near the *x* axis denote significant differences from control on respective days. Labels on the *x* axis denote control (C) and low (L), medium (M), and high (H) particle treatment levels.



Effects on growth, condition, and metabolism

Exposure of rainbow trout to suspended sediment pulses over 8 days caused a marked reduction of the specific growth rate. Particle exposure-related gill damage, which could have reduced fitness and growth, was apparently absent (see below). An alternative explanation could be reduced food uptake. Reduced food uptake is consistent with a notably retarded feeding reaction in our particle-exposed rainbow trout during the first days of the experiment. These responses have been previously reported in salmonid fish exposed to suspended mineral particles (Sweka and Hartman 2001*a*, 2001*b*; De Robertis et al. 2003). Exposure to placer mining sediments (1000 mg·L^{-1} for 42 days) resulted in 33% mass gain reduction in Arctic grayling (*Thymallus arcticus*; McLeay et al. 1987). In rainbow trout, reduced feeding rates (1.5% to 0.5% body mass·day^{-1}) over 11 days caused a slight decrease in somatic growth (Boujard et al. 2000). We used total masses to calculate specific growth rates. Hence, we believe the more than 75% reduction observed in our experiment on day 8 not only reflects decreased somatic growth, but also reduced food intake. In any case, our results support the idea that sediment pulses can cause food deprivation in rainbow trout (Shaw and Richardson 2001). This is consistent with the decreased condition factor discussed below.

Exposure to daily pulses of small-sized mica particles for 24 days caused a slightly decreased condition factor in rainbow trout. This most likely reflects impaired somatic growth (i.e., mass) compared with structural growth (i.e., length), which is common in fish under restricted food conditions (Broekhuizen et al. 1994). Similar responses have been observed in rainbow trout when feeding rates were reduced from 1% to 0.3% of their body mass (Storebakken et al. 1991). The decreased serum protein and ASAT activity are consistent with food deprivation and adaptations in energy metabolism (Hevroy et al. 2011). The ASAT is an important aminotransferase in fish (Cowey and Walton 1989). Serum activities of this protein can positively correlate with growth in salmonid fish (Hevroy et al. 2004), and altered levels may indicate changes in organ function (Sandnes et al. 1988). Reduced serum protein and ASAT activities are common in food-deprived salmonids (Sauer and Haider 1979; Storebakken et al. 1991; Hevroy et al. 2011). Therefore, the negative relationship between hepato-

Fig. 5. Relationship between organ indices (A: spleen-somatic index; B: hepato-somatic index) and hematology parameters in rainbow trout exposed to suspended mica particles. Symbols denote control (open circles) and particle-exposed (solid circles) fish. Lines are regression lines (solid) with 95% pointwise confidence intervals (dashed) predicted from the linear model fit. (Note for ASAT activity, 1 Unit = 16.67 nkat.)

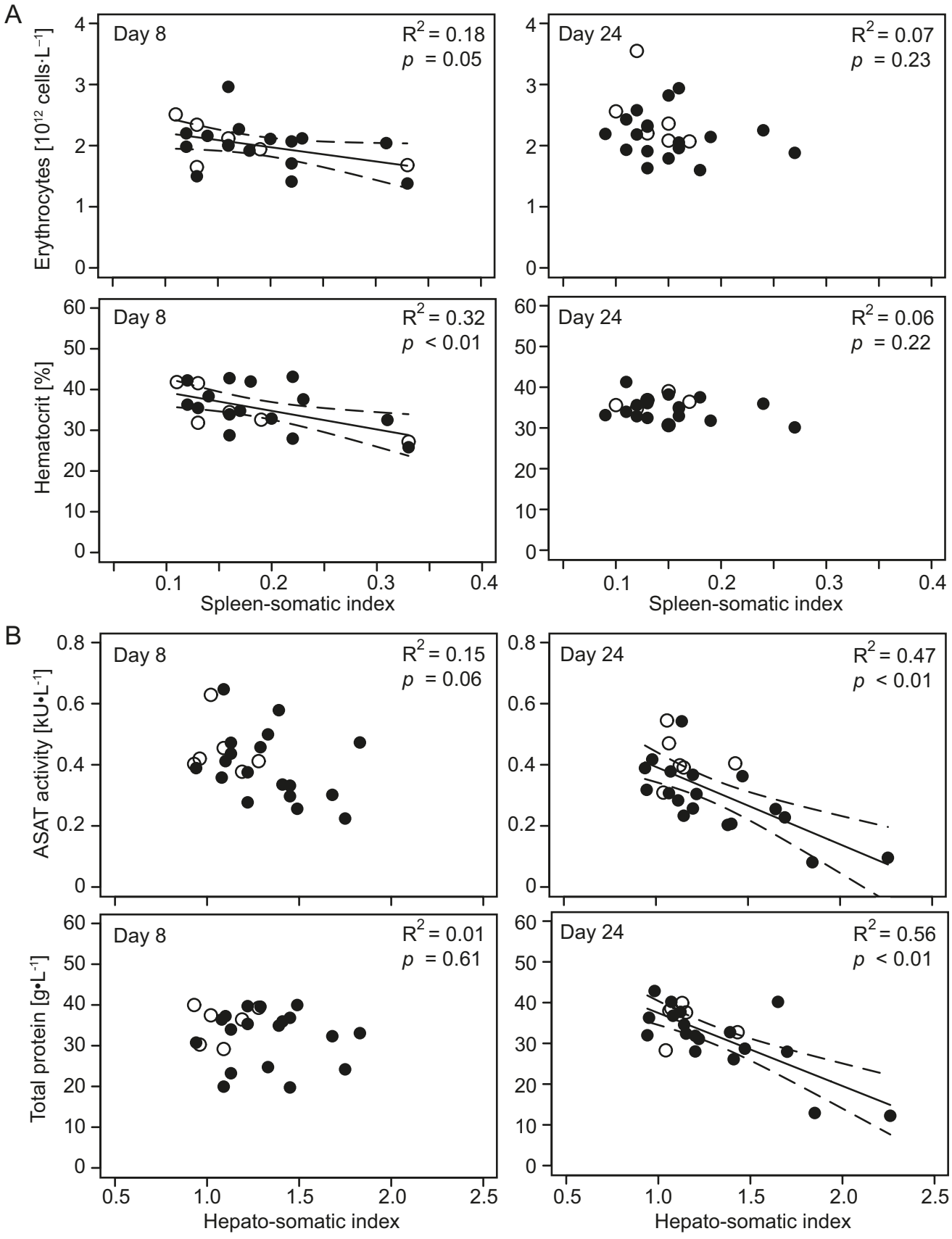


Table 4. Plasma parameters in rainbow trout exposed to suspended mica particles.

		Treatment level			
Parameter	Day	Control	Low	Medium	High
Na (mmol·L^{-1})	8	149.83±0.65	151.50±0.85	152.50±1.05	150.40±0.93
	24	151.50±0.43	152.00±1.21	152.83±0.90	150.33±0.67
K (mmol·L^{-1})	8	1.93±0.51	2.40±0.27	1.65±0.23	1.90±0.41
	24	1.32±0.12	1.43±0.13	1.85±0.23	1.70±0.12
Ca (mmol·L^{-1})	8	2.13±0.04	2.15±0.04	2.16±0.06	2.19±0.09
	24	2.17±0.03	2.17±0.07	2.20±0.07	2.07±0.06
Cl (mmol·L^{-1})	8	134.83±1.25	137.33±1.41	138.50±1.15	135.40±0.87
	24	137.33±0.61	137.00±0.68	137.33±1.28	137.50±0.72
ALAT (U·L^{-1})	8	18.33±4.06	14.50±1.57	22.33±6.46	17.50±1.65
	24	14.00±2.21	10.33±1.36	13.50±2.43	10.60±1.50
ASAT (kU·L^{-1})	8	0.45±0.04	0.41±0.06	0.39±0.03	0.39±0.05
	24	0.42±0.03	0.30±0.04	0.34±0.05	0.23±0.04*
Protein (g·L^{-1})	8	35.43±1.88	34.37±3.04	32.97±1.90	29.00±3.17
	24	35.85±1.81	33.25±1.73	35.40±2.42	32.13±2.23
Glucose (mmol·L^{-1})	8	3.98±0.49	3.18±0.18	3.12±0.15	3.68±0.45
	24	3.17±0.21	3.70±0.40	4.12±0.38	3.48±0.30

Note: Data are mean ± SE; asterisk (*, $p < 0.05$) denotes significant difference from control on respective day. 1 Unit = 16.67 nkat.

somatic index and plasma protein as well as plasma ASAT activity might reflect that particle-exposed rainbow trout conserved liver energy stores at the expense of other energy sources. The latter has previously been demonstrated in food-deprived rainbow trout (Moon et al. 1989; Simpkins et al. 2003; Harmon et al. 2011). The unspecific hyaline degeneration in kidney tubular cells as well as the decreased number of erythrocytes on day 24 have also been observed in fish experiencing food deprivation and metabolic stress (Weinberg et al. 1973; Lim and Klesius 2003; Ferguson 2006). Together these data document that pulsed particle exposure over 24 days can cause noticeable structural and metabolic changes in rainbow trout, even when apical effects on mass and condition are absent or moderate, respectively. Additional studies are needed to elucidate the exact mechanisms behind the growth effect and metabolic responses observed here, and it would be of interest to disentangle the relative roles of turbidity-induced physiological stress versus reductions in food uptake and related metabolic stress, which we cannot completely resolve here.

Effects in target organs

Histological damage in the gills was absent. This result agrees with some studies in salmonid fish using suspended mineral particles of a similar size range as ours (Redding et al. 1987; Shrimpton et al. 2007). Redding et al. (1987) suggested that their clay-sized mineral particles irritated the gills to an extent that hematocrit was increased, despite no histological damage. Our analyses, which applied additional end points to investigate this hypothesis of gill irritation, revealed no major effects of the particle exposure on anaerobic metabolism (LDH activity), cell homeostasis (Na^+K^+-ATPase activity), or lipid peroxidation in the gills. Also, no systemic effects potentially related to an impaired gill function, such as altered plasma electrolytes, erythrocyte numbers, or hemoglobin content, were observed. Hence, the small-sized mica particles most likely did not cause severe respiratory impairment.

As with the gills, no large-scale histo-pathological effects were observed in liver or spleen. Thus, the increase of liver and spleen indices in particle-exposed fish was not related to inflammatory cell infiltration or other proliferative changes, at least at the time of sampling. Nonetheless, we observed noticeable effects on splenic melano-macrophages, which were generally increased in particle-exposed trout. Increases in pigmented macrophages could be related to stress (Peters and Schwarzer 1985) and food deprivation (Agius and Roberts 2003). However, under these conditions we would have expected this effect to also occur in the kidney (Peters and Schwarzer 1985; Mizuno et al. 2002). This was not the case in our experiment. Instead kidney MAs were unchanged or even slightly decreased in particle-exposed rainbow trout (data not shown). An alternative explanation could be particle uptake. Splenic macrophages represent a major deposit for particulates (Ellis et al. 1976; Ziegenfuss and Wolke 1991; Furukawa et al. 2002). In juvenile Pacific salmon, mineral particle uptake in gills and distribution to the spleen occurred in less than 4 days (Goldes et al. 1986; Martens and Servizi 1993). In our experiment, splenic MAs were increased on day 8, followed by an increase of individual granular macrophages on day 24. This temporal pattern resembles patterns observed in studies investigating particle distribution in teleost fish (Ellis et al. 1976; Wolke 1992; Furukawa et al. 2002). We conclude from this that similar to previous studies with salmonids (Goldes et al. 1986; Martens and Servizi 1993), particle uptake likely also occurred in our experiment. In this context, our results document for the first time that small-sized mineral particle exposure can affect the macrophage system of salmonid fish. Potential effects on the nonspecific immune response remain to be evaluated, especially with natural particles that can also carry contaminants (cf. Newcombe and Jensen 1996).

Systemic effects

The initial increase of immature erythrocytes in particle-exposed rainbow trout indicates release from storage organs, which suggests a previous stress response (Pearson and Stevens 1991; Houston 1997). The negative correlations between spleen-somatic index and primary red blood cell parameters on day 8 further corroborate this. During the first days of exposure, rainbow trout in particle exposed tanks also reacted to sediment pulses with sporadic swimming spurts. Similar reactions to sediment pulses have been reported in juvenile coho salmon (*Oncorhynchus kisutch*; Berg and Northcote 1985). Therefore, the sediment pulses were most likely stressful for our rainbow trout, at least during the first days of exposure. This agrees with studies indicating that acute sediment exposure can cause physiological stress in salmonids (Redding et al. 1987; Servizi and Martens 1992; Lake and Hinch 1999). Given that gill function was most likely not severely impaired, we suspect that this initial stress response was more related to the sudden increase in turbidity rather than a threatened respiratory homeostasis.

Interestingly, our results also indicate that salmonid fish might adapt their acute stress response when challenged with daily sediment pulses over longer time periods. On day 8, primary hematological parameters were unaltered, and most immature erythrocytes were in a late stage of development (i.e., late pro-erythrocytes; Lehmann and Stürenberg 1974). Hence, erythrocytes

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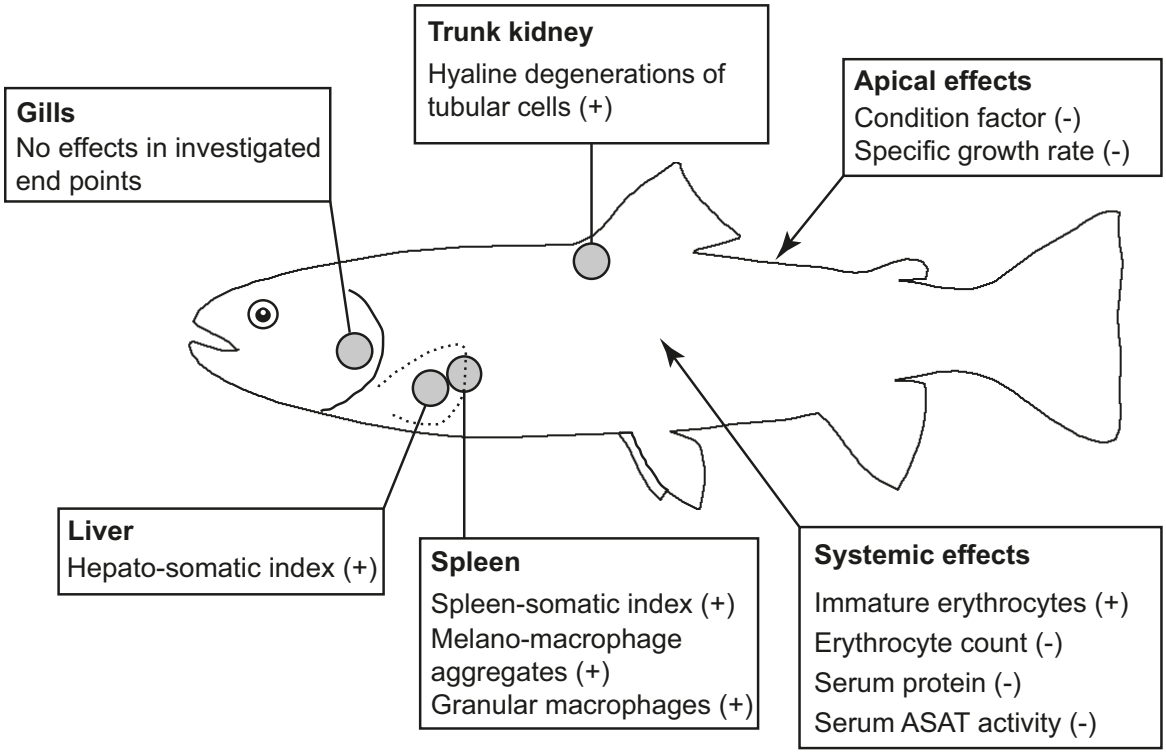
Fig. 6. Schematic overview of responses in rainbow trout exposed to suspended mica particles. No effects were observed in gills, and for all other end points only significant effects are listed. Here symbols in parentheses indicate an increase (+) or decrease (–) of the respective end point.



were likely released early in the exposure and the hematological homeostasis was already reestablished (Murad et al. 1990). In addition, approximately 1 week after the exposure started, rainbow trout no longer reacted to the sediment pulses with swimming spurts. Finally, on day 24 no indications of erythrocyte release were observed. This indicates that rainbow trout have adapted to the sediment pulses, which would agree with the general adaptation syndrome (Selye 1973). It predicts that fish challenged with mild stressors exhibit an initial phase of physiological alarm followed by adaptation (Beyers et al. 1999). Similar responses have been observed in salmonids with other repetitive stressors (Barton et al. 1987; Schreck 2000).

We were surprised to see such rapid behavioral and physiological adaptations in our rainbow trout. We cannot conclude when exactly before our first sampling day rainbow trout began to adapt, and clearly this should be clarified in future studies. Nonetheless, our results illustrate that the daily sediment pulses applied here did not challenge the homeostasis of juvenile rainbow trout to an extent that survival was affected. Rather they represented a stressor the fish could adapt to, albeit with increased energy demands that might have contributed to the decreased condition observed on day 24 (cf. Beyers et al. 1999).

Conclusions and environmental implications

Our results show that small-sized suspended mineral particles such as used here affect salmonid fish mostly via turbidity, rather than by direct physical damage. Hence, we expect that short suspension events of uncontaminated, small-sized mineral particles, for example during dredging (Harvey and Lisle 1998) or flood events (Tramblay et al. 2010; Schindler Wildhaber et al. 2011), do not affect individual rainbow trout to a large extent. Of course, they can elicit a stress response, but our results also suggest that rainbow trout could adapt if sediment pulses are sporadic or separated by a day or more. More prolonged exposure to small-sized mineral particles could occur in lakes and estuaries (Kröger et al. 2013; Mitchell 2012) but also during industrial operations (Wilber and Clarke 2001; Crosa et al. 2010). Chronic exposure is more likely with smaller particle sizes that persist longer in suspension (Newcombe 2003). Our results indicate that rainbow trout can withstand this, at least over a few weeks. Nonetheless, our results also indicate that even when physical gill damage and pronounced mass effects are absent, notable structural and physiological effects can occur. In our rainbow trout, this was reflected in cellular effects in the spleen and kidney, metabolic changes, and a decreased condition. These effects clearly indicate that possible adaptive responses of the organism need to be considered by fisheries biologists when evaluating suspended sediment effects in salmonid fish. Future studies are needed to better understand the mechanisms behind the adaptive responses observed here. This would further advance our understanding of how suspended mineral particles interact with other environmental stressors, such as contaminants and pathogens, when affecting salmonid health (cf. Newcombe and Jensen 1996; Brinkmann et al. 2010).

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References

Agius, C., and Roberts, R.J. 2003. Melano-macrophage centres and their role in fish pathology. Journal of Fish Diseases, **26**(9): 499–509. doi:10.1046/j.1365-2761.2003.00485.x. PMID:14575368.

Alabaster, J.S., and Lloyd, L. 1982. Water quality criteria for freshwater fish. Butterworths, London.

Asselman, N.E.M., Middelkoop, H., and vanDijk, P.M. 2003. The impact of changes in climate and land use on soil erosion, transport and deposition of suspended sediment in the River Rhine. Hydrol. Process. **17**(16): 3225–3244. doi:10.1002/hyp.1384.

Atteia, O., Perret, D., Adatte, T., Kozel, R., and Rossi, P. 1998. Characterization of natural colloids from a river and spring in a karstic basin. Environ. Geol. **34**(4): 257–269. doi:10.1007/s002540050277.

Baayen, R.H., Davidson, D.J., and Bates, D.M. 2008. Mixed-effects modeling with crossed random effects for subjects and items. Journal of Memory and Language, **59**(4): 390–412. doi:10.1016/j.jml.2007.12.005.

Barton, B.A., Schreck, C.B., and Barton, L.D. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Dis. Aquat. Org. **2**(3): 173–185.

Berg, L., and Northcote, T.G. 1985. Changes in territorial, gill-flaring, and feeding behavior in juvenile coho salmon (*Oncorhynchus kisutch*) following short-term pulses of suspended sediment. Can. J. Fish. Aquat. Sci. **42**(8): 1410–1417. doi:10.1139/f85-176.

Bergmeyer, H.U. 1974. Methods of enzymatic analysis. Verlag Chemie, Weinheim.

Beyers, D.W., Rice, J.A., Clements, W.H., and Henry, C.J. 1999. Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish. Can. J. Fish. Aquat. Sci. **56**(5): 814–822. doi:10.1139/f99-006.

Bilotta, G.S., and Brazier, R.E. 2008. Understanding the influence of suspended solids on water quality and aquatic biota. Water Res. **42**(12): 2849–2861. doi:10.1016/j.watres.2008.03.018. PMID:18462772.

Bisson, P.A., and Bilby, R.E. 1982. Avoidance of suspended sediment by juvenile coho salmon. N. Am. J. Fish. Manage. **2**(4): 371–374. doi:10.1577/1548-8659(1982)2<371:AOSSBJ>2.0.CO;2.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., and White, J.S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. **24**(3): 127–135. doi:10.1016/j.tree.2008.10.008. PMID:19185386.

Boujard, T., Burel, C., Médale, F., Haylor, G., and Moisan, A. 2000. Effect of past nutritional history and fasting on feed intake and growth in rainbow trout *Oncorhynchus mykiss*. Aquat. Living Resour. **13**(3): 129–137. doi:10.1016/S0990-7440(00)00149-2.

Brinkmann, M., Hudjetz, S., Cofalla, C., Roger, S., Kammann, U., Giesy, J.P., Hecker, M., Wiseman, S., Zhang, X.W., Wolz, J., Schuttrumpf, H., and Hollert, H. 2010. A combined hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events. Part I — multiple biomarkers in rainbow trout. Journal of Soils and Sediments, **10**(7): 1347–1361.

Broekhuizen, N., Gurney, W.S.C., Jones, A., and Bryant, A.D. 1994. Modelling compensatory growth. Funct. Ecol. **8**(6): 770–782. doi:10.2307/2390237.

Collins, T.J. 2007. ImageJ for microscopy. Biotechniques, **43**(S1): S25–S30. PMID:17936939.

Cowey, C.B., and Walton, C.J. 1989. Intermediary metabolism. *In* Fish nutrition. *Edited by* J.E. Halver. Academic Press, San Diego, Calif. pp. 259–329.

Crosa, G., Castelli, E., Gentili, G., and Espa, P. 2010. Effects of suspended sediments from reservoir flushing on fish and macroinvertebrates in an alpine stream. Aquat. Sci. **72**(1): 85–95. doi:10.1007/s00027-009-0117-z.

DeRobertis, A., Ryer, C.H., Veloza, A., and Brodeur, R.D. 2003. Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. Can. J. Fish. Aquat. Sci. **60**(12): 1517–1526. doi:10.1139/f03-123.

Ellis, A.E., Munroe, A.L.S., and Roberts, R.J. 1976. Defence mechanisms in fish. 1: A study of the phagocytic system and the fate of intraperitoneally injected particulate material in the plaice (*Pleuronectes platessa* L). J. Fish Biol. **8**(1): 67–78. doi:10.1111/j.1095-8649.1976.tb03908.x.

Federici, G., Shaw, B.J., and Handy, R.D. 2007. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. Aquat. Toxicol. **84**(4): 415–430. doi:10.1016/j.aquatox.2007.07.009. PMID:17727975.

Ferguson, H.W. 2006. Systemic pathology of fish. Iowa State University Press, Ames.

Foster, I.D.L., and Lees, J.A. 1999. Changes in the physical and geochemical properties of suspended sediment delivered to the headwaters of LOIS river basins over the last 100 years: a preliminary analysis of lake and reservoir bottom sediments. Hydrol. Process. **13**(7): 1067–1086. doi:10.1002/(SICI)1099-1085(199905)13:7<1067::AID-HYP790>3.0.CO;2-Y.

Furukawa, T., Nakamura, O., Suzuki, Y., Atsuta, S., Nakamura, H., and Watanabe, T. 2002. Entrapment and transport of foreign material in the spleen and kidney of Japanese conger *Conger myriaster*. Fish. Sci. **68**(6): 1219–1225. doi:10.1046/j.1444-2906.2002.00558.x.

Gallaugher, P., and Farrell, A.P. 1998. Hematocrit and blood oxygen-carrying capacity. *In* Fish respiration: Volume 17 in the Fish physiology series. *Edited by* S.F. Perry and B.L. Tufts. Academic Press, San Diego, Calif. pp. 185–227.

Goldes, S.A., Ferguson, H.W., Daoust, P.Y., and Moccia, R.D. 1986. Phagocytosis of the inert suspended clay kaolin by the gills of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. **9**(2): 147–151. doi:10.1111/j.1365-2761.1986.tb00996.x.

Goldes, S.A., Ferguson, H.W., Moccia, R.D., and Daoust, P.Y. 1988. Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. **11**(1): 23–33. doi:10.1111/j.1365-2761.1988.tb00520.x.

Handy, R.D., and Depledge, M.H. 1999. Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. Ecotoxicology, **8**(5): 329–349. doi:10.1023/A:1008930404461.

Harmon, K.J., Bolinger, M.T., and Rodnick, K.J. 2011. Carbohydrate energy reserves and effects of food deprivation in male and female rainbow trout. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **158**(4): 423–431. doi:10.1016/j.cbpa.2010.11.017.

Harvey, B.C., and Lisle, T.E. 1998. Effects of suction dredging on streams: a review and an evaluation strategy. Fisheries, **23**(8): 8–17. doi:10.1577/1548-8446(1998)023<0008:EOSDOS>2.0.CO;2.

Hevroy, E.M., Sandnes, K., and Hemre, G.I. 2004. Growth, feed utilisation, appetite and health in Atlantic salmon (*Salmo salar* L.) fed a new type of high lipid fish meal, Sea Grain®, processed from various pelagic marine fish species. Aquaculture, **235**(1–4): 371–392.

Hevroy, E.M., Azpeleta, C., Shimizu, M., Lanzen, A., Kaiya, H., Espe, M., and Olsvik, P.A. 2011. Effects of short-term starvation on ghrelin, GH-IGF system, and IGF-binding proteins in Atlantic salmon. Fish Physiol. Biochem. **37**(1): 217–232. doi:10.1007/s10695-010-9434-3. PMID:20878468.

Holt, S., Gunderson, M., Joyce, K., Nayini, N.R., Eyster, G.F., Garitano, A.M., Zonia, C., Krause, G.S., Aust, S.D., and White, B.C. 1986. Myocardial tissue iron delocalization and evidence for lipid peroxidation after two hours of ischemia. Ann. Emerg. Med. **15**(10): 1155–1159. doi:10.1016/S0196-0644(86)80857-5. PMID:3752645.

Houston, A.H. 1997. Review: Are the classical hematological variables acceptable indicators of fish health? Trans. Am. Fish. Soc. **126**(6): 879–894. doi:10.1577/1548-8659(1997)126<0879:RATCHV>2.3.CO;2.

Houston, A.H., and Murad, A. 1992. Erythrodynamics in goldfish, *Carassius auratus* L.: temperature effects. Physiol. Zool. **65**(1): 55–76.

Kemp, P., Sear, D., Collins, A., Naden, P., and Jones, I. 2011. The impacts of fine sediment on riverine fish. Hydrol. Processes, **25**(11): 1800–1821.

Kröger, R., Dibble, E., Brandt, J.B., Fleming, J.P., Huenemann, T., Stubbs, T., Prevost, J.D., Tietjen, T., Littlejohn, K.A., Pierce, S.C., and Spickard, M. 2013. Spatial and temporal changes in total suspended sediment concentrations in an oxbow lake after implementing agricultural landscape management practices. River Res. Appl. **29**(1): 56–64. doi:10.1002/rra.1579.

Lake, R.G., and Hinch, S.G. 1999. Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **56**(5): 862–867. doi:10.1139/f99-024.

Lehmann, J., and Stürenberg, F.J. 1974. Haematologisch-serologische Substratuntersuchungen an der Regenbogenforelle (*Salmo gairdneri* Rich.) I. Methodik zur Blutentnahme und Blutuntersuchung bei Fischen. Gewässer und Abwässer, **53/54**: 114–132.

Lim, C., and Klesius, P.H. 2003. Influence of feed deprivation on hematology, macrophage chemotaxis, and resistance to *Edwardsiella ictaluri* challenge of channel catfish. J. Aquat. Anim. Health, **15**(1): 13–20.

Martens, D.W., and Servizi, J.A. 1993. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). Can. J. Fish. Aquat. Sci. **50**(3): 586–590. doi:10.1139/f93-067.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can. J. Fish. Aquat. Sci. **50**(3): 656–658. doi:10.1139/f93-075.

McLeay, D.J., Birtwell, I.K., Hartman, G.F., and Ennis, G.L. 1987. Responses of Arctic grayling (*Thymallus arcticus*) to acute and prolonged exposure to Yukon placer mining sediment. Can. J. Fish. Aquat. Sci. **44**(3): 658–673. doi:10.1139/f87-080.

Mitchell, S.B. 2012. Turbidity maxima in four macrotidal estuaries. Ocean and Coastal Manage. doi:10.1016/j.ocecoaman.2012.05.030.

Mizuno, S., Misaka, N., Miyakoshi, Y., Takeuchi, K., and Kasahara, N. 2002. Effects of starvation on melano-macrophages in the kidney of masu salmon (*Oncorhynchus masou*). Aquaculture, **209**(1–4): 247–255. doi:10.1016/S0044-8486(01)00716-5.

Moon, T.W., Foster, G.D., and Plisetskaya, E.M. 1989. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. Can. J. Zool. **67**(9): 2189–2193. doi:10.1139/z89-309.

Murad, A., Houston, A.H., and Samson, L. 1990. Haematological response to reduced oxygen-carrying capacity, increased temperature and hypoxia in goldfish, *Carassius auratus* L. J. Fish Biol. **36**(3): 289–305. doi:10.1111/j.1095-8649.1990.tb05610.x.

Newcombe, C.P. 2003. Impact assessment model for clear water fishes exposed

to excessively cloudy water. J. Am. Water Resour. Assoc. **39**(3): 529–544. doi:10.1111/j.1752-1688.2003.tb03674.x.

Newcombe, C.P., and Jensen, J.O.T. 1996. Channel suspended sediment and fisheries: a synthesis for quantitative assessment of risk and impact. N. Am. J. Fish. Manage. **16**: 693–727. doi:10.1577/1548-8675(1996)016<0693:CSSAFA>2.3.CO;2.

Pearson, M.P., and Stevens, E.D. 1991. Size and hematological impact of the splenic erythrocyte reservoir in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. **9**(1): 39–50. doi:10.1007/BF01987610.

Peters, G., and Schwarzer, R. 1985. Changes in hemopoietic tissue of rainbow trout under influence of stress. Diseases of Aquatic Organisms, **1**(1): 1–10. doi:10.3354/dao001001.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rau, M.A., Whitaker, J., Freedman, J.H., and DiGiulio, R.T. 2004. Differential susceptibility of fish and rat liver cells to oxidative stress and cytotoxicity upon exposure to prooxidants. Comp. Biochem. Physiol. C Toxicol. Pharmacol. **137**(4): 335–342. doi:10.1016/j.cca.2004.03.001. PMID:15228951.

Redding, J.M., and Schreck, C.B. 1982. Mount St. Helens ash causes sublethal stress responses in steelhead trout. Mt. St. Helens: effects on water resources, Jantzen Beach, Oregon.

Redding, J.M., Schreck, C.B., and Everest, F.H. 1987. Physiological effects on coho salmon and steelhead of exposure to suspended solids. Trans. Am. Fish. Soc. **116**(5): 737–744. doi:10.1577/1548-8659(1987)116<737:PEOCSA>2.0.CO;2.

Sandnes, K., Lie, O., and Waagbo, R. 1988. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. J. Fish Biol. **32**(1): 129–136. doi:10.1111/j.1095-8649.1988.tb05341.x.

Sauer, D.M., and Haider, G. 1979. Enzyme activities in the plasma of rainbow trout, *Salmo gairdneri* Richardson: the effects of nutritional status and salinity. J. Fish Biol. **14**(4): 407–412. doi:10.1111/j.1095-8649.1979.tb03536.x.

Scheurer, K., Alewell, C., Bänninger, D., and Burkhardt-Holm, P. 2009. Climate and land-use changes affecting river sediment and brown trout in alpine countries — a review. Environ. Sci. Pollut. Res. **16**(2): 232–242. doi:10.1007/s11356-008-0075-3.

Schindler Wildhaber, Y., Michel, C., Burkhardt-Holm, P., Baenninger, D., and Alewell, C. 2011. Measurement of spatial and temporal fine sediment dynamics in a small river. Hydrol. Earth Syst. Sci. **16**(6): 1501–1515.

Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. *In* The biology of animal stress. *Edited by* G.P. Moberg and J.A. Mench. CABI Publishing, Wallingford, UK. pp. 147–158.

Schubert, S., Peter, A., Burki, R., Schönenberger, R., Suter, M.J.F., Segner, H., and Burkhardt-Holm, P. 2008. Sensitivity of brown trout reproduction to long-term estrogenic exposure. Aquat. Toxicol. **90**(1): 65–72. doi:10.1016/j.aquatox.2008.08.002. PMID:18804294.

Schwindt, A.R., Truelove, N., Schreck, C.B., Fournie, J.W., Landers, D.H., and Kent, M.L. 2006. Quantitative evaluation of macrophage aggregates in brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss*. Dis. Aquat. Org. **68**(2): 101–113. doi:10.3354/dao068101. PMID:16532602.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. **61**(6): 692–699. PMID:4746051.

Servizi, J.A., and Martens, D.W. 1987. Some effects of suspended Fraser River sediments on sockeye salmon, *Oncorhynchus nerka*. *In* Sockeye salmon, *Oncorhynchus nerka*, population biology and future management. *Edited by* H.D. Smith, L. Margolis, and C.C. Wood. Can. Spec. Publ. Fish. Aquat. Sci. pp. 254–264.

Servizi, J.A., and Martens, D.W. 1992. Sublethal responses of coho salmon (*Oncorhynchus kisutch*) to suspended sediments. Can. J. Fish. Aquat. Sci. **49**(7): 1389–1395. doi:10.1139/f92-154.

Shaw, E.A., and Richardson, J.S. 2001. Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. Can. J. Fish. Aquat. Sci. **58**(11): 2213–2221. doi:10.1139/f01-160.

Shrimpton, J.M., Zydlewski, J.D., and Heath, J.W. 2007. Effect of daily oscillation in temperature and increased suspended sediment on growth and smolting in juvenile chinook salmon, *Oncorhynchus tshawytscha*. Aquaculture, **273**(2–3): 269–276. doi:10.1016/j.aquaculture.2007.10.009.

Sigler, J.W., Bjornn, T.C., and Everest, F.H. 1984. Effects of chronic turbidity on density and growth of steelheads and coho salmon. Trans. Am. Fish. Soc. **113**(2): 142–150. doi:10.1577/1548-8659(1984)113<142:EOCTOD>2.0.CO;2.

Simpkins, D.G., Hubert, W.A., DelRio, C.M., and Rule, D.C. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: Effects on body composition and condition indices. Trans. Am. Fish. Soc. **132**(3): 576–589. doi:10.1577/1548-8659(2003)132<0576:PROJRT>2.0.CO;2.

Storebakken, T., Hung, S.S.O., Calvert, C.C., and Plisetskaya, E.M. 1991. Nutrient partitioning in rainbow trout at different feeding rates. Aquaculture, **96**(2): 191–203. doi:10.1016/0044-8486(91)90149-2.

Sweka, J.A., and Hartman, K.J. 2001*a*. Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. Can. J. Fish. Aquat. Sci. **58**(2): 386–393. doi:10.1139/f00-260.

Sweka, J.A., and Hartman, K.J. 2001*b*. Influence of turbidity on brook trout reactive distance and foraging success. Trans. Am. Fish. Soc. **130**(1): 138–146. doi:10.1577/1548-8659(2001)130<0138:IOTOBT>2.0.CO;2.

Syvitski, J.P.M., Vörösmarty, C.J., Kettner, A.J., and Green, P. 2005. Impact of humans on the flux of terrestrial sediment to the global coastal ocean. Science, **308**(5720): 376–380. doi:10.1126/science.1109454. PMID:15831750.

Tramblay, Y., Saint-Hilaire, A., Ouarda, T.B.M.J., Moatar, F., and Hecht, B. 2010. Estimation of local extreme suspended sediment concentrations in California Rivers. Sci. Total Environ. **408**(19): 4221–4229. doi:10.1016/j.scitotenv.2010.05.001. PMID:20570317.

US EPA. 2009. National Water Quality Inventory: Report to Congress (2004 reporting cycle). United States Environmental Protection Agency, Office of Water, Washington, D.C.

Venables, W.N., and Ripley, B.D. 1994. Modern applied statistics with S-Plus (MASS). Springer, New York.

Waters, T.F. 1995. Sediment in streams. Sources, biological effects, and control. American Fisheries Society, Bethesda, Md.

Weinberg, S.R., Siegel, C.D., and Gordon, A.S. 1973. Studies on peripheral blood cell parameters and morphology of red paradise fish, *Macropodus opercularis*: effect of food deprivation on erythropoiesis. Anat. Rec. **175**(1): 7–13. doi:10.1002/ar.1091750103. PMID:4682842.

Wilber, D.H., and Clarke, D.G. 2001. Biological effects of suspended sediments: A review of suspended sediment impacts on fish and shellfish with relation to dredging activities in estuaries. N. Am. J. Fish. Manage. **21**(4): 855–875. doi:10.1577/1548-8675(2001)021<0855:BEOSSA>2.0.CO;2.

Wolke, R.E. 1992. Piscine macrophage aggregates: a review. Annu. Rev. Fish Dis. **2**: 91–108. doi:10.1016/0959-8030(92)90058-6.

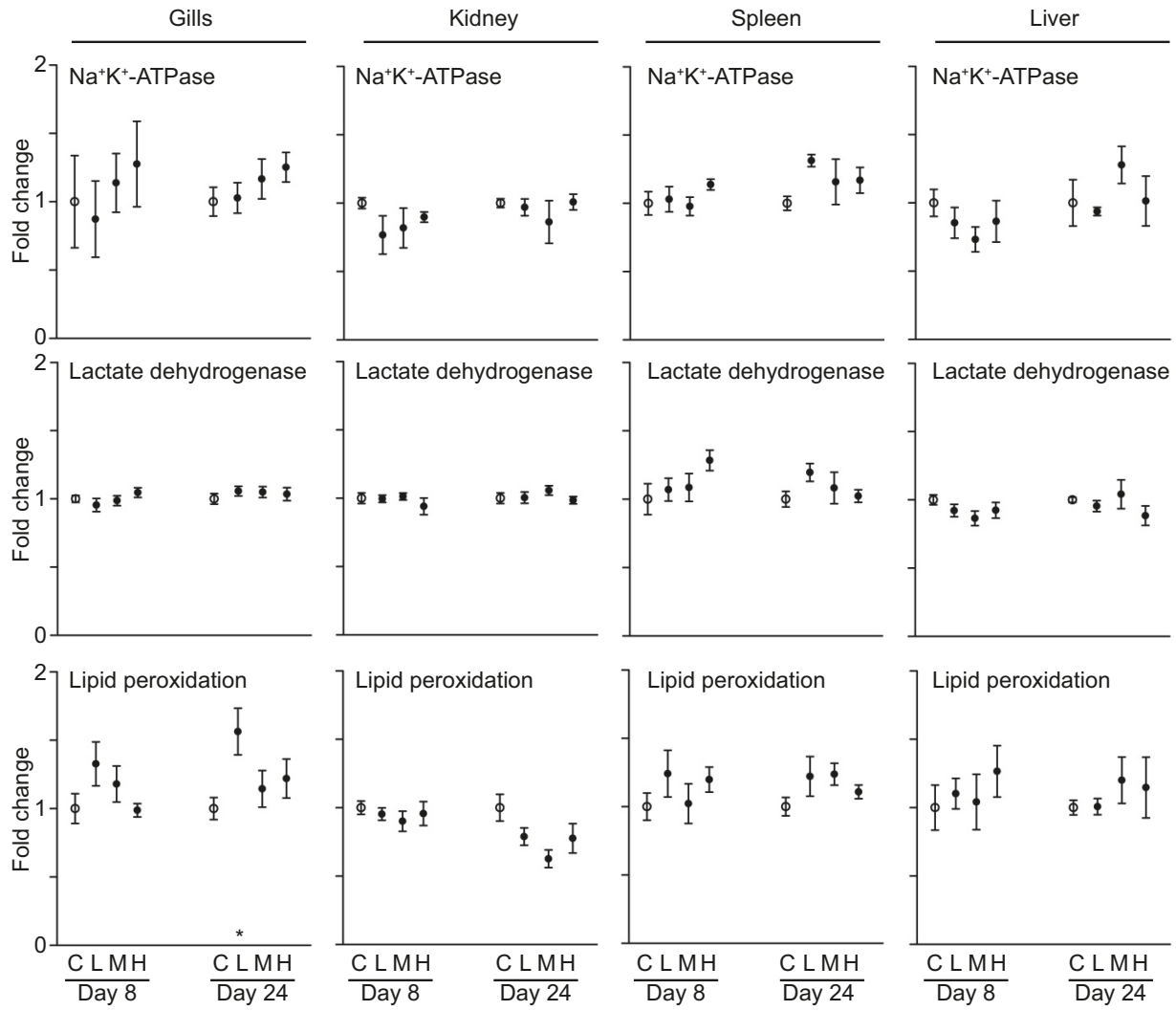
Ziegenfuss, M.C., and Wolke, R.E. 1991. The use of fluorescent microspheres in the study of piscine macrophage aggregate kinetics. Dev. Comp. Immunol. **15**(3): 165–171. doi:10.1016/0145-305X(91)90007-L. PMID:2060686.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., and Smith, G.M. 2009. Mixed effects models and extensions in ecology with R. Springer Publishing, New York.

Appendix A

Figure A1 appears on the following page.

Fig. A1. Biochemical responses in gills, kidney, spleen, and liver of rainbow trout exposed to suspended mica particles. Data show Na^+K^+-ATPase activity (top row), lactate dehydrogenase activity (middle row), and lipid peroxidation (TBARS (thiobarbituric acid-reactive substances) assay; bottom row). Shown are the fold-changes compared with the control on the respective day. Symbols denote mean ± SE; asterisk (*, $p < 0.01$) near the *x* axis denotes significant difference from control in respective day. Labels on *x* axis denote control (C) and low (L), medium (M), and high (H) particle treatment levels.



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Chapter 3

Natural mineral particles cause cytotoxicity in rainbow trout gill epithelial cells *in vitro*

This chapter is nearing submission as a full paper to *PlosONE*:

Michel C*, Herzog S*, de Capitani C, Burkhardt-Holm P, and Pietsch C. Natural mineral particles cause cytotoxicity in rainbow trout gill epithelial cells *in vitro*. *shared first authorship

Abstract

We provide the first empirical investigation about uptake and cytotoxicity of natural mineral particles in salmonid gill epithelial cells. It has been suggested that natural clay particles might induce comparable cytotoxic effects than synthetic nanoparticles (SNPs). Thus, our results are further discussed in the context of SNP toxicity in gill epithelial cells. In our experiment, we evaluated uptake and cytotoxicity of four common fluvial mineral particles (quartz, feldspar, mica, and kaolin; 10, 50, and 250 mg L^{-1} each) using the rainbow trout epithelial gill cell-line RTgill-W1. Cytotoxicity assays for cell membrane permeability, oxidative stress and metabolic activity were applied. These assays were complemented with direct cell counts and transmission electron microscopy to investigate particle uptake and ultrastructural effects. Particle exposure had negligible effects on cell numbers *per se*. Particles of up to two micrometer diameter were phagocytized by the cells, regardless of mineral type. Our results document that, firstly, natural mineral particles can cause cytotoxicity in gill epithelial cells, and secondly, that the cytotoxic potential differs between mineral species. The clays mica and kaolin were distinctly more cytotoxic than the framework silicates quartz and feldspar, most likely related to the higher specific surface area of the clay particles. Moreover, the clay particles differed in the kind of cytotoxic effect induced: mica caused oxidative and endoplasmic reticulum stress, while kaolin caused increased cell membrane damage. Cytotoxic effects and particle uptake occurred in cells exposed with and without 10% fetal bovine serum. In summary, we demonstrate that natural mineral particles can induce cytotoxic effects in gill epithelial cells, and that these effects vary between different clay particles. Comparisons with published SNP studies in gill epithelial cells indicate that natural clay particles cause comparable cytotoxic effects, but also that they might be less cytotoxic. Our findings call for further experiments, firstly, to identify particle properties and cellular effect pathways related to clay particle cytotoxicity, and, secondly, to test if SNPs are more cytotoxic and/or induce different toxicity pathways than natural clay particles. These data would clearly contribute to a better eco-toxicological risk assessment of both natural mineral and synthetic nanoparticles in aquatic organisms.

Keywords: fine sediment, clay, nanoparticles, salmonid fish, risk assessment, human impact, oxidative stress

Introduction

The fish gill is considered a primary target organ for suspended mineral particle effects. However, especially for the smaller particle sizes of silt (≤ 63 µm) and clay (≤ 2 µm) evidence about physical gill damage is inconclusive (Waters, 1995). For these particle sizes some studies reported structural gill damage after exposure (270–4887 mg L-1, Herbert and Merkens, 1961; Goldes et al., 1988; Servizi and Martens, 1991), while other studies did not find effects with similar concentrations and exposure durations (McLeay et al., 1987; Redding et al., 1987; Michel et al., 2013). One possible explanation could be differences in the geochemical composition of the tested particles. For example, it has been suggested that clay particles could have a stronger impact because of their smaller size and higher surface reactivity (Waters, 1995). We recently documented that small sized clay particles (mica/muscovite, < 30 µm) caused lipid peroxidation, but no structural damage, in the gill of rainbow trout (Figure A1, Michel et al., 2013). Altogether, these data suggest that silt and clay sized mineral particles can cause cytotoxic effects in gill epithelia, and that these effects might differ between particles of different geochemical composition.

Whether natural mineral particles induce similar cytotoxic effects than synthetic nanoparticles (SNPs) is an important question in SNP risk assessment (Handy et al., 2008a; Wiesner et al., 2009). SNPs are defined as particles with at least one dimension below 100 nm (Roco, 2003; Moore, 2006). For eco-toxicological risk assessment also larger SNP and agglomerates need to be considered (Handy and Shaw, 2007; Handy et al., 2008b). In water, the size of SNP agglomerates ranges from a few hundred nanometers to a few micrometers (Zhang et al., 2008; Jiang et al., 2009; Keller et al., 2010). Aquatic organisms have been exposed to similar sized mineral particles ever since ("colloids"; Handy et al., 2008a; Wiesner et al., 2009). One example are clay particles commonly transported in the suspended load of rivers (Lead and Wilkinson, 2007; Bernhardt et al., 2010). The amount to these natural particles present in aquatic ecosystems most likely by far outweighs the amount of anthropogenic SNPs released to the environment (Handy et al., 2008a; Bernhardt et al., 2010). A key question in SNP risk assessment is therefore if toxic effects and toxicity mechanisms differ between SNPs and natural mineral particles, since the impact of SNPs on aquatic organisms might be higher when they exert novel effects (Handy et al., 2008a; Wiesner et al., 2009).

Many of the particle properties considered important for cytotoxic effects of mineral particles and SNPs are comparable (Donaldson and Borm, 2007; Handy et al., 2008b). For both,

particle size and shape, which jointly determine the surface area of a particle sample, are important for cellular effects to develop (Guthrie, 1997; Donaldson and Borm, 2007; Delay and Frimmel, 2012). The cytotoxic potential usually increases for samples with smaller particle sizes since they have a larger surface area per unit mass ("specific surface area", Guthrie, 1997). Moreover, the biological activity of the particles is also related to functional surface groups, which affect particle surface charges and reactivity (Fubini, 1997; Schwarze et al., 2007; Nel et al., 2009). The influence of surface structure and composition on cytotoxicity usually increases with smaller particle sizes (Guthrie, 1997; Schwarze et al., 2007). Toxic compounds bound to the particle surfaces could further contribute to effects (Fubini, 1997; Christian et al., 2008). Yet, it has been also suggested that SNPs with novel tailor-made surface properties might induce different cytotoxic pathways than natural particles (Owen and Handy, 2007; Bernhardt et al., 2010). The cytotoxic potential of some SNPs has been investigated in gill epithelial cells (Kühnel et al., 2009; Hildebrand et al., 2010; Van Hoecke et al., 2011), but so far no data are available for natural silt and clay sized mineral particles.

To this end, we applied an *in vitro* testing procedure with the rainbow trout epithelial gill cell line RTgill-W1 to investigate (*i*.) if small-sized mineral particles cause cytotoxic and/or ultrastructural effects in gill epithelial cells, (*ii*.) if they are taken up by the cells and (*iii*.) if the particle exposure affects cell numbers. The latter could indicate particle induced cell death. Moreover, we investigated if the kind of effect varies between different mineral species and if concentration and exposure duration alters cytotoxic responses. For our investigations we selected the four silicate minerals quartz, feldspar, mica and kaolin, which are common in European and North American watersheds (Irion, 1991; Martens and Servizi, 1993; Atteia et al., 1998; Evans et al., 2006). These particles represent the two bulk structures of tectosilicates (quartz, feldspar; “framework silicates”) and phyllosilicates (mica, kaolin; “clays”). Particle sizes (Figure 1) were selected to be similar to particles transported in rivers (Irion, 1991), and to comprise the size range of natural colloids and SNP agglomerates in water (Figure 1; Lead and Wilkinson, 2007; Zhang et al., 2008; Jiang et al., 2009; Keller et al., 2010). Our results provide first empirical data on cytotoxic effects of natural silt and clay sized mineral particles in gill epithelial cells. Further, by comparing our results with studies investigating SNP cytotoxicity in gill epithelial cells we provide new insights for SNP risk assessment in aquatic ecosystems.

Materials and methods

Cell culture

The epithelial gill cell line RTgill-W1 established from non-tumorigenic gill cells of rainbow trout was used (Bols et al., 1994). Cells were cultivated in Leibovitz`s L15 medium (LuBio Science GmbH, Switzerland) containing penicillin-streptomycin (100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin, Sigma-Aldrich GmbH, Switzerland) and supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich GmbH, Switzerland). Cells were maintained at 19°C in normal atmosphere and sub-cultured weekly.

Preparation and characterization of particles

Commercial mineral particles (quartz (silica), potassium feldspar (orthoclase), mica (muscovite), and kaolin (kaolinite)) were used because of their purity and defined mineral composition. Particle samples were selected to have comparable size distributions, and the particle size distribution of each sample was quantified with a Mastersizer 2000 laser particle size analyzer (Malvern Instruments Ltd., United Kingdom).

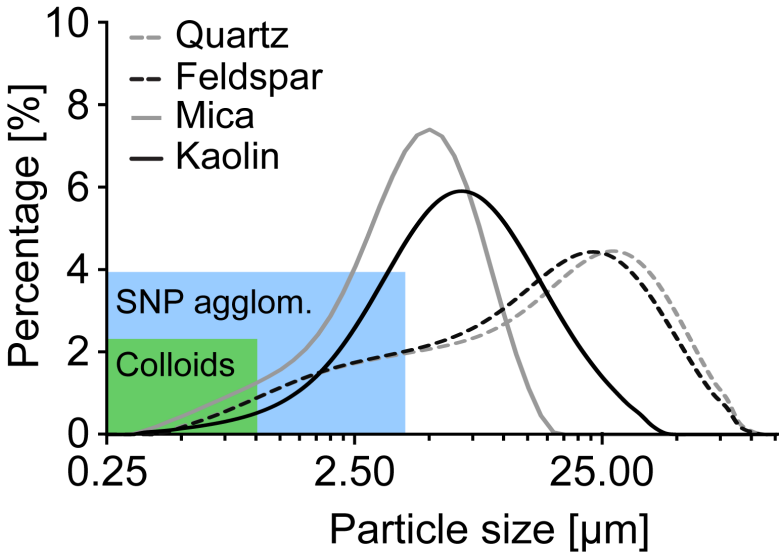

Figure 1 – Particle size distributions of the studied mineral particles. Boxes mark the size range of synthetic nanoparticle agglomerates (SNP agglom.) and natural colloidal particles in water (cf. Introduction).

According to manufacturer's data the specific surface area of the clay samples is 21 $m^2 g^{-1}$ (mica) and 11 $m^2 g^{-1}$ (kaolin). For the framework silicate samples no data was available. Yet, quartz particle samples of the same source (D_{95} < 8.2 µm) have a specific surface area of 4.22

$m^2 g^{-1}$ (Cakmak et al., 2004). For feldspar samples ($D_{95} < 20$ µm) a specific surface area of 3.6 $m^2 g^{-1}$ was reported (Hetland et al., 2000). The specific surface area of a mineral particle sample is negatively related to the particle size (Schaetzl and Anderson, 2005). Hence, these data indicate that our clay samples ($D_{95} < 20$ µm) had at least a three-fold higher specific surface area compared to the framework silicates ($D_{95} < 63$ µm). Prior to using the particles samples in exposure experiments, the particles were pre-weighed in 1.5 ml reaction tubes and sterilized by γ irradiation (100Gy, 42h; Gammacell 40 Extactor, Theratronics Inc., Canada). This is a common method to sterilize nanoparticles, since it has minimal effects on particle properties (Schulze et al., 2008). Mineralogy and purity of the particle samples was confirmed before and after gamma irradiation using x-ray diffractometry (Diffractometer D5000, Siemens, Germany).

Cytotoxicity assays

For cytotoxicity assays, cells were seeded in L15 medium (10% FBS, with pen/strep) in 96-well tissue culture plates (TPP AG, Switzerland) at a concentration of 35000 cells per well, and grown to confluence at 19°C in normal atmosphere for 48h (Dayeh et al., 2005). Then the medium was exchanged and cells were exposed to particle suspensions for 24, 48, 72, and 96h. All assays were conducted in L15 medium with pen/strep once with and once without 10% FBS (henceforth termed "serum").

To obtain particle working suspensions, a stock suspension (1250 mg L^{-1}) was prepared in Leibovitz`s L15 medium just before cell exposure. Particles in the stock suspension were dispersed twice by ultrasound sonication for one minute (Sonorex, Bandelin GmbH, Germany). Particle working suspensions of 10 mg L^{-1} (low), 50 mg L^{-1} (medium) and 250 mg L^{-1} (high) were then prepared by dilution from the stock suspension. On every plate a blank without cells and particles, and a control containing only cells was included. For each of three experiments, one plate with eight wells per treatment level per day were applied.

Membrane permeability was assessed with the cell-membrane-impermeable fluorescent dye propidium iodide (PI assay). Upon cell membrane damage this dye enters the nucleus and binds to the DNA, which results in increased light emission upon excitation. Hence, an increase in signal indicates increased cell membrane permeability, and hence an impaired cell membrane. Cell viability and metabolic activity were evaluated with the MTT assay (Mosmann, 1983). This assay is based on the metabolic reduction of the soluble yellow thiazolyl blue tetrazolium bromide (MTT) salt to a purple insoluble formazan product in

viable cells. Here an increase in signal indicates either (*i*.) increased metabolic and mitochondrial activity of viable cells and/or (*ii*.) an increase in viable cell numbers (Kupcsik, 2011). Oxidative stress after particle exposure in cells was quantified with the H_2DCF-DA assay. Oxidation of this dye by free radicals causes an increased emission upon excitation. Hence, an increase in signal indicates increased free radicals and hence oxidative stress. All cytotoxicity assays were conducted as described in Pietsch et al. (2011). Only in our experiment, each well was washed once with the buffer used in the respective dye solution before application of the indicator dye. This was done to reduce particle interference with the cytotoxicity assays. All chemicals were obtained from Sigma-Aldrich (Switzerland).

To compare the relative cytotoxicity of the four mineral species studied, the following ranking procedure was applied: For each assay, the maximum fold-change in the 250 mg L^{-1} particle concentration was identified (Figure S1 and S2). Then, the range spanning from no response (i.e. control ± 10%) to the maximum fold-change was divided into three equal intervals. These intervals were used to rank the effect size of each mineral particle studied (Table S1). To be conservative, only significant changes beyond ± 10% from controls were considered biologically relevant in the ranking, since the controls commonly spread in this range. Moreover, particle interference was not taken into account for the ranking.

Particle interference assays

To test if the mineral particles *per se* affect the applied cytotoxicity assays, particle suspensions of 10 mg L^{-1} (low), 50 mg L^{-1} (medium) and 250 mg L^{-1} (high) were prepared as described above, and again once with and once without serum. Suspensions were added in 96-well tissue culture plates (TPP AG, Switzerland) with control wells containing no particles included on every plate. For each of three experiments, one plate each with three wells per mineral per treatment level was measured. Plates were incubated at 19°C in normal atmosphere for 72h, and cytotoxicity assays were conducted as described for cell assays.

Cell numbers

To assess effects of the mineral particle exposure on cell numbers, RTgill-W1 cells in L15 medium (10% FBS, with pen/strep) were seeded in 24-well tissue culture plates (Greiner BioOne, Germany) at a concentration of 140000 cells per well. Cells were grown to confluence at 19°C in normal atmosphere for 48h (Dayeh et al., 2005). The medium was then exchanged, and cells were exposed to 250 mg L^{-1} particles suspended in L15 medium with pen/strep, and again once with and once without serum. Three plates, each with four wells per

treatment level were applied. After 72h cell nuclei were stained with the cell-membrane-permeable fluorescent stain Hoechst 33342 (5 μg mL^{-1} for 10 minutes; Molecular Probes, Invitrogen Inc.). Eight random pictures (400× magnification) were taken in each well along two central transects using a DMI 6000B inverted microscope and the LAS AF software v2.2.0 (Leica Microsystems GmbH, Germany). Cell numbers were quantified by counting cell nuclei in two random fields per picture using the Fiji distribution of ImageJ (Schindelin et al., 2012). The counting order was randomized and sample names were blinded to avoid subjective bias. To complement these direct cell counts, changes of viable cell numbers in controls with and without serum were approximated for each time-point using the metabolic activity in controls (Kupcsik, 2011). For each of three experiments, one plate each with four wells per mineral per treatment level was counted.

Particle uptake and cellular effects

Particle uptake and the ultra-structure of cells exposed to the particles were studied by transmission electron microscopy (TEM). Then cells were exposed to 250 mg L^{-1} mineral particles (L15 medium, with pen/strep), and again once with and once without serum. In parallel, control cells were grown under similar conditions in particle free medium. For this approximately 7.7×10^6 cells were seeded into 75 cm^2 culture flasks (TPP AG, Switzerland) and grown to confluence for 48h (Dayeh et al., 2005). The growth medium was discarded, 12.5 mL exposure solution was added to the flask, and cells were incubated for 72 h. After this time cells were washed twice, once with the exposure medium and once with serum-free Earle's medium. Cells were fixed in 3% Karnofski parafomaldehyde containing 0.5 % glutaraldehyde in PBS for 1 h and then washed with PBS. Subsequently, cells were post-fixed in 1% osmium-tetroxide containing 1.5% potassium ferrocyanide for 1.2h and washed with water. Cells were dehydrated in an ascending ethanol series and acetone, stained with 6% uranyl acetate and embedded in epoxy resin (Spurr, 1969). The resin was hardened for 48h at 60°C and samples were sectioned at 60nm thickness on a microtom (Jung Ultracut E, Reichert Microscope Services, USA), equipped with a diamond knife (Diatome AG, Switzerland). TEM examinations were made with a Morgagni 286(D) transmission electron microscope (FEI Company, USA). All TEM sample preparations and analyses were conducted at the Center for Microscopy and Image Analysis (ZMB) of the University of Basel. With the applied method, the detection limit for mineral particles in the cells was in the range of 20–30nm (M. Dürrenberger, ZMB, University of Baesel, pers. comm.)

Statistical analysis

All statistical analyses were conducted in the open-source statistics software R v2.12.0 (R Development Core Team, 2011). Significance was accepted at $p \leq 0.05$. Continuous response variables were analyzed with linear mixed effect models, with plates included as random factor to account for spatial clustering of wells (function: lmer, lme4 package; Zuur et al., 2009). The fixed effect part of the model consisted of the treatment level as categorical explanatory variable, and the fold-change (all time-points) or the raw response data (72h exposure) as continuous response variable. Significance was tested *via* likelihood ratio tests (Zuur et al., 2009). Once a significant main effect was detected, each treatment level was compared to the respective control using the Markov chain Monte Carlo (MCMC) re-sampling approach implemented in the function pvals.fnc (languageR package; Baayen et al., 2008). Cell count data were analyzed with generalized linear mixed effect models (glmm) fitted with a poisson error distribution and a $\log_e$ link function (function: glmmPQL, MASS package; Venables and Ripley, 1994). Fixed and random effect terms were included as described above. Significance was tested with Wald χ^2 tests (function: wald.test, aod package). Once a significant main effect was detected, each particle treatment level was compared to the respective control using Wald *t*-tests (Bolker et al., 2009). If outliers were observed, the model was fitted with and without these data-points, and significance was only accepted if supported in both analyses. Model fit and assumptions were evaluated according to standard procedures, and no violations were observed (Zuur et al., 2009). Finally, to test for changes in metabolic activity of the controls over time the Pearson product-moment correlation analysis (function: cor.test) was applied.

Results

Particle characterization

The size distributions of the two clay particle (mica, kaolin) samples were distinctly smaller than for the two framework silicate samples (quartz, feldspar; Figure 1). Altogether, 20% of the quartz and 19% of the feldspar particles were smaller 6.3 µm; whereas, 57% of the mica and 42% of the kaolin particles were smaller 6.3 µm. X-ray diffractometry confirmed the mineralogical structure and purity of all mineral particle mixtures (≥ 95% crystalline purity). No changes in mineral structure and cristallinity could be found after γ-irradiation.

Particle uptake and cellular effects

Irrespective of the presence of serum control cells appeared elongated (Figure 2A) with a centrally located nucleus and cell organelles distributed throughout the cytoplasm (Figure 2B). In all cells ribosomes were abundant, mostly near the endoplasmic reticulum (rough ER). Lysosomes of up to one µm diameter were common, with some of them showing layered membrane structures and high electron densities, which are most likely myelin bodies (Figure 2B). Microfilament bundles aligned with the elongated cells appeared regularly in the cytoplasm. Altogether, our cells appeared similar to the RTgill-W1 cells described in the original publication (Bols et al., 1994)

Regardless of the mineral particle studied and the presence of serum, all cells appeared intact and without pronounced ultrastructural effects. All particles studied were found in membrane bound vacuoles within the cytoplasm (Figure 2C–F). Occasionally evidence for phagocytic uptake could be observed (Figure 3A). The size of intracellular particles ranged from 0.1 to 2 µm, and uptake occurred irrespective of the presence of serum. Qualitatively, mica and kaolin particles were more often found in cells. Kaolin particles were occasionally found without a surrounding membrane (Figure 3B). Mica particles caused a slightly dilated endoplasmic reticulum (Figure 3C).

Cytotoxicity assays

Membrane permeability – The quartz, feldspar and mica particles had only minimal effects on membrane permeability (Figures 4A, S1, and S2). In contrast, kaolin caused a marked increase in cell membrane permeability at concentrations of 50 mg L^{-1} and above, indicating a damaging effect of these particles on the cell membrane. This response pattern was observed at all time-points and regardless of serum present in the medium (Figures S1 *vs* S2).

Oxidative stress – The quartz, feldspar and kaolin particles caused some oxidative stress with effect strength being mostly within ± 20% of controls, indicating a slight effect of these particles on free radical levels (Figures 4B, S1 and S2). In contrast, the highest mica particle concentration (250 mg L^{-1}) caused a consistent increase in reactive oxygen species at all time-points (Figures 4B, S1 and S2). This general increase of free radicals caused by the mica particles was observed at all time-points and regardless of serum present in the medium (Figures S1 vs S2).

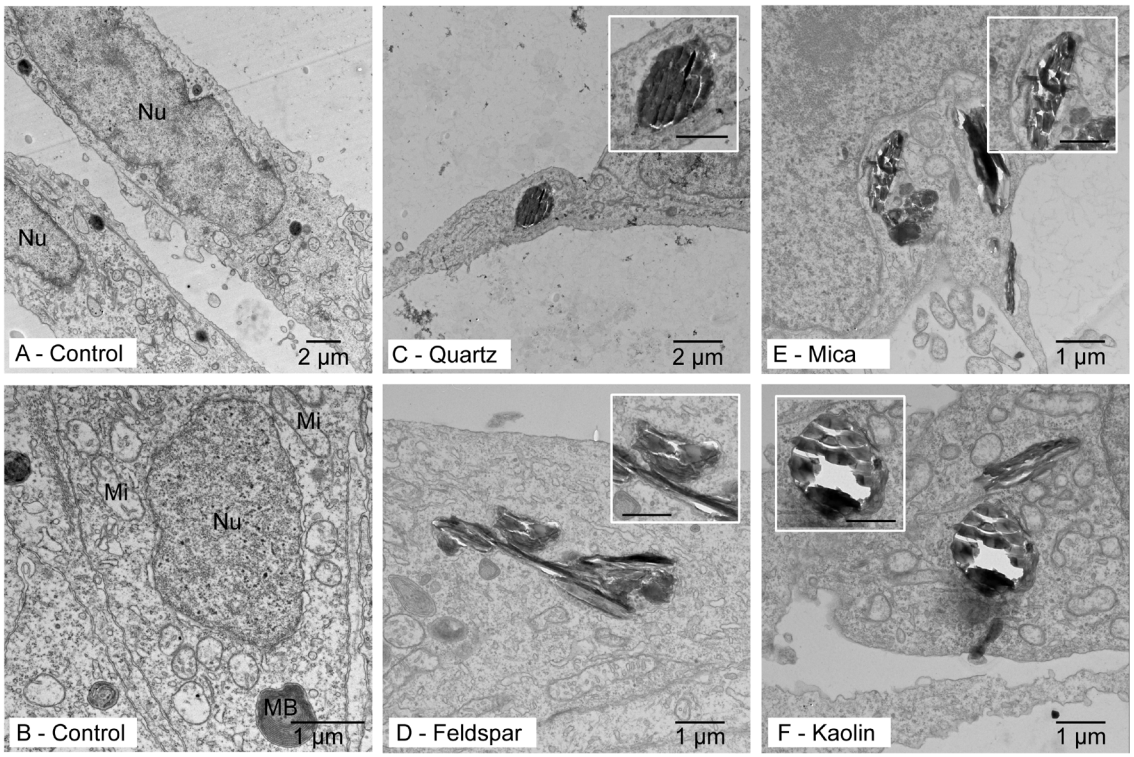

Figure 2 – **Control and particle containing RTgill-W1 cells.** Pictures were taken after 72h exposure either without (control) or with 250 mg L^{-1} of the respective particle. Shown are examples for A.) Control cells, with marked nucleus (Nu). B.) Control cell detail, with nucleus (Nu), mitochondria (Mi) and a myelin body (MB). Pictures C to D show examples for mineral particle phagocytosis with insets showing details of internalized particles surrounded by a membrane (0.5 µm bar). Note: white areas in pictures with mineral particles represent artifacts introduced during sectioning.

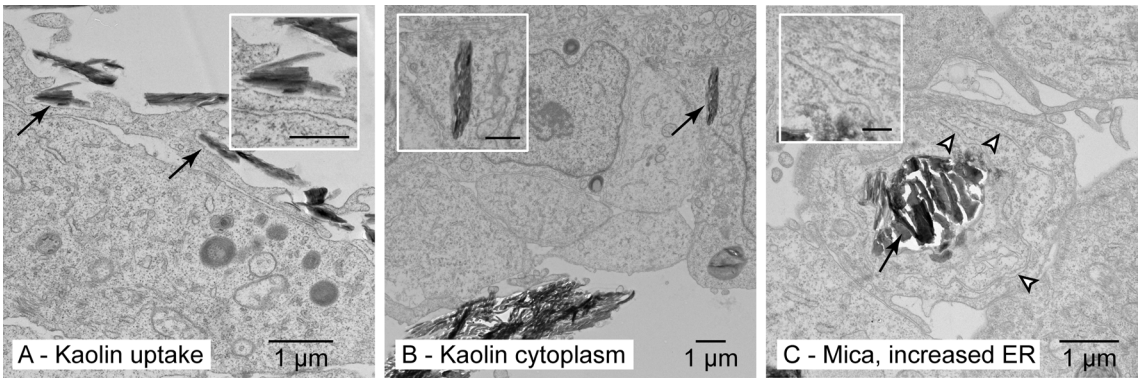

Figure 3 – **Particle phagocytosis and effects in cells.** Pictures were taken after 72h exposure to 250 mg L^{-1} of the respective particle. Shown are A.) kaolin particle phagocytosis (arrows), inset shows particle marked on upper left (0.5 µm bar). B.) Kaolin particle in cytoplasm without surrounding membrane, inset shows detail (0.5 µm bar). C.) Increased endoplasmic reticulum (ER, open arrowheads) in a cell containing mica particles (black arrow), inset shows detail of the ER marked by the upper two arrowheads (0.5 µm bar). Note: white areas around particle in picture C represent artifacts introduced during sectioning.

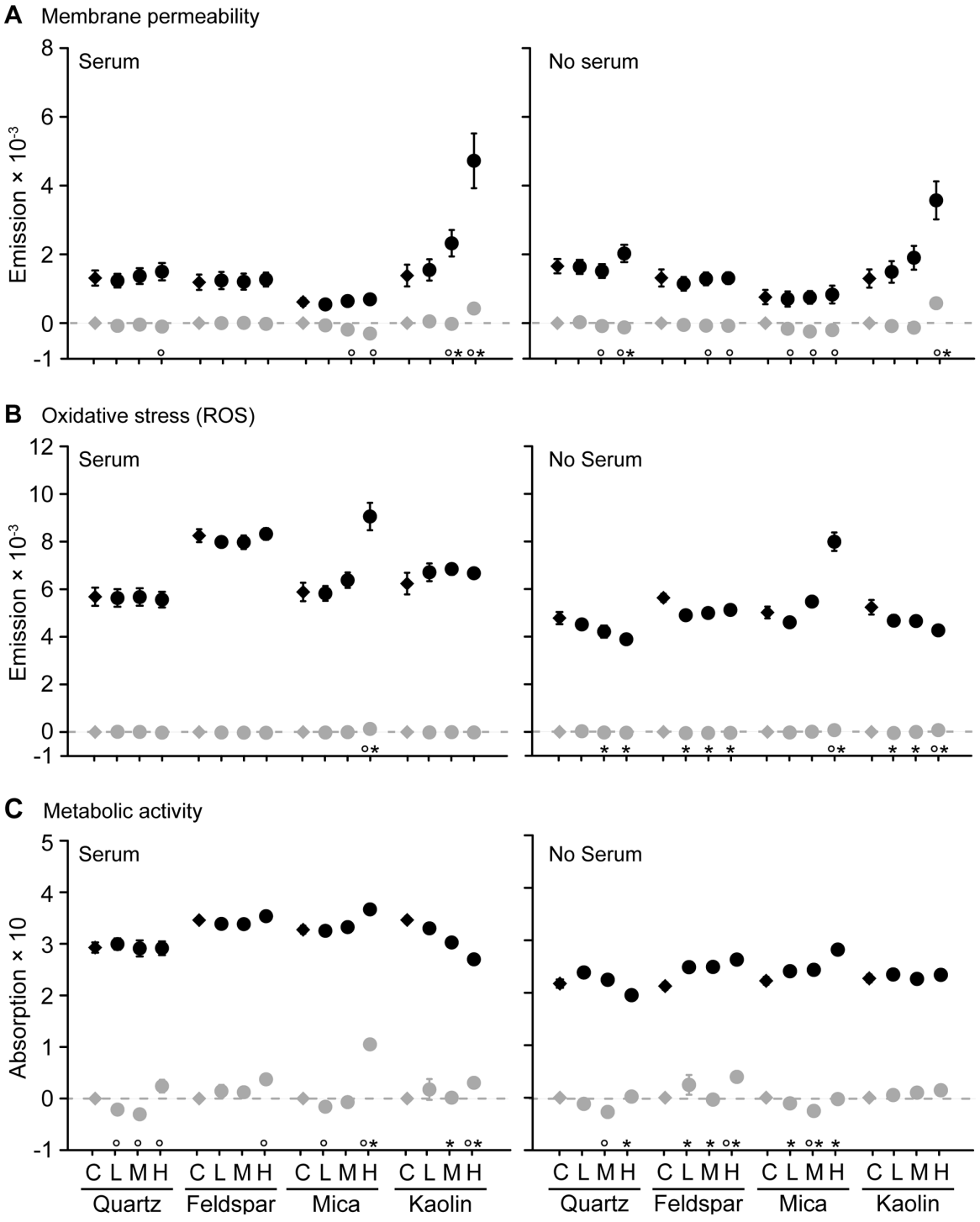

Figure 4 – Cytotoxic effects and particle interference in the applied cytotoxicity assays. Shown are cytotoxic effects of the particles in cells (black symbols) and particle interference with the respective assay (grey symbols), all data after 72h exposure. Symbols near the x-axis denote significant differences ($p < 0.05$) to respective control for cytotoxicity (asterisks) and particle interference (circles) data. Labels on x-axis denote control (C), as well as low (L), medium (M) and high (H) treatment level, grouped according to mineral species. Data points are mean ± SE (cell assays: $n = 24$, i.e. 3 plates, 8 wells each; particle interference assays: $n = 9$, i.e. 3 plates, 3 wells each). Note: Shown are summary statistics calculated from raw-data, while significance was tested with mixed-effect models to adjust for clustering of the wells on plates.

Metabolic activity – The quartz particles caused a slight increase in metabolic activity when cells were exposed ≤ 48 h with serum, which returned to control levels at 72h and 96h (Figures 4C and S1). Without serum quartz caused a slight decrease in metabolic activity, mostly in the highest particle concentration (Figures 4C and S2). Feldspar caused almost no effects in cells exposed with serum (Figures 4C and S1), but without serum the metabolic activity was slightly increased, which was most pronounced at 72h (Figure 4C and S2). For the mica particles the cell assay indicated an increase in metabolic activity, which appeared to be stronger in cells exposed without serum (Figure 4C, S1 and S2). The kaolin particles caused a concentration depended decrease in metabolic activity in cells exposed ≥ 72h with serum (Figure 4C and S1); without serum no decrease could be observed (Figure 4C and S2).

Relative cytotoxicity

Comparing the relative cytotoxicity (Table 1) revealed that quartz, feldspar and mica hardly affected cell membrane permeability, while kaolin caused strong effects. In the oxidative stress assay, quartz and feldspar caused occasional slight effects, regardless of exposure duration and presence of serum in the medium. Mica caused consistent moderate to strong increases, regardless of exposure duration and presence of serum in the medium. With serum present in the medium kaolin caused slight increases in cells exposed ≤ 72h, which reversed to a slight decrease at 96h. Without serum kaolin induced a slight increase at all time-points ≤ 72h. In the metabolic activity assay, quartz and feldspar caused slight increases in cells exposed ≤ 72h. Mica caused slight to moderate increases in cells exposed ≤ 72h with serum. Without serum mica caused moderate to strong responses at all time-points. Kaolin caused moderate to strong decreases in cells exposed ≥ 72h with serum. Without serum the metabolic activity was slightly increased in cells exposed ≤ 48h. Altogether, comparing the relative cytotoxicity of all investigated mineral particles (Tables 1 and S1) suggests the following order feldspar < quartz << mica ≤ kaolin.

Particle interference assays

All mineral particles had minimal effects on the cell membrane permeability (PI) and oxidative stress (H_2-DCF-DA) assays (Figure 4A–C). Yet, the metabolic activity assay (MTT) was markedly affected by all particles, except when kaolin was tested without serum (Figure 4C). Here, effect strength regularly exceeded the values observed in cell assays, which was most prominent with 250 mg L^{-1} mica particles suspended in medium with serum (Figure 4C).

Cell numbers

After 72h particle exposure cell numbers counted in treatments with serum where distinctly higher than in treatments without serum (Figure 5). Even the highest particle concentration (250 mg L^{-1}) had no marked effect on cell numbers after 72 h exposure (Figure 5A). Only when cells were exposed without serum, the feldspar particles caused a slight increase and the kaolin particles a slight decrease in cell numbers (Figure 5A). The metabolic activity confirmed thie general increase in viable cell numbers in controls with serum compared to without serum (Figure 5B). In addition, it demonstrated that the numbers of viable cells increased over the exposure period, and that this increase was two-fold stronger for cells in medium with serum (Figure 5B). These data indicate that the difference in viable cell numbers was minimal at 24h and reached a maximum at 96h. At this time-point an almost two-fold increase could be observed (Figure 5B).

Table 1 – Relative cytotoxicity of the mineral particles. Given are effects on membrane permeability (PI assay), metabolic activity (MTT assay), and oxidative stress (ROS assay). Ranked effect strength was inferred relative to the maximum fold-change in the 250 mg L^{-1} particle concentration (Figures S1 and S2) without taking particle interference into account. Symbols denote response intensity as slight (+/-), moderate (++/--) or strong (+++/---). Empty cells denote no effect beyond ± 10% from the control. Fold-change intervals linked to the respective response intensities are given Table S1.

Serum	Time	Quartz			Feldspar			Mica			Kaolin		
		PI	ROS	MTT	PI	ROS	MTT	PI	ROS	MTT	PI	ROS	MTT
Yes	24h		-	+			+		++	+	+	+	
	48h		+	+					+++	++	+++	+	
	72h								++	+	+++	+	--
	96h								++		+++	-	---
No	24h								++	+++	+	-	+
	48h						+		++	+++	++	-	+
	72h			-			++		++	++	+++	-	
	96h								++	+++	+++		

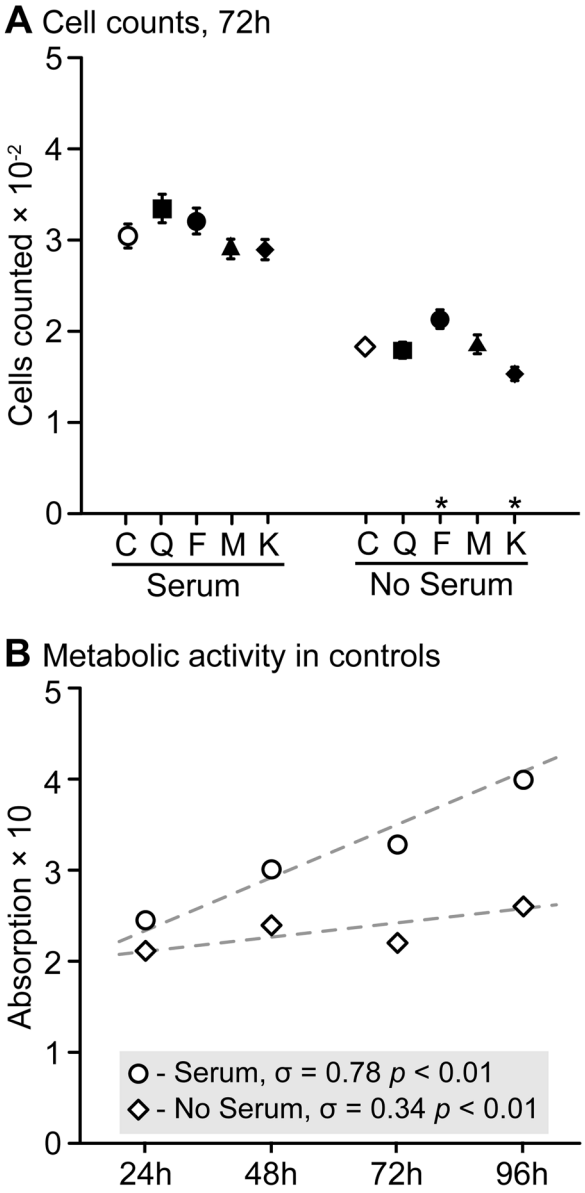

Figure 5 – **Effects of particle exposure and serum addition on cell numbers**. A.) Cell counts after 72h exposure to 250 mg L^{-1} mineral particles. Shown are exposure with (left group) and without (right group) serum. Open symbols are controls (C), and filled symbols denote mineral particle (quartz, Q; feldspar, F; mica, M; kaolin, K). Asterisks near the x-axis denote significant (p < 0.05) differences to respective control; B.) Changes in metabolic activity of controls during the experiment, shown are controls with (circles) and without (diamonds) serum. Dashed lines are best fit lines with respective Pearson product-moment correlation coefficient σ given in grey box. In both graphs data points are mean ± SE (cell counts: n = 12, i.e.3 plates, 4 wells each; metabolic activity: n = 96, i.e. 12 plates, 8 wells each).

Discussion

We provide an empirical investigation about cytotoxicity and uptake of different natural mineral particles in salmonid gill epithelial cells. Thereby, we also report first benchmark data for comparing the cytotoxic potential of natural mineral particles with synthetic nanoparticles (SNPs). Finally, our results indicate that natural mineral particles can interfere with common cytotoxicity assays and this has important methodological implications.

Methodological implications

Serum addition had some effects on the responses observed in the cell assays. We see two main possibilities for this: First, serum can coat particles and thereby affect particle surface properties and agglomeration, which could alter the interaction with cells and probably their cytotoxicity (e.g. Schulze et al., 2008; Kühnel et al., 2009). Yet we have no indication that serum addition affected particle uptake and/or exerted strong effects on cytotoxic responses in the cell membrane permeability (PI) and oxidative stress (H_2-DCF-DA) assays. We therefore believe serum interaction with the particles were of less importance in our experiment. However, serum addition had a marked effect on cell numbers in wells and their metabolic status. This has been previously demonstrated, also for the RTgill-W1 cell line (Bols et al., 1994; Gstraunthaler, 2003). The most pronounced effects of serum addition could be seen for cells exposed to the two clay particles: Kaolin caused a concentration dependent decrease in metabolic activity only in cells exposed with serum. Similarly, the highest mica particle concentration caused a decrease in metabolic activity only with serum (once particle interference was taken into account). Without serum, mica tended to provoke an increased metabolic activity at all time-points. Kaolin might have also caused a slight increase, yet this might have been masked by the slightly decreased cell numbers. Altogether, it appears to us that a decreased metabolic activity as response to the particle exposure was more pronounced with serum present in the medium. A similar pattern could be observed in RTgill-W1 cells exposed to cobalt doped tungsten carbide nanoparticles for 72h (Fig.4 in Kühnel et al., 2009). One possible explanation could be that faster proliferating cells, such as the RTgill-W1 cells in medium with serum, are more susceptible to particle-induced toxicity in the applied MTT assay (Brunner et al., 2006). Moreover, when the indicator dye needs to be actively metabolized by the cells (e.g. MTT and Alamar blue assays) the number of viable cells and their metabolic status might affect assay results more strongly. In support of this notion, the propidium iodide assay, where the indicator dye only binds to nuclear DNA, was least

affected by serum addition. In our experiment, serum addition increased the number of viable cells and likely also their metabolic status latest after 48h exposure. Therefore, studies investigating particle effects in cell culture experiment at time-points beyond 24h, or even earlier in case of faster proliferating cells, should complement cytotoxicity data with an assessment of cell numbers to avoid misinterpretation of cell assay results.

We also demonstrated that mineral particles can interfere with common cytotoxicity assays applied in human toxicology research (e.g. Stringer and Kobzik, 1998; Schins et al., 2002; Camatini et al., 2012). Particle interference with cytotoxicity assays is a known problem in SNP research, where marked effects can occur with concentrations as low as 10 and 30 mg L^{-1} (MTT and H2-DCF-DA assays; Wang et al., 2011; Holder et al., 2012; Kroll et al., 2012). Our mineral particles interfered mostly at concentrations ≥ 50 mg L^{-1}. Moreover, effect strengths of the mineral particles in our assays were smaller than for SNPs (Kroll et al., 2012). This could be related to the smaller particle size of SNPs, which increases their specific surface area and hence particle reactivity (Fig. 1 in Delay and Frimmel, 2012). Finally. the washing step included in our experiment likely reduced particle interference (Kroll et al., 2012). Altogether, our results indicate that mineral particles interfere less, at least in the cell permeability (PI) and oxidative stress (H_2-DCF-DA) assays. Yet, in the metabolic activity assay (MTT) the mineral particles caused sufficient interference to confound the interpretation of effects measured in cell assays. Particularly with serum, the highest mica particle concentration (250 mg L^{-1}) tampered with the metabolic activity measured in cell assays. In the light of this data the MTT assay, an often applied standard test for particle cytotoxicity, appeared to be least reliable. In conclusion, interference of mineral particles with cytotoxicity assays needs to be considered in cell culture experiments. We see our particle interference controls, which allowed us to disseminate interference from particle effects in cells, as a necessary prerequisite to validate the results of such experiments.

Particle uptake and cellular effects

Regardless of their mineralogy, particles between 0.1 and 2 µm diameter were regularly internalized in RTgill-W1 cells. Given the size of the incorporated particles, we postulate that internalization occurred most likely via phagocytosis (cf. Foster et al., 2001; Gehr et al., 2011). In support of this notion, most mineral particles were observed in membrane-bound vesicles in the cytoplasm. The observed particle uptake clearly indicates that mineral particles came into contact and interacted with the cells. Similar uptake of nano-particle agglomerates

has been previously demonstrated in RT-gill W1 cells (Kühnel et al., 2009). Moreover, similar-sized particles are regularly phagocytized in bird, mammalian and human respiratory epithelium cells *in vitro* (Foster et al., 2001; Schins et al., 2002; Kiama et al., 2008). *In vivo*, particle uptake in gill epithelia has been demonstrated in the blue mussel (ferric iron and plastic particles; George et al., 1976; von Moos et al., 2012) and in juvenile pacific salmon (mineral particles; Goldes et al., 1986; Martens and Servizi, 1993). Altogether, these data clearly suggest that mineral particle uptake is common in gill epithelia of aquatic animals.

We cannot judge from our data if the number of particles incorporated by the cells was different between the different mineral particles studied. This has been demonstrated for nanoparticles of different composition (Busch et al., 2011) and also for different sized albumin coated microspheres (diameters: 0.5–3 µm; Foster et al., 2001). What we could demonstrate is that three out of four mineral particles did not cause visible ultra-structural alterations in the RTgill-W1 cells. Only mica caused a slight dilation of the endoplasmic reticulum. This effect was previously observed in cells experiencing free radical stress (Hitomi et al., 2004; Long et al., 2005). Hence, it corroborates that the mica particles caused oxidative stress in our cells (see below). The ultra-structural investigations also documented that regardless of the cytotoxic effects triggered by some of the particles, none of them caused pronounced cell disintegration or death. This is in accordance with our finding that the particle exposure did not cause marked changes in cell numbers.

Geochemical composition affects cytotoxicity

Our experiment demonstrates remarkable differences in the cytotoxicity of four silicate minerals common in rivers worldwide in a rainbow trout gill cell-line. Feldspar exhibited the least cytotoxic potential, while compared to both framework silicates the clay particles (mica, kaolin) caused distinctly stronger cytotoxic effects. We believe that the generally increased cytotoxicity of the two clays is at least partly related to their smaller particle sizes and higher specific surface area (Guthrie, 1997). In human lung epithelial cells and alveolar macrophages an increase in cellular effects has been repeatedly documented for mineral particle samples with increased specific surface areas (Gao et al., 2002; Schwarze et al., 2007). In our experiment, cell numbers were not affected by the particle exposure, and hence cytotoxic responses are most likely not related to changes in cell numbers. Therfore, our results document that small-sized natural mineral particles can cause cytotoxic effects in gill epithelial cells, and that especially clay particles have a strong cytotoxic potential.

In addition to the generally increased cytotoxic potential of the clay particles, our results also demonstrate that kaolin and mica affected the cells differently: kaolin caused cell membrane damage at concentrations of 50 mg L^{-1} (at 48h and 72h) and 250 mg L^{-1} (all time-points). This could also explain why only kaolin particles were occasionally found without a surrounding membrane in the cytoplasm. The mica particles did not affect cell membrane permeability. Instead, the highest mica particle concentration (250 mg L^{-1}) induced consistent free radical stress. As pointed out above, this also caused endoplasmic reticulum stress. For two reasons it appears unlikely to us that this marked difference observed between mica and kaolin is only related to differences in the specific surface area: First, the particles caused markedly different cytotoxic effects, either by impairing the cell membrane (kaolin) or related to energy metabolism (mica). Second, mica had a two-fold higher specific surface compared to kaolin, indicating two-fold higher surface area dose of the mica particles. However, neither in the cell membrane permeability nor in the oxidative stress assay a two-fold difference can explain the differences in cytotoxicity between mica and kaolin. Evidence for cell membrane damage was minimal for the mica particles. These data altogether suggest that, like for other mineral particles in the low µm size range, additional contributing factors have to be assumed (Guthrie, 1997). This would agree with studies in lung epithelial cells investigating mineral particles of different geochemical composition (Schwarze et al., 2007) or synthetic nano- and micron-sized particles (Warheit et al., 2006; Karlsson et al., 2009). Among the most important factors that could have contributed are different functional surface groups but also toxic compounds bound to the particles (Fubini, 1997; Guthrie, 1997). Moreover, shape differences could have contributed to the increased cell membrane damage caused by the kaolin particles (Doshi and Mitragotri, 2010). An extensive study of all potential contributing factors was out of scope for our study. Nonetheless, our results indicate that the geochemical composition and surface properties affect the cytotoxic potential of clay particles in gill epithelia of aquatic biota.

Comparison with synthetic nanoparticles

Similar to our results, *in vitro* studies indicate that synthetic nanoparticles (SNPs) can cause cytotoxic effects in gill epithelial cells, and that their effects differ with particle composition. The cytotoxic potential of some SNPs has been investigated in RTgill-W1 cells: For example, 25 mg L^{-1} palladium-magnetite nanoparticles did not affect cell-viability in RTgill-W1 cells after 72h exposure (Hildebrand et al., 2010). However, when exposed for the same time-period cobalt doped tungsten carbide nanoparticles (30 mg L^{-1}) decreased membrane integrity

and metabolic activity (Kühnel et al., 2009). Similarly, 45 mg L^{-1} gold nanoparticles decreased the metabolic activity in RTgill-W1 cells after 24h exposure (Van Hoecke et al., 2011). In an *in vivo* experiment, TiO_2 nanoparticles caused lipid peroxidation in the gills of rainbow trout (1 mg L^{-1}, 14 days exposure; Federici et al., 2007). Likewise, waterborne exposure to suspended fine sediment pulses (200 mg L^{-1} max, 120 days exposure; Debes et al., 2012) and silver nanoparticles (0.1 mg L^{-1}, 10 days exposure; Scown et al., 2010) induced the expression of genes in salmonid gills involved in protecting the tissue from oxidative damage. We recently demonstrated that suspended mica clay pulses can induce lipid peroxidation in the gill of rainbow trout (300 mg L^{-1} max, 24 days exposure; Michel et al., 2013). These *in vivo* results agree with the notion that oxidative stress is a common of both mineral particles and SNPs in cells (Donaldson and Borm, 2007; Handy and Shaw, 2007). Altogether, it appears therefore plausible that natural clay particles induce cytotoxic effects comparable to some SNPs in gill epithelial cells, and our *in vitro* results provide first empirical evidence for this.

It is a remarkable result of our experiment that natural clay particles induced cytotoxic effects comparable to SNPs in gill epithelial cells. For eco-toxicological risk assessment it would be important to know, firstly, if synthetic nanoparticles are more cytotoxic than natural clay particles, and, secondly, if effect pathways differ between the two (Handy et al., 2008a). With our results, we cannot address question two. However, for question one our results together with the research summarized in the previous paragraph suggests that natural clay particles might be less cytotoxic. In the above cited *in vitro* experiments, effect concentrations for SNPs were consistently lower compared to our study. Also, natural clay particles (Herbert and Merkens, 1961; Goldes, 1983; Redding et al., 1987; Michel et al., 2013) induce less structural damage in fish gills compared to some SNPs (Federici et al., 2007; Griffitt et al., 2009). One reason for the stronger cytotoxic potential of SNPs could be their increased specific surface area (e.g. 57–188 m^2 g^{-1}; Brunner et al., 2006), but also novel surface properties and hence a different surface reactivity (Handy et al., 2008b). Therefore, while our results clearly indicate that some clay particles induce similar cytotoxic effects than SNPs, the relative cytotoxicity of SNPs compared to natural clay particles with similar specific surface area remains to be evaluated. These data would also allow understanding if the tailor-made surface modifications of some SNPs induce stronger or different cytotoxic effects, which is an important aspect for eco-toxicological risk-assessment of SNPs (Handy et al., 2008a).

Conclusion

We investigated uptake and cytotoxicity of natural mineral particles (quartz, feldspar, mica, and kaolin) in salmonid gill epithelial cells. Mineral particle exposure hardly affected cell numbers. Particles with up to 2 µm diameter were regularly phagocytized by the cells, indicating that mineral particle uptake is common in salmonid gills. Among the different mineral species studied, the clay particles mica and kaolin were distinctly more cytotoxic than the framework silicates quartz and feldspar. This was most likely related to the smaller particle sizes and higher specific surface area of the clay particles. In addition, the kind of cytotoxic effect differed between mica and kaolin: mica caused free radical and endoplasmic reticulum stress, whereas kaolin caused increased cell membrane damage. Thus, our results demonstrate that cytotoxic effects vary, firstly, between framework silicate and clay particles, and secondly between clay particles of different geochemical composition. Among clay particles differences could be related to differences in functional surface groups and shape, but also bound toxic substances. Therefore, care has to be taken when concluding about cytotoxic effects between different mineral species. Most interesting, our results in comparison to published studies indicate that natural clay particles can induce comparable cytotoxic effects in gill epithelial cells than synthetic nanoparticles. Future research is therefore needed: Firstly, to identify particle properties and cellular pathways related to cytotoxic responses induced by clay particles, and, secondly, to evaluate if synthetic nanoparticles are more cytotoxic and/or induce different toxicity pathways than natural clay particles. These data would clearly contribute to a better eco-toxicological risk assessment of both natural mineral and synthetic nanoparticles.

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Supplementary information

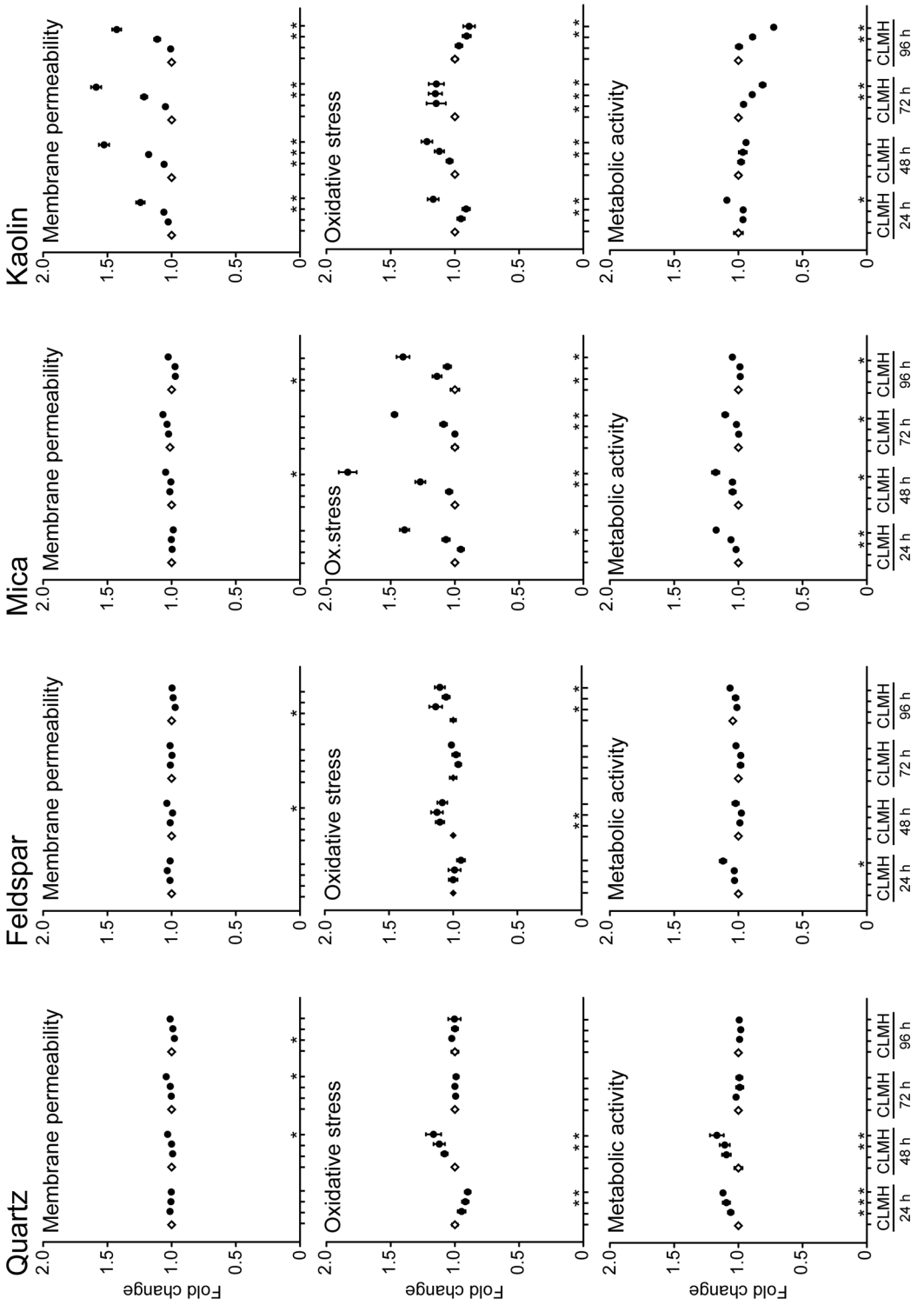


Figure S1 – Cytotoxic effects in RTgill-W1 cells exposed with serum. Shown are effects on membrane permeability (PI assay), metabolic activity (MTT assay) and oxidative stress (ROS assay). Grouped data points in each graph represent exposure times (24, 48, 72 and 96h), with symbols from left to right denoting control (open diamonds) and particle exposed cells (filled dots) in increasing particle concentration. Data points show mean ± SE (n = 24; i.e. 3 plates with 8 wells each). Asterisks above x-axis denote significant differences to respective control (p < 0.05). Note: Data points show values calculated from raw-data, while significance was tested with mixed-effect models to adjust for

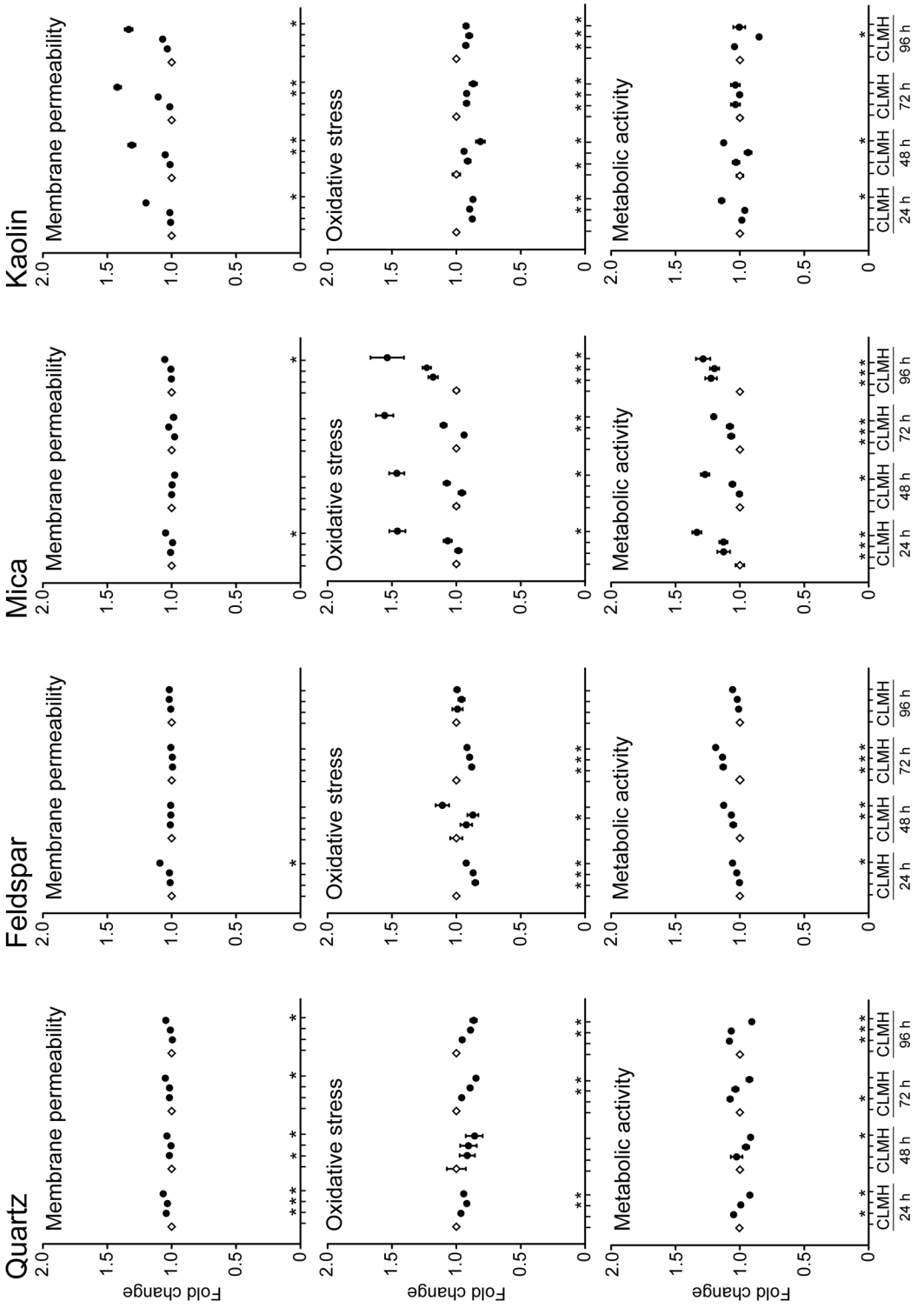


Figure S2 – Cytotoxic effects in RTgill-W1 cells exposed without serum. Shown are effects on membrane permeability (PI assay), metabolic activity (MTT assay) and oxidative stress (ROS assay). Grouped data points in each graph represent exposure times (24, 48, 72 and 96h), with symbols from left to right denoting control (open diamonds) and particle exposed cells (filled dots) in increasing particle concentration. Data points show mean ± SE (n = 24; i.e. 3 plates with 8 wells each). Asterisks above x-axis denote significant differences to respective control ($p < 0.05$). Note: Data points show values calculated from raw-data, while significance was tested with mixed-effect models to adjust for the spatial clustering of the wells on plates into account.

Table S1 – Fold-change intervals for relative cytotoxicity ranking in Table 1.

Assay	Ranked effect strength					
	---	--	0	+	++	+++
PI	0.41-0.56	0.57-0.73	0.91-1.09	1.10-1.25	1.26-1.32	1.33-1.59
ROS	0.17-0.40	0.41-0.65	0.91-1.09	1.10-1.33	1.34-1.58	1.59-1.83
MTT	0.67-0.73	0.74-0.81	0.91-1.09	1.10-1.17	1.18-1.25	1.26-1.33

References

Atteia O, Perret D, Adatte T, Kozel R, Rossi P. Characterization of natural colloids from a river and spring in a karstic basin. Environmental Geology 1998; 34: 257-269.

Baayen RH, Davidson DJ, Bates DM. Mixed-effects modeling with crossed random effects for subjects and items. Journal of Memory and Language 2008; 59: 390-412.

Bernhardt ES, Colman BP, Hochella MF, Cardinale BJ, Nisbet RM, Richardson CJ, et al. An ecological perspective on nanomaterial impacts in the environment. Journal of Environmental Quality 2010; 39: 1954-1965.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, et al. Generalized linear mixed models: A practical guide for ecology and evolution. Trends in Ecology & Evolution 2009; 24: 127-135.

Bols NC, Barlian A, Chirinotrejo M, Caldwell SJ, Goegan P, Lee LEJ. Development of a cell-line from primary cultures of rainbow trout, *Oncorhynchus mykiss* (Walbaum), gills. Journal of Fish Diseases 1994; 17: 601-611.

Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, et al. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. Environmental Science & Technology 2006; 40: 4374-4381.

Busch W, Bastian S, Trahorsch U, Iwe M, Kuhnel D, Meissner T, et al. Internalisation of engineered nanoparticles into mammalian cells in vitro: influence of cell type and particle properties. Journal of Nanoparticle Research 2011; 13: 293-310.

Cakmak GD, Schins RPF, Shi TM, Fenoglio I, Fubini B, Borm PJA. In vitro genotoxicity assessment of commercial quartz flours in comparison to standard DQ12 quartz. International Journal of Hygiene and Environmental Health 2004; 207: 105-113.

Camatini M, Corvaja V, Pezzolato E, Mantecca P, Gualtieri M. PM10-biogenic fraction drives the seasonal variation of proinflammatory response in A549 cells. Environmental Toxicology 2012; 27: 63-73.

Christian P, Von der Kammer F, Baalousha M, Hofmann T. Nanoparticles: structure, properties, preparation and behaviour in environmental media. Ecotoxicology 2008; 17: 326-343.

Dayeh V, Schirmer K, Lee L, Bols N, Blaise C, Férard JF. Rainbow trout gill cell line microplate cytotoxicity test. In: Blaise C, Férard JF, editors. Small-scale freshwater toxicity investigations. Kluwer Academic, Netherlands, 2005, pp. 473-503.

Debes PV, Normandeau E, Fraser DJ, Bernatchez L, Hutchings JA. Differences in transcription levels among wild, domesticated, and hybrid Atlantic salmon (*Salmo salar*) from two environments. Molecular Ecology 2012; 21: 2574-2587.

Delay M, Frimmel FH. Nanoparticles in aquatic systems. Analytical and Bioanalytical Chemistry 2012; 402: 583-592.

Donaldson K, Borm P. Particle Toxicology. Boca Raton, FL CRC Press, 2007.

Doshi N, Mitragotri S. Needle-shaped polymeric particles induce transient disruption of cell membranes. Journal of the Royal Society Interface 2010; 7: S403-S410.

Evans DJ, Gibson CE, Rossell RS. Sediment loads and sources in heavily modified Irish catchments: A move towards informed management strategies. Geomorphology 2006; 79: 93-113.

Federici G, Shaw BJ, Handy RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (*Oncorhynchus mykiss*): Gill injury, oxidative stress, and other physiological effects. Aquatic Toxicology 2007; 84: 415-430.

Foster KA, Yazdanian M, Audus KL. Microparticulate uptake mechanisms of *in-vitro* cell culture models of the respiratory epithelium. Journal of Pharmacy and Pharmacology 2001; 53: 57-66.

Fubini B. Surface reactivity in the pathogenic response to particulates. Environmental Health Perspectives 1997; 105: 1013-1020.

Gao N, Keane MJ, Ong T, Ye J, Martin J, Miller WE, et al. Respirable quartz and kaolin alumino-silicate induction of *in vitro* cytotoxicity and apoptosis in the presence of surfactant or serum: caveats to bioassay interpretation. Annals of Occupational Hygiene 2002; 46: 50-52.

Gehr P, Clift MJD, Brandenberger C, Lehmann A, Herzog F, Rothen-Rutishauser B. Endocytosis of environmental and engineered micro- and nanosized particles. Comprehensive Physiology. 1. John Wiley & Sons, Inc., 2011.

George SG, Pirie BJS, Coombs TL. Kinetics of accumulation and excretion of ferric hydroxide in *Mytilus edulis* (L.) and its distribution in tissues. Journal of Experimental Marine Biology and Ecology 1976; 23: 71-84.

Goldes SA. Histological and ultrastructural effects of the inert clay kaolin on the gills of rainbow trout (*Salmo gairdneri* Richardson). Master thesis. University of Guelph, Guelph, Ontario., 1983.

Goldes SA, Ferguson HW, Daoust PY, Moccia RD. Phagocytosis of the inert suspended clay kaolin by the gills of rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 1986; 9: 147-151.

Goldes SA, Ferguson HW, Moccia RD, Daoust PY. Histological effects of the inert suspended clay kaolin on the gills of juvenile rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases 1988; 11: 23-33.

Griffitt RJ, Hyndman K, Denslow ND, Barber DS. Comparison of Molecular and Histological Changes in Zebrafish Gills Exposed to Metallic Nanoparticles. Toxicological Sciences 2009; 107: 404-415.

Gstraunthaler G. Alternatives to the use of fetal bovine serum: Serum-free cell culture. Altex-Alternativen Zu Tierexperimenten 2003; 20: 275-281.

Guthrie GD. Mineral properties and their contributions to particle toxicity. Environmental Health Perspectives 1997; 105: 1003-1011.

Handy RD, Owen R, Valsami-Jones E. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 2008a; 17: 315-325.

Handy RD, Shaw BJ. Toxic effects of nanoparticles and nanomaterials: Implications for public health, risk assessment and the public perception of nanotechnology. Health Risk & Society 2007; 9: 125-144.

Handy RD, von der Kammer F, Lead JR, Hassellov M, Owen R, Crane M. The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology 2008b; 17: 287-314.

Herbert DWM, Merkens JC. The effect of suspended mineral solids on the survival of trout. International Journal of Air and Water Pollution 1961; 5: 46-55.

Hetland RB, Refsnes M, Myran T, Johansen BV, Uthus N, Schwarze PE. Mineral and/or metal content as critical determinants of particle-induced release of IL-6 and IL-8 from A549 cells. Journal of Toxicology and Environmental Health-Part A 2000; 60: 47-65.

Hildebrand H, Kuhnel D, Potthoff A, Mackenzie K, Springer A, Schirmer K. Evaluating the cytotoxicity of palladium/magnetite nano-catalysts intended for wastewater treatment. Environmental Pollution 2010; 158: 65-73.

Hitomi J, Katayama T, Taniguchi M, Honda A, Imaizumi K, Tohyama M. Apoptosis induced by endoplasmic reticulum stress depends on activation of caspase-3 via caspase-12. Neuroscience Letters 2004; 357: 127-130.

Holder AL, Goth-Goldstein R, Lucas D, Koshland CP. Particle-induced artifacts in the MTT and LDH viability assays. Chemical Research in Toxicology 2012; 25: 1885-1892.

Irion G. Minerals in rivers. In: Degens ET, Kempe S, Richey JE, editors. Biogeochemistry of majorworld rivers. John Willey & Sons, New York, 1991, pp. 265-283.
Jiang JK, Oberdorster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. Journal of Nanoparticle Research 2009; 11: 77-89.
Karlsson HL, Gustafsson J, Cronholm P, Moller L. Size-dependent toxicity of metal oxide particles-A comparison between nano- and micrometer size. Toxicology Letters 2009; 188: 112-118.
Keller AA, Wang HT, Zhou DX, Lenihan HS, Cherr G, Cardinale BJ, et al. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environmental Science & Technology 2010; 44: 1962-1967.
Kiama SG, Adekunle JS, Maina JN. Comparative in vitro study of interactions between particles and respiratory surface macrophages, erythrocytes, and epithelial cells of the chicken and the rat. Journal of Anatomy 2008; 213: 452-463.
Kroll A, Pillukat MH, Hahn D, Schnekenburger J. Interference of engineered nanoparticles with in vitro toxicity assays. Archives of Toxicology 2012; 86: 1123-1136.
Kühnel D, Busch W, Meissner T, Springer A, Potthoff A, Richter V, et al. Agglomeration of tungsten carbide nanoparticles in exposure medium does not prevent uptake and toxicity toward a rainbow trout gill cell line. Aquatic Toxicology 2009; 93: 91-99.
Kupcsik L. Estimation of cell number based on metabolic activity: The MTT reduction assay. In: Stoddart MJ, editor. Mammalian Cell Viability: Methods and Protocols. 740. Springer, 2011.
Lead JR, Wilkinson KJ. Environmental colloids and particles: current knowledge and future development. In: Wilkinson KJ, Lead JR, editors. Environmental colloids and particles: behaviour, aeparation and characterisation. 1
Wiley, New York, 2007, pp. 1-15.
Long JF, Waldman WJ, Kristovich R, Williams M, Knight D, Dutta PK. Comparison of ultrastructural cytotoxic effects of carbon and carbon/iron particulates on human monocyte-derived macrophages. Environmental Health Perspectives 2005; 113: 170-174.
Martens DW, Servizi JA. Suspended sediment particles inside gills and spleens of juvenile Pacific salmon (*Oncorhynchus* spp.). Canadian Journal of Fisheries and Aquatic Sciences 1993; 50: 586-590.
McLeay DJ, Birtwell IK, Hartman GF, Ennis GL. Responses of Arctic grayling (*Thymallus arcticus*) to acute and prolonged exposure to Yukon placer mining sediment. Canadian Journal of Fisheries and Aquatic Sciences 1987; 44: 658-673.
Michel C, Schmidt-Posthaus H, Burkhardt-Holm P. Suspended sediment pulse effects in rainbow trout *Oncorhynchus mykiss* – relating apical and systemic responses. Canadian Journal of Fisheries and Aquatic Sciences 2013; 70: 630-641.
Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment International 2006; 32: 967-976.
Mosmann T. Rapid colorimetric assay for cellular growth and survival - application to proliferation and cyto-toxicity assays. Journal of Immunological Methods 1983; 65: 55-63.
Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysicochemical interactions at the nano-bio interface. Nature Materials 2009; 8: 543-557.
Owen R, Handy R. Formulating the problems for environmental risk assessment of nanomaterials. Environmental Science & Technology 2007; 41: 5582-5588.

Pietsch C, Bucheli TD, Wettstein FE, Burkhardt-Holm P. Frequent biphasic cellular responses of permanent fish cell cultures to deoxynivalenol (DON). Toxicology and Applied Pharmacology 2011; 256: 24-34.

R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2011.

Redding JM, Schreck CB, Everest FH. Physiological effects on coho salmon and steelhead of exposure to suspended solids. Transactions of the American Fisheries Society 1987; 116: 737-744.

Roco MC. Nanotechnology: convergence with modern biology and medicine. Current Opinion in Biotechnology 2003; 14: 337-346.

Schaetzl RJ, Anderson SN. Soils: Genesis and Geomorphology. Cambridge: Cambridge University Press, 2005.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nature Methods 2012; 9: 676-682.

Schins RPF, Duffin R, Hohr D, Knaapen AM, Shi TM, Weishaupt C, et al. Surface modification of quartz inhibits toxicity, particle uptake, and oxidative DNA damage in human lung epithelial cells. Chemical Research in Toxicology 2002; 15: 1166-1173.

Schulze C, Kroll A, Lehr CM, Schafer UF, Becker K, Schnekenburger J, et al. Not ready to use - overcoming pitfalls when dispersing nanoparticles in physiological media. Nanotoxicology 2008; 2: 51-U17.

Schwarze PE, Ovrevik J, Hetland RB, Becher R, Cassee FR, Lag M, et al. Importance of size and composition of particles for effects on cells *in vitro*. Inhalation Toxicology 2007; 19: 17-22.

Scown TM, Santos EM, Johnston BD, Gaiser B, Baalousha M, Mitov S, et al. Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicological Sciences 2010; 115: 521-534.

Servizi JA, Martens DW. Effect of temperature, season, and fish size on acute lethality of suspended sediments to coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 1991; 48: 493-497.

Spurr AR. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 1969; 26: 31-&.

Stringer B, Kobzik L. Environmental particulate-mediated cytokine production in lung epithelial cells (A549): Role of preexisting inflammation and oxidant stress. Journal of Toxicology and Environmental Health-Part A 1998; 55: 31-44.

Van Hoecke K, De Schamphelaere KAC, Ali Z, Zhang F, Elsaesser A, Rivera-Gil P, et al. Ecotoxicity and uptake of polymer coated gold nanoparticles. Nanotoxicology 2011; 7: 37-47.

Venables WN, Ripley BD. Modern Applied Statistics with S-Plus (MASS). New York: Springer, 1994.

von Moos N, Burkhardt-Holm P, Köhler A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environmental Science & Technology 2012; 46: 11327-11335.

Wang SG, Yu HT, Wickliffe JK. Limitation of the MTT and XTT assays for measuring cell viability due to superoxide formation induced by nano-scale TiO2. Toxicology in Vitro 2011; 25: 2147-2151.

Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL. Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: Toxicity is not dependent upon particle size and surface area. Toxicological Sciences 2006; 91: 227-236.

Waters TF. Sediment in streams. Sources, biological effects, and control. Bethesda, MD: American Fisheries Society, 1995.

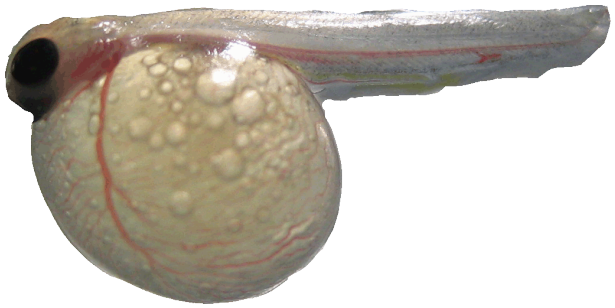
Wiesner MR, Lowry GV, Jones KL, Hochella MF, Di Giulio RT, Casman E, et al. Decreasing Uncertainties in Assessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials. Environmental Science & Technology 2009; 43: 6458-6462.

Zhang Y, Chen YS, Westerhoff P, Hristovski K, Crittenden JC. Stability of commercial metal oxide nanoparticles in water. Water Research 2008; 42: 2204-2212.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. Mixed effects models and extensions in ecology with R. New York: Springer Pub., 2009.

Part 2

Effects on salmonid embryo survival



Chapter 4

Measurement of spatial and temporal fine sediment dynamics in a small river

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Hydrology and Earth System Sciences

Measurement of spatial and temporal fine sediment dynamics in a small river

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Abstract. Empirical measurements on fine sediment dynamics and fine sediment infiltration and accumulation have been conducted worldwide, but it is difficult to compare the results because the applied methods differ widely. We compared common methods to capture temporal and spatial dynamics of suspended sediment (SS), fine sediment infiltration and accumulation and tested them for their suitability in a small, canalized river of the Swiss Plateau. Measurement suitability was assessed by data comparison, relation to hydrological data and in the context of previously published data. SS concentration and load were assessed by optical backscatter (OBS) sensors and SS samplers. The former exhibit a better temporal resolution, but were associated with calibration problems. Due to the relatively low cost and easy mounting of SS samplers, they can provide a higher spatial distribution in the river's cross section. The latter resulted in a better correlation between sediment infiltration and SS load assessed by SS samplers than SS concentrations measured with OBS sensors. Sediment infiltration baskets and bedload traps capture the temporal and spatial distribution of fine sediment infiltration. Data obtained by both methods were positively correlated with water level and SS. In contrast, accumulation baskets do not assess the temporal behaviour of fine sediment, but the net accumulation over a certain time period. Less fine sediment accumulated in upwelling zones and within areas of higher mean water level due to scouring of fine sediments. Even though SS and sediment infiltration assessed with the bedload traps increased from up- to downstream, less fine sediment accumulated downstream. This is probably also attributable to more scouring downstream.

1 Introduction

Fine sediment (<2 mm) load in rivers are generally increasing throughout the world in catchments that are impacted both directly and indirectly by human activities (Owens et al., 2005). Sediment supply in the alpine Rhine basin is estimated to increase between 220 % and 284 % by the year 2100 due to climate and land use change (Asselman et al., 2003). These observed and anticipated changes in fine sediment dynamics in rivers can provide a serious threat to aquatic ecosystems, including phytoplankton, aquatic invertebrates and fish (for a review see Bilotta and Brazier, 2008). Salmonid fish can be affected by suspended sediments (SS) in several ways. While SS can directly impact health and fitness of free swimming fish (Newcombe and Jensen, 1996), fine sediment deposition in the gravel bed can induce siltation of the riverbed resulting in a decrease in hydraulic conductivity (Schälchli, 1995). This affects the oxygen supply to the developing salmonid embryos in the redd negatively, which inhibits the incubation success (Greig et al., 2005). The consequences of climate and land use change on the transport of sediment into rivers, on sediment transport in the river and on clogging processes are poorly known. Studies for the Alps, pre-Alps and the hilly regions of the Swiss Plateau are rare. This includes small rivers, which are habitats for gravel spawning fish (Scheurer et al., 2009).

Several studies have shown a strong correlation between sediment deposition and the occurrence of fine sediment in the water column. Higher fine sediment load in rivers generally lead to increased fine sediment infiltration into the riverbed (Acornley and Sear, 1999; Zimmermann and Lapointe, 2005), while periods of low flow and smaller SS

concentration and load trigger low sediment infiltration rates with finer grain sizes (Sear, 1993; Soulsby et al., 2001). Consequently, direct measurements of SS concentration and load may be a straight forward method to assess sediment deposition. The estimation of SS concentrations from turbidity measurements with optical backscatter (OBS) sensors depends on the content of fine particulate organic matter as well as grain size distribution of the SS and colour and shape of the grains (Packman et al., 1999). Accordingly, OBS turbidity measurements require calibration at individual test sites.

Deposition of fine sediments is not only controlled by SS concentration, but also by flow hydraulics and inter-gravel flow. These specific hydraulic conditions, influenced by the topography and the permeability of the riverbed, can have a large influence on sediment deposition (Brunke, 1999; Seydell et al., 2009). Seydell et al. (2009) found significantly higher fine sediment infiltration rates in downwelling zones than upwelling zones. Furthermore, sediment infiltration is dependent on flow velocity (Brunke, 1999). Rivers of the hilly regions of the Swiss Plateau and other regions in Europe are generally canalized and laterally stabilized by terraces for land drainage and flood control. These terraces lower the flow velocity and trigger downwelling processes upstream of the terraces, resulting in an increase of fine sediment infiltration (Bucher, 2002). Additionally, terraces may impede desiltation, i.e., processes that increase hydraulic conductivity attributable to higher bed-shear stress (Schälchli, 1995).

Numerous studies have been conducted on fine sediment dynamics and fine sediment infiltration and accumulation in Canada (e.g. Julien and Bergeron, 2006; Levasseur et al., 2006; Zimmermann and Lapointe, 2005), the USA (e.g. Lisle and Lewis, 1992), and the United Kingdom (e.g. Greig et al., 2005; Heywood and Walling, 2007; Sear, 1993; Soulsby et al., 2001). The results of those empirical studies of fine sediment infiltration rates are difficult to generalize mostly due to different measurement methodologies (Sear et al., 2008). Hence, there is a need to compare methodologies as well as data on sediment input and riverbed clogging to achieve a better comparability of results from different studies and to increase knowledge on the interaction between fine sediment dynamics and fine sediment infiltration and accumulation (Scheurer et al., 2009). The aim of this study was to (i) compare results obtained by different methods used to capture temporal and spatial dynamics of suspended sediment and fine sediment infiltration and accumulation, (ii) test their suitability for a river in the Swiss Plateau, (iii) compare the results with hydrological data, and (iv) compare the results with literature data. Because these questions are crucial for gravel spawning salmonid embryos, the study was conducted in artificial redds.

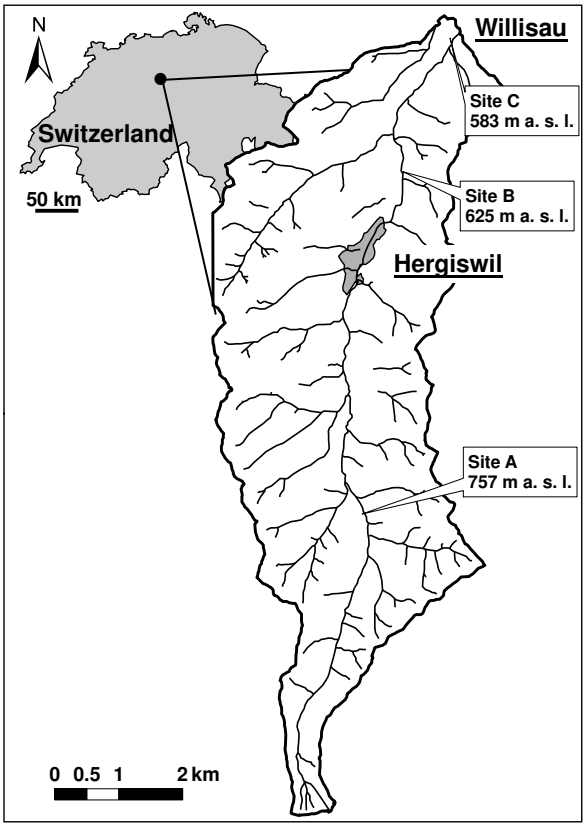

Fig. 1. Watershed of the river Enziwigger with the three field sites A, B and C and the towns Willisau and Hergiswil (Canton of Lucerne, Switzerland).

2 Materials and methods

2.1 Study site and general setup

The river Enziwigger is a small canalized river located near Willisau (Canton of Lucerne, Switzerland) with a total watershed area of about 31 km^2 (Fig. 1). The flow regime of the Enziwigger is not affected by hydro-power and no waste water treatment plant is located above Willisau. Like most rivers in the Swiss Plateau, its morphology is strongly modified: only 5 % of the ecomorphology is close to natural or natural, 21 % is little affected and 74 % is strongly affected or even artificial, including terraces that have been inserted to prevent deep channel erosion and scouring of the bed during flood events (classified with the Swiss modular stepwise procedure for ecomorphology after Hütte and Niederhauser (1998) (EBP-WSB-Agrofutura, 2005)). In spite of these strong modifications its biological condition (classified with the macrozoobenthos module of the Swiss modular stepwise procedure (Stucki, 2010)) is considered good (EBP-WSB-Agrofutura, 2005). The only fish species in the Enziwigger is the brown trout, *Salmo trutta* (EBP-WSB-Agrofutura, 2005).

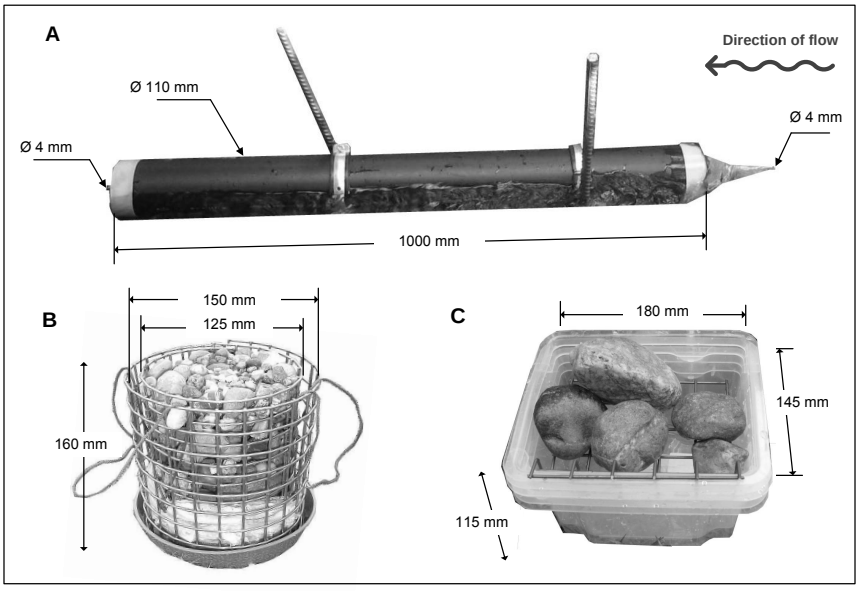

Fig. 2. Devices used to measure fine sediment dynamics in the redds. **(A)**: suspended sediment sampler, **(B)**: sediment infiltration/accumulation basket, **(C)**: bedload trap.

The bedrock of the watershed consists of Upper Freshwater Molasse. The soil types are mainly (stagnic) Cambisol and Leptosol (classified according to WRB; IUSS, 2006). The mean annual temperature in Willisau is 8.5 °C, with a mean annual rainfall of 1050 mm. Mean annual rainfall on the peak of the mountain Napf, where the river Enziwigger originates, is 1700 mm per year (1961–2007; data from MeteoSwiss). Discharge was measured in Willisau from November 2007 until November 2008 by the Canton of Lucerne. Mean discharge was 2.1 $m^3 s^{-1}$, minimum discharge was 1.1 $m^3 s^{-1}$, and maximum 10.1 $m^3 s^{-1}$.

Measurements were set up in artificial salmonid redds located at three experimental sites along the river named A, B and C (from up- to downstream; Fig. 1) at altitudes of 757, 625 and 583 m above sea level (for site characteristics see Table 1). Each site was equipped with six artificial redds in places where natural brown trout redds had been mapped in November 2008. The locations of the redds are mostly consistent over years (Philip Amrein, fish warden of the Canton of Lucerne, personal communication, 2009). Data were assessed during two spawning seasons (Season 1: November 2009 to end of March 2010; Season 2: November 2010 to end of March 2011) in 18 artificial redds per year ($n_{tot} = 36$).

2.2 OBS sensors and time integrated samplers to measure suspended sediment

Turbidity was measured continuously every 15 s during both field periods at each site with one optical back scatter (OBS) probe (Campbell Scientific, OBS-3+). The median from 40 measurements was logged every 10 min. The probes were mounted about 5 cm above the riverbed (about 20 cm depth during baseflow conditions). To calibrate the nephelometric turbidity unit (NTU) to suspended sediment concentration (SSC_{NTU}) in mg l^{-1}, water samples were taken every seven hours with an automatic water sampler (ISCO 6700, Isco Inc., USA). Because of freezing of the suction hose during the first field season, manual water samples were taken weekly during the second field season. The latter were complemented with samples collected by local habitants during storm events. Water samples were taken to the laboratory to asses the total SSC (see Sect. 2.7).

Time-integrated suspended sediment (SS) samplers following Phillips et al. (2000) were installed behind each redd and emptied at weekly intervals to determine the spatial variation of the SS load (Fig. 2a). The SS samplers were one metre long and consisted of commercially available PE pipes with an outer diameter of 110 mm and a wall thickness of 4.2 mm. They were sealed with a plugged polyethylene funnel at the inlet and a cap at the outlet. An aluminum tube with an inner diameter of 4 mm was passed through the funnel and the cap as inlet and outlet. The SS samplers were mounted parallel to the riverbed at two upright steel bars driven into the channel bed, with the inlet tube pointing directly into the direction of the flow. The greater cross-sectional area of the main cylinder compared to that of the inlet tube reduces the flow velocity within the samplers by a factor of 600 relative to that of the ambient flow. This reduction in flow velocity induces sedimentation of the SS particles as the water moves through the cylinder towards the outlet tube (Phillips et al., 2000). The SS samplers collect a statistically representative sample under field conditions (Phillips et al., 2000).

Table 1. Site characteristics: D_{50} of the riverbed sediment was defined by freeze core samples and with line-number-analyses (Fehr, 1987). Data are given as mean ± standard deviation.

Site	A	B	C
Altitude (m a.s.l.)	757	625	583
Watershed area (km^2)	5.5	22.6	28.9
Mean watershed slope (°)[a]	26.0	20.3	19.5
River slope at the site (°)[b]	5.0	1.5	1.4
River slope of riffle between 2 terraces (°)	0.27	0.24	0.23
D_{50} (freeze core) (mm)	20 ± 4	19 ± 6	16 ± 1
D_{50} (line-nr-analysis) (mm)	25 ± 8	25 ± 4	16 ± 4
Channel width (m)	3–3.5	4–4.5	4.5–5
Water depth above redds (cm)	10.9 ± 3.9	23.2 ± 6.0	20.9 ± 7.9
Step length (m)	11–15	9–12	7–10
Mean bed shear stress above redds (Pa)[c]	5.0	9.5	8.2

[a] Calculation based on the slope value for each pixel from a digital elevation model of the watershed.
[b] Based on the slope value from a digital elevation model. [c] Calculated by the reach-average bed shear stress formula: $\tau_0 = \rho g R S$, where τ_0 is bed shear stress, ρ is water density, g is acceleration due to gravity, R is hydraulic radius at mean water level and S is the slope.

2.3 Sediment baskets to measure fine sediment infiltration and accumulation

Fine sediment infiltration and accumulation was determined with sediment baskets (Fig. 2b; cf. Acornley and Sear, 1999; Heywood and Walling, 2007; Greig et al., 2005). They consisted of two baskets made of 20 × 20 mm wire mesh with 2.5 mm wire and a solid bottom. The inner baskets had a diameter of 125 mm and were 160 mm deep. They were filled with riverbed sediment >4 mm to start with initial conditions without fine sediments. A second basket with a diameter of 150 mm was dug in the riverbed as a placeholder. A polyethylene bag with two long handles was placed around the inner baskets and stuffed between the two baskets. The bag was pulled over the inner basket during sampling to prevent loss of fine sediment during removal of the inner basket.

Each redd was equipped with two sediment baskets. One of them was emptied at weekly intervals to investigate the weekly fine sediment infiltration rates (= sediment infiltration basket). The baskets' sediment was sieved with a 4 mm sieve and refilled with the same sediment during each sampling event. Sediment <4 mm was taken to the laboratory for grain size analyses. The second set of sediment baskets was emptied only at the end of the spawning season to asses the total net accumulation of fine sediment during the incubation period (= accumulation basket; Sear et al., 2008). During Season 1 (2009/2010) 10 of the initial 18 accumulation baskets were washed away at high flow. Two accumulation baskets in each redd were, therefore, installed during Season 2 (2010/2011).

2.4 Bedload traps to measure sediment transported along the bed

The volume of the described sediment infiltration baskets is small and most of the space within the trap is taken up by coarse bed material. Thus, these baskets can fill very quickly in situations where sediment loads are high, resulting in an underestimation of the sediment infiltration rate (Bond, 2002). Bedload traps similar to Bond (2002) were designed to overcome this problem. They consisted of two nestable 180 × 145 × 115 mm dug boxes with a 25 × 25 mm wire lid, above which coarse bed material was placed to avoid resuspension of the settled material in the trap (Fig. 2c). To empty the box, it was covered by a lid and the inner box was removed. The coarse bed material above the trap caused turbulence; consequently, part of the settled fine sediment might not be material transported as bedload, but also as suspension. We still call the described traps "bedload samplers" to clearly distinct them from the sediment infiltration baskets and to use the same nomenclature as Bond (2002). During the first field season, each redd was equipped with one bedload trap, which was emptied weekly. No bedload traps were installed during the second field season.

2.5 Hydraulic conditions

The temporal dynamic of the water level at the three sites was measured every 15 s during both seasons with pressure transmitter probes (STS, Sensor Technik Sirnach, Switzerland). Average values were logged at 10 min intervals. The water level above each redd was measured weekly to assess its spatial and temporal variability within a site.

The vertical hydraulic gradient in the redds was measured weekly within mini piezometers designed after Baxter et al. (2003) and installed in the pit and tail of each redd.

The piezometers had a length of 300 mm and consisted of a 25 mm diameter polypropylene (PP) pipe with an inner diameter of 21.4 mm. They were perforated with approximately 30 evenly spaced holes in the lower 160 mm and plugged at the bottom. The vertical hydraulic gradient is a unitless measure that is positive under upwelling conditions and negative under downwelling condition. It is calculated by the formula

$$\mathrm{VHG} = \Delta h / \Delta l \quad (1)$$

where VHG is the vertical hydraulic gradient, Δh is the difference in head between the water level in the piezometer and the level of the stream surface and Δl is the depth from the streambed surface to the first opening in the piezometer sidewall (Baxter et al., 2003).

2.6 Freeze core samples

Freeze core samples were taken with a copped and plugged 400 mm diameter steel pipe. The pipe was pounded in the river sediment to a depth of approximately 350 mm and filled with liquid nitrogen. Freeze cores with a length of roughly 350 mm and a diameter of about 150 mm were removed and divided vertically in 100 mm wide layers. Sediment from the cores was taken to the laboratory, dried and sieved.

2.7 Sample analyses

The grain size distributions of the sediments were determined with the standardized sieve technique using sieves of different mesh sizes. Grains with a diameter <32 µm were measured with a sedigraph (Micrometrics 100, Coulter Electronics, Germany). Grain size fractions were named according to the German soil taxonomy: Sand: 63 µm–2 mm, silt: 2 µm–63 µm and clay: <2 µm (Sponagel et al., 2005). Water samples for determination of suspended sediment concentrations were filtered through pre-weighed Whatman-filters with 11 µm pore diameter, dried at 40 °C and weighed. Organic carbon concentration was measured with a CHN-Analyzer (Leco, USA).

3 Results and discussion

3.1 Suspended sediment

3.1.1 Turbidity measured by optical backscatter sensors

The calibration of NTU values to suspended sediment concentration (SSC_{NTU}) was difficult and associated with a high variance (Fig. 3). The calibration curve has an offset to the zero-point, indicating a systematic measurement error. Some general statements, however, were possible: SSC_{NTU} varied at all sites between 2 and 10 mg l^{-1} during low flow conditions and increased at high flow ranging from around 150 mg l^{-1} (site A) to around 300 mg l^{-1} (site C). Only small

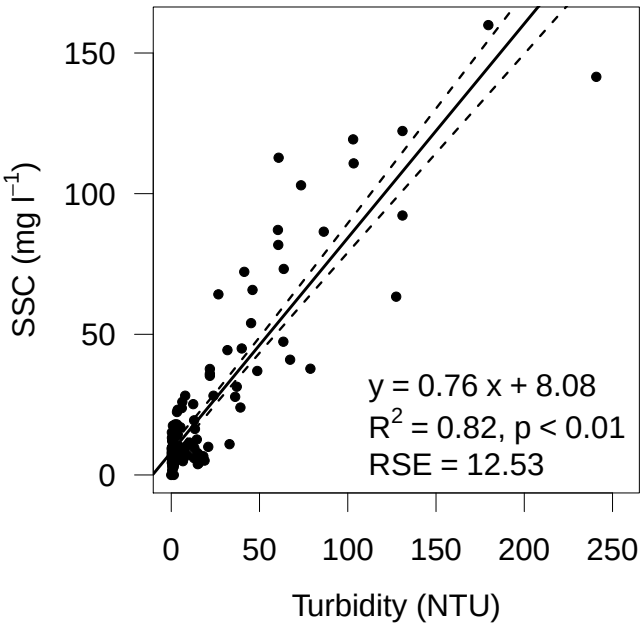

Fig. 3. Correlation between turbidity in NTU and suspended sediment concentration (SSC). Dashed lines are the 95 % confidence intervals; RSE = residual standard error (degree of freedom = 154)

floods occurred during the second field season, resulting in significant smaller mean SSC_{NTU} at all sites with an overall mean of 17.0 mg l^{-1} compared to an overall mean of 42.7 mg l^{-1} during the first season (t-test, $p < 0.01$; Table 2). SSC_{NTU} increased significantly from upstream (site A) to the two downstream sites (B and C) during both seasons (ANOVA, $p < 0.01$; Table 2). The high mean SSC_{NTU} at site B for the second season might partly be related to measurement artifacts since the OBS sensor at this site was often shielded by leaves. Even though obvious outliers were excluded from the dataset, many high value data points remained in the dataset. These values could not be excluded with certainty, but might still be influenced by measurement artifacts.

The high temporal resolution in SSC_{NTU} data is an advantage of the OBS sensors. SSC_{NTU} increased rapidly with increasing water level at all sites and there is evidence of sediment exhaustion during the falling limb of flood events (Fig. 4). The observed simultaneous peaks in discharge and SSC_{NTU} correspond to the remobilization and transport of in-channel sediments (e.g. Duvert et al., 2010). Although OBS sensors are widely used for turbidity measurements, their handling is an often underestimated problem (for a review see Downing, 2006). The most frequent problems with OBS sensors are their signal dependence on grain size distribution and on sediment composition (shape of particles) as well as algal growth on the sensor windows (Downing, 2006; Minella et al., 2008; Packman et al., 1999). An infinite number of combinations of sediment characteristics,

Table 2. Mean and standard deviation of suspended sediment concentration (SSC_{NTU}) measured with the OBS sensors and suspended sediment (SS) load caught by suspended sediment samplers at the three sites during the two field seasons.

	Field season 1 (2009/2010)		Field season 2 (2010/2011)	
Site	SSC_{NTU} (mg l^{-1})	SS (g week^{-1})	SSC_{NTU} (mg l^{-1})	SS (g week^{-1})
A	28.0 ± 37.8**	14.4 ± 3.5*	12.9 ± 7.6**	7.0 ± 1.7**
B	49.1 ± 56.5	16.8 ± 3.3	21.4 ± 12.8*	11.5 ± 0.4*
C	54.9 ± 62.8*	20.3 ± 2.5**	16.2 ± 23.3	11.2 ± 0.5
mean	42.7 ± 53.3	17.2 ± 3.9	17.0 ± 16.5	9.9 ± 2.3

Differences between seasons are highly significant for all sites and both measurement devices (t-test, $p < 0.01$).
* Differs significantly from the two other sites (ANOVA, $p < 0.05$). ** Differs highly significantly from the two other sites (ANOVA, $p < 0.01$).

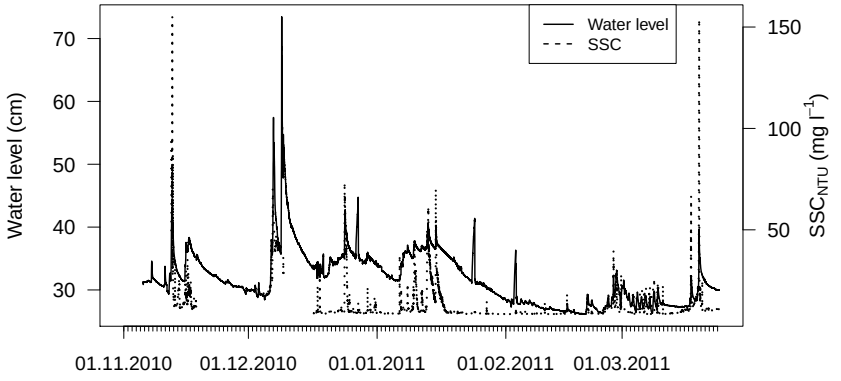

Fig. 4. Example of the temporal variation of the suspended sediment concentration (SSC) and water level (Site A, Season 1).

including size, shape, mineral compositions and surface texture, is possible. Each combination produces a unique signal and each metre has a unique emitter-detector geometry that samples the signal in a particular way (Downing, 2006). NTU is consequently an arbitrary unit, incomparable to NTU measured at other times and places or with different turbidity metres (Downing, 2006). A calibration of NTU to SSC_{NTU} in mg l^{-1} is necessary for the comparison to other studies. Measurement uncertainty is, however, introduced into the SSC_{NTU} data when converting NTU to SSC_{NTU} (Downing, 2006; Navratil et al., 2011).

Several problems with the OBS sensors were observed during the two field seasons. Drifting leaves were caught by the sensors in the fall months, resulting in abnormally high NTU values. This was particularly the case at site B during Season 2. More frequent checks at the field site, similar to the SS samplers (see Sect. 3.1.2), could partly counterbalance this shortcoming. Moreover, freezing of the suction hose of the ISCO samplers during the winter interrupted sediment concentration sampling. Regular sediment concentration samples are, however, necessary for a good calibration. Finally, the D_{50} of the SS (50th percentile grain size diameter; data assessed by SS samplers, see Sect. 3.1.2) fluctuated strongly during the field season with a minimum of 6.7 µm at low flow with low SSC_{NTU} and a maximum of 110.5 µm at high flow associated with high SSC_{NTU} (Fig. 5). The large effect of the change in grain size distribution on the OBS signal has been documented in numerous studies (for a review see Downing, 2006). Organic carbon concentrations of the suspended sediment were also highly variable with minimum values around 1.5 % at high flow and maximum values around 10.5 % at low flow. This change in organic carbon concentrations has again an influence on the conversion of NTU to SSC_{NTU} values (Downing, 2006).

3.1.2 Suspended sediment samplers

Results from the SS samplers paralleled the observations with the OBS sensors, showing significant higher SS loads during the first season than the second season (t-test, $p < 0.01$) and a SS increase from upstream to downstream (ANOVA, $p < 0.01$; Table 2). The SS loads captured during one week by the six SS samplers per site varied highly with coefficients of variation between 12 and 100 %. This might represent the well known variation in suspended sediment concentration through the cross-section of rivers (Horowitz et al., 1990; Spreafico et al., 2005). The D_{50} of the SS varied highly across the channel and with time, again representing the variation of SS within a river both with low and high mean SS concentrations in the water column (Fig. 5).

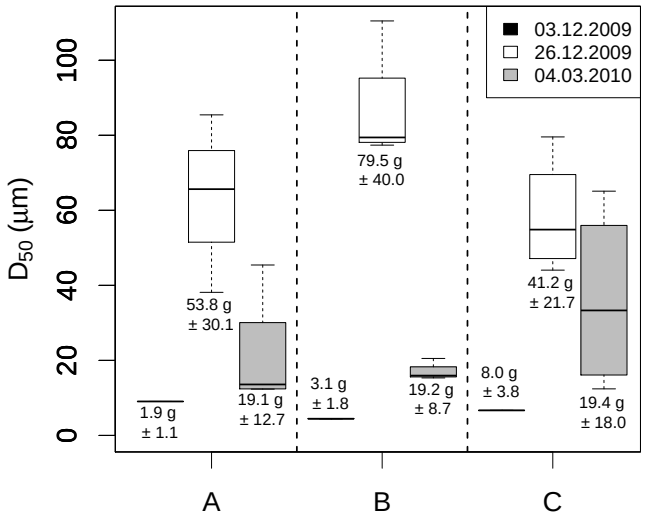

Fig. 5. Weekly D_{50} of the suspended sediment (SS) caught by the SS samplers ($n = 6$/site) during three weeks at the three sites A, B and C. Mean total amount of SS load ± standard deviation is given below/above the boxes. The 6 samples of 3 December 2009 had to be merged for grain size analysis because of the small quantity of SS.

The described differences could also partly be attributable to instrumental biases.

The deposited sediments can be retained for further analyses of their composition, which is a major advantage of the SS samplers. In addition, the SS samplers can be installed in a relatively dense sampling network because they are inexpensive and easily fabricated. An installation at specific test sites, for example behind individual artificial redds, is possible. Thus, they can provide information about cross-sectional differences of SS loads and about the SS load at a specific test locations. Problems of this method include clogging of the inlet with leaves and the difficulty of placing the samplers horizontally with the inlet tube directly pointing into the flow direction. Consequently, distinguishing instrumental and sampling errors from spatial and temporal heterogeneity can be difficult. It is, therefore, suggested to closely monitor the samplers to ensure their proper performance, especially during fall when a large number of leaves drift in the river.

3.2 Sediment infiltration

A strong temporal variation of fine sediment infiltration with values ranging between $0.01\,\mathrm{kg\,m^{-2}\,d^{-1}}$ during low flow conditions and $10.36\,\mathrm{kg\,m^{-2}\,d^{-1}}$ during peak discharge was found (Table 3). Our results confirm the conclusions of previous field studies showing maximum fine sediment infiltration during peak discharge when sediment transport is high (Soulsby et al., 2001; Zimmermann and Lapointe, 2005; Acornley and Sear, 1999; Greig et al., 2005). At all sites, an exponential increase in sediment infiltration with increasing water level was found. Sediment infiltration rates below a certain water level threshold (site A: about 15 cm, site B and C: about 25 cm) were very small (Fig. 6). At site B and C, sediment infiltration reached a maximum at a water level around 45 cm. This indicates a saturation or equilibrium of input and scouring at higher water level. This equilibrium was never reached at site A, most likely due to an overall lower water level and, therefore, less scouring (Table 1, Fig. 6).

The sediment infiltration baskets were not filled with homogeneous gravel, but with riverbed gravel collected during redd construction; consequently, the D_{50} (27.1 ± 2.1 mm; note here and in the following all values are given as mean ± sd) as well as the sorting coefficient ($\mathrm{SO} = (D_{75}/D_{25})^{0.5}$; 1.6 ± 0.1) among the cleaned sediment baskets differed. Spearman rank correlation tests showed, however, that these differences had no influence on the amount of fine sediment infiltration ($p = 0.5$ and 0.2 respectively).

Sediment infiltration rates during the first season were significantly higher at all sites with a mean of $1.54 \pm 0.24\,\mathrm{kg\,m^2\,day^{-1}}$ compared to the second season with a mean of $0.74 \pm 0.21\,\mathrm{kg\,m^2\,day^{-1}}$ (t-test, $p < 0.01$). This is attributable to numerous high flows during the first field season. No significant differences of infiltration rates between the three sites were found (ANOVA, $p < 0.3$–0.8). The variation of sediment infiltration rates among infiltration baskets at each site was very high with coefficients of variation up to 100 % (Table 4). The most likely explanation for these differences is the cross-channel variation due to the differences in flow velocity caused by bank roughness effects (Acornley and Sear, 1999).

Overall, the observed sediment infiltration rates are relatively high compared to other sediment infiltration studies conducted with sediment baskets (Table 3). These high sediment infiltration rates can partly be explained by the high input of fine sediment from the molasse bedrock in the catchment. Furthermore, the sampling was conducted at a higher frequency than in the other studies (Table 3). The efficiency of newly cleaned gravel in trapping fine sediments is at its maximum with initial conditions and decreases with time (Heywood and Walling, 2007). Hence, weekly sampled sediment baskets will yield higher daily means compared to monthly sampled baskets. Additionally, part of the deposited sediments might be washed out again. The difference between unequal sampling intervals can also be seen in the large discrepancy between the sediment infiltration rates calculated from the weekly obtained sediment infiltration data and those calculated from the accumulation baskets, which were only sampled at the end of the seasons after four months (Table 3). As such, quantitative comparisons of sediment infiltration rates from studies with different sampling intervals have to be done with caution.

Grain size analyses showed an increase of silt and clay with increasing fine sediment infiltration in absolute values

Table 3. Range or mean ± standard deviation of infiltration rate (IR) of sediment < 2 mm in permeable sediment baskets.

Reference	Study site	IR ($kg\,m^{-2}\,d^{-1}$)	Sampling interval
This study	River Enziwigger, Lucerne, Switzerland	0.01–10.36	weekly
This study	River Enziwigger, Lucerne, Switzerland	0.21–0.70	4 month
Acornley and Sear (1999)	River Test, Hampshire, UK	0.02–1.00	monthly
Acornley and Sear (1999)	Wallop Brook, UK	0.04–0.40	monthly
Sear (1993)	North Tyne, Northumberland, UK	0.005–1.60	monthly
Seydell et al. (2009)	River Lahn, near Marburg, Germany	0.16 ± 0.07	two weeks interval
Zimmermann and Lapointe (2005)	Cascapédia River, Québec, Canada	0.006–6.80	after suspension event

Table 4. Mean and range of daily sediment <2 mm infiltration rate (IR) during the two seasons at the three sites and of the coefficient of variation (CV) of the weekly values within the six samplers per site.

	Field season 1 (2009/2010)		Field season 2 (2010/2011)	
Site	IR ($kg\,m^2\,day^{-1}$)	CV (%)	IR ($kg\,m^2\,day^{-1}$)	CV (%)
A	1.67 (0.02–10.36)	32.9 (7.6–58.4)	0.68 (0.02–7.57)	31.1 (10.5–67.3)
B	1.29 (0.01–8.22)	40.6 (17.2–75.1)	0.62 (0.03–5.31)	27.5 (14.7–50.0)
C	1.55 (0.06–7.46)	38.3 (0–86.4)	0.66 (0.05–7.38)	48.7 (15.5–106.1)

Differences between seasons are significant for all sites (t-test, $p < 0.01$). There are no significant differences between the sites (ANOVA).

(Fig. 7, left), but a decrease in relative values (i.e., fraction of silt and clay of the total fine sediment deposition; Fig. 7, right). During periods with low sediment infiltration rates, up to 94 % of the sediment consisted of sediment <0.25 mm; thus, sediments of a size most likely to be transported in suspension. This agrees with Acornley and Sear (1999) and Sear (1993). They found mainly sediment infiltration composed of sediments transported in suspension (<0.25 mm) during low flow and a greater proportion of sediments with a diameter between 0.25 and 4 mm during high flow. This fraction is large enough to be in intermittent contact with the bed, yet small enough to pass through small interstices of the weekly cleaned sediment infiltration baskets (Lisle, 1989).

Sediment infiltration baskets do not represent natural conditions. Sediments <4 mm is removed in a regular interval, causing an overestimation of the real capacity of sediment infiltration occurring in the undisturbed riverbed. The suitability of the infiltration baskets strongly depends on the purpose of the research question. They are a quasi standardized method to obtain spatial and temporal differences of fine sediment infiltration or to assess the time needed for siltation of a freshly cut redd. To assess sediment infiltration rates close to natural conditions, Greig et al. (2005) assessed the temporal sediment accumulation by installing multiple small, porous infiltration pots. At each time step (2 weeks), two small pots were randomly removed. This allowed them to conduct seven measurements during the spawning period. Problems with this method are the possible spatial variability among the pots and the loss of pots at high flow (see Sect. 3.3). Another measurement strategy could consist of measuring sediment infiltration from week to week without removing the fine sediment trapped in the baskets, but this also does not represent true natural conditions, since the sediment structure would get disturbed while measuring the infiltrated sediment.

3.3 Sediment accumulation

The highest values for fine sediment accumulation over the two seasons were observed at site A, the most upstream site (ANOVA, $p < 0.05$). On average, 20.1 % of the sediment basket consisted of particles <2 mm at site A, 18.7 % at site B and 13.9 % at site C (Table 5). The decrease in fine sediment accumulation downstream could be related to higher scouring of fine sediment down the stream due to the higher water level. Sediment accumulation at site B and C did not differ significantly between the two seasons (t-test, $p = 0.3$ and 0.5 respectively), despite the significant higher fine sediment infiltration during the first field season at all sites. Only at site A, significantly higher fine sediment accumulation rates were obtained during the first season (t-test, $p < 0.01$, Table 5). Consequently, downstream scouring of fine sediment seems to have a greater effect on sediment accumulation than SS load and sediment infiltration on sediment accumulation.

The sediment accumulation baskets were not filled with standardized gravel but with riverbed gravel to represent natural conditions. These differences in D_{50} as well as in sorting coefficient of the accumulation baskets had no influence on the amount of sediment accumulation (Spearman rank correlation, $p = 0.5$).

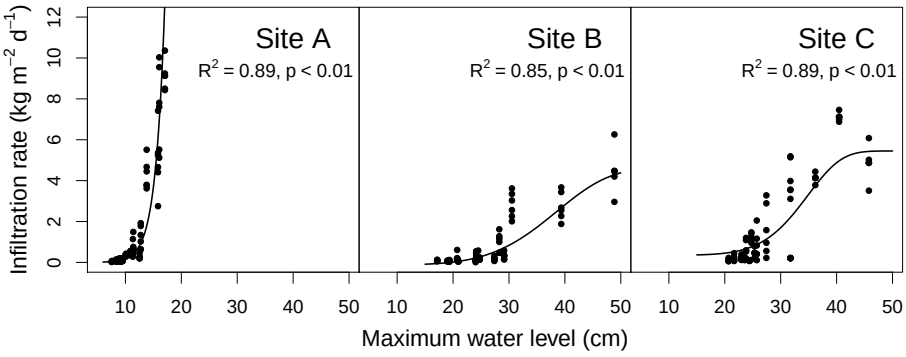

Fig. 6. Sediment infiltration rate in relation to the highest mean daily water level above the redds during the measurement week. The relationship at site B and C is described by a Weibull growth function.

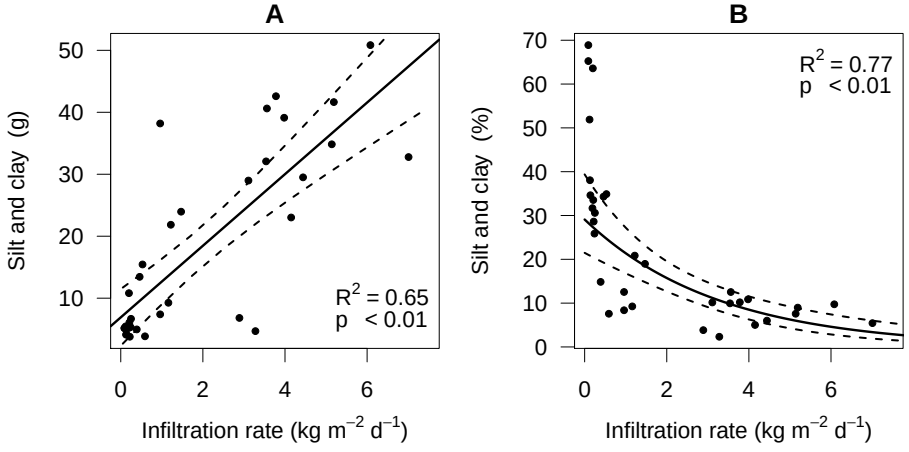

Fig. 7. Weekly silt and clay infiltration at site C in absolute values **(A)** and relative values (i.e., fraction of silt and clay of the total fine sediment deposition; **(B)**) in relation to the daily infiltration rate of sediment <2 mm. Dashed lines are the 95 % confidence intervals.

Comparisons with other studies revealed similar rates of sediment accumulation to those reported in this study (Table 6). 90 ± 2.6 % of the accumulated fine sediment was sand and 67 ± 5.6 % had a diameter >0.25 mm. Thus, the size most likely carried in suspension (<0.25 mm) accounted for only 33 % of the sediment accumulated in the sediment basket. This is in the same range as found by Lisle (1989). During high flow the main component of the infiltrated sediment is in the bedload fraction (see Sect. 3.2). This fraction is deposited and accumulated at all depths down to the bottom of the basket as long as size distributions of transported sediment and the riverbed particles do not overlap (Lisle, 1989).

The fine sediment fraction (<2 mm) in the accumulation baskets was greater than in the riverbed sediment obtained by freeze core samples (Table 5). Because of the high variation among the accumulation baskets and among the freeze core samples, the differences were only significant at site A (t-test, $p < 0.01$). According to Zimmermann et al. (2005), these differences could reflect the influence of the effective size of the pore spaces available in the substrate on sediment infiltration. The overestimation of fine sediment in the baskets in this study could also be due to the small gap of about 4 mm between the inner and the outer sediment basket, in which the fine sediment (mainly in the bedload fraction) was able to infiltrate. This gap accounts for about 13 % of the volume of the inner baskets and was entirely filled with fine sediment at the end of the spawning season. The differences could also reflect an overestimation of the coarse fraction by freeze cores since individual pieces of coarse gravel and cobbles protruding out of the freeze cores can result in a smaller percentage of fine sediment of the sample (Young et al., 1991; Zimmermann et al., 2005).

Comparisons with the freeze core samples showed significantly higher silt and clay fractions of the total fine sediment in the accumulation baskets with 7.8 to 10.5 % compared to 4.8 to 5.1 % in the freeze core samples (t-test, $p < 0.05$; Table 5). This high fraction in the accumulation baskets is probably attributable to silt and clay particles which would have infiltrated to deeper layers in a natural environment. At the beginning of the measurement campaign, the sediment in

Table 5. Mean values ± standard deviation of the fraction of fine sediment (<2 mm) in the accumulation baskets and the fraction of sediment <63 µm of the accumulated fine sediment during the two spawning seasons S1 (2009/2010) and S2 (2010/2911) and in freeze cores (FC) taken in winter 2008/2009 at the three sites.

Site	% <2 mm S1	% <2 mm S2	% <2 mm mean	% <2 mm in FC	% <63 µm S1	% <63 µm S2	% <63 µm mean	% <63 µm in FC
A	25.5 ± 1.4** ($n = 4$)	18.0 ± 3.3 ($n = 10$)	20.1 ± 4.5* ($n = 14$)	$13.6 \pm 4.1^{++}$ ($n = 6$)	8.2 ± 1.3 ($n = 4$)	11.3 ± 2.1** ($n = 10$)	10.4 ± 2.4 ($n = 14$)	$5.1 \pm 1.7^{++}$ ($n = 6$)
B	16.0 ± 4.3 ($n = 2$)	20.1 ± 4.4 ($n = 4$)	18.7 ± 4.5 ($n = 6$)	13.3 ± 4.5 ($n = 6$)	9.3 ± 2.4 ($n = 2$)	7.0 ± 1.0** ($n = 4$)	7.8 ± 1.8 ($n = 6$)	$4.8 \pm 1.1^{+}$ ($n = 6$)
C	15.4 ± 3.3 ($n = 2$)	13.1 ± 2.6 ($n = 4$)	13.9 ± 2.8* ($n = 6$)	12.5 ± 4.1 ($n = 6$)	13.8 ± 4.1* ($n = 2$)	8.9 ± 0.6 ($n = 4$)	10.5 ± 3.1 ($n = 6$)	$5.0 \pm 2.5^{+}$ ($n = 6$)

* Differs significantly from the two other sites (ANOVA, $p < 0.05$). ** Differs highly significantly from the two other sites (ANOVA, $p < 0.01$). $^{+}$ Differs significantly from the mean fraction of the accumulation baskets (t-test, $p < 0.05$). $^{++}$ Differs highly significantly from the mean fraction of the accumulation baskets (t-test, $p < 0.01$).

Table 6. Fine sediment (<2 mm) and silt and clay (<63µm) accumulation in the accumulation baskets as % of the whole baskets and the silt and clay fraction of the sediment <2 mm. Range (mean) or mean ± standard deviation.

Reference	Study site	<2 mm (%)	<63 µm (%)	<63 µm of <2 mm (%)
This study	River Enziwigger, Lucerne	9.6–26.7 (18.3)	0.9–2.4 (1.7)	6.1–16.7 (9.8)
Greig et al. (2005)	River Test and Blackwater, Hampshire	10.0, 12.2		
Greig et al. (2005)	River Ithon and Aran, Wales	28.9, 15.7		
Heywood and Walling (2007)	Avon catchment, Hampshire	1.3–17.2		31 ± 14
Levasseur et al. (2006)	Sainte Margerite River, Quebec	0.4–27 (13.2)	0.04–0.72 (0.16)	
Lisle (1989)	Coast Range of northern California			4.8–5.9
Julien and Bergeron (2006)	Sainte Margerite River, Quebec	3.3–29.2*	0.03 ± 0.02–0.41 ± 0.2	
Zimmermann and Lapointe (2005)	Cascapédia River watershed, upper reaches; Québec	3.5–10		4–9

* Sediment <1 mm.

the sediment baskets is comparable to a freshly cut redd. This cleaned gravel is vulnerable to deep infiltration by fines before a seal is formed during entrainment of the armor layer (Lisle, 1989). Sediments can only infiltrate to the bottom of the baskets. Freeze core data support this assumption indicating a significant higher silt and clay fraction at a depth of 10–20 cm and 20–30 cm with silt and clay content of 6.0 ± 2.0 % and 6.3 ± 2.5 %, respectively, compared to the upper layer (0–10 cm) with a silt and clay content of 3.6 ± 2.4 % (ANOVA, $p < 0.01$). The fraction of particles <63 µm of the accumulated sediment within a site and between the two seasons varied greatly (Table 5). Therefore, no general conclusions concerning the differences between the three sites and the two seasons can be drawn. The hydraulic differences within a site and the forming of a surface seal of sand (Lisle, 1989) influences the deposition and accumulation of silt and clay particles probably to a larger extent than their abundance in the water column. Silt and clay fractions assessed in other studies were also highly variable, making a comparison difficult (Table 6).

3.4 Fine sediment transported along the bed

Mean sediment caught by the bedload samplers increased along the river from $1.93\,kg\,m^2\,d^{-1}$ at site A to $2.24\,kg\,m^2\,d^{-1}$ at site C (Table 7). This pattern parallels the data from the SS samplers and OBS sensors and could be related to an increasing shear stress attributable to higher water levels down the stream or/and to a higher input of fine sediments from the arable corn fields in the lower part of the catchment.

Table 7. Mean and range of daily bedload (BL) <2 mm, of the percentage of BL < 2 mm of the total BL and of the coefficient of variation (CV) of the weekly values within the six samplers at the three sites.

Site	BL < 2 mm (kg m^2 d^{-1})	CV (%)	% < 2 mm of BL	CV % < 2 mm of BL (%)
A	1.93 (0.02–14.26)	72.0 (10.7–193.4)	73.8 (32.2–98.3)**	45.0 (0–86.6)
B	2.01 (0.01–10.80)	79.8 (0–183.2)	30.3 (4.0–60.6)*	64.2 (24.3–96.3)
C	2.24 (0.02–8.5)	61.9 (0–178.2)	58.7 (23.7–92.5)	27.5 (0–62.8)

* Differs significantly from the two other sites (ANOVA, $p < 0.05$). ** Differs highly significantly from the two other sites (ANOVA, $p < 0.01$).

At all sites, bedload rates were very small until a certain water level (data not shown). Above this level, bedload increased exponentially with increasing water level. This matches the pattern we found with the sediment infiltration baskets (Fig. 6).

The percentage of fines in the total captured bedload was highest at site A (ANOVA, $p < 0.01$). This is probably also attributable to the low water level compared to the other two sites and the relatively small slope due to the frequent artificial terraces, resulting in lower shear stress (Table 1). The fraction of the bedload smaller than 2 mm decreased with higher water level and total bedload (Spearman rank correlation, $p < 0.01$; Table 8).

Bedload rates and the percentage of fine sediment of the total bedload caught by the six bedload samplers varied highly per site (Table 7). This variations can be partly accounted for by cross-channel differences also observed in the SS load, sediment infiltration and accumulation data. The higher coefficients of variation of the bedload data compared to the other methods is likely explained by (i) the variation in precision in placing the traps flush with the sediment surface. If the border of the trap is not flush with the bed, fine sediment transported along the bed was not trapped and (ii) the turbulence caused by the coarse bed material above the trap, which differs between traps and triggers different trapping efficiencies.

In total 26 bedload traps were lost at the 18 research plots during the first field season. Thus, on average, at every sampling spot the traps were lost 1.5 times. Hence, we found that a major disadvantage of the bedload samplers is their big contact surface, making them more susceptible to scouring at high discharge. For those reasons, no bedload traps were installed during the second field season.

3.5 Comparison of the different methods

3.5.1 SS samplers and OBS turbidity sensors

Results clearly suggest that both the SS samplers and the OBS turbidity sensors are suitable to capture large scale spatial and temporal variations in suspended sediment concentrations or loads (Table 2). The two methods revealed a significant increase in suspended sediment (both SS load and SS concentration) along the river and significantly higher suspended sediment in the 2009/1010 season than the 2010/2011 season. Similarly, both methods showed a significantly positive Spearman correlation between SS and water level (Table 8). The weak correlation between SSC_{NTU} and water level at site B was probably related to measurement problems with the OBS sensor due to leaves caught by the sensor (see Sect. 3.1.1). Even though the methods differ in their quantitative results, correlation analysis showed a significant correlation between SS load caught by the sampler during one week and the average SSC_{NTU} that week (Table 8).

The advantage of the SS samplers is their relatively low cost and their easy mounting, making a high spatial distribution across the channel possible. Therefore, the correlation between SS loads obtained with the SS samplers and the sediment infiltration rate was better than the correlation between SSC_{NTU} assessed by OBS sensors and the sediment infiltration rates (Table 8). SS sampler data are positively correlated to fine sediment infiltration (Table 8, Fig. 8). At a deposition of about 40 kg m^{-2}, saturation or equilibrium of input and scouring was reached at site B and C. At site A, deposition increased until about 60 kg m^{-2}. This can be explained by less scouring at site A attributable to lower water levels. OBS data are only weakly correlated with sediment infiltration (Table 8) probably due to the cross channel differences in SS or measurement biases discussed earlier. Cross channel differences cannot be assessed with only one point measurement of SSC_{NTU} per site. Certainly, a higher cross channel resolution could also be obtained by mounting multiple OBS sensors across the channel. But the installation of the sensors across the channel could be quite difficult and would also be connected with high costs.

3.5.2 Sediment infiltration baskets and bedload traps

Fine sediment infiltration rates measured with the sediment baskets correlated significantly with sediment transported as bedload measured with the bedload traps (Table 8). A nonlinear regression (saturation curve) describes the observed relationship best (Fig. 9, left). Values above a sediment infiltration rate of 2 kg m^{-2} d^{-1} are highly variable, probably attributable to higher scouring. While sediment infiltration baskets reach a saturation around 10 kg m^{-2} d^{-1}, bedload traps can capture sediments until about 15 kg m^{-2} d^{-1} because of their large volume (Fig. 9, left). Consequently,

Table 8. Spearman rank correlation coefficients (ρ) between the measured parameters of both seasons for the three sites with mean weekly SSC_{NTU} measured with OBS sensors, total weekly suspended sediment (SS) load measured by SS samplers, daily fine sediment infiltration rate, fine sediment accumulation, daily bedload of fine sediment, the percentage of fine sediment of the total bedload, highest mean daily water level of a week and vertical hydraulic gradient (VHG). The accumulation baskets were correlated with the mean values of the parameters during the whole field seasons. The sample size (n) is given in parentheses.

	SS	Infiltration	Accu.	Bedload	Bedload (%)	Water level	VHG
SSC_{NTU}	0.8 (212)**	0.8 (218)**	0.8 (14)**	0.7 (104)**	−0.4 (104)**	0.7 (196)**	0.2 (131)*
($mg\,l^{-1}$)	0.2 (204)*	0.2 (204)**	0.2 (6)	−0.4 (96)*	0.1 (96)	0.2 (204)**	0.1 (121)
	0.7 (204)**	0.5 (204)**	0.6 (6)	0.8 (90)**	−0.5 (90)**	0.9 (180)**	0.2 (90)
SS		0.9 (212)**	0.8 (14)**	0.8 (104)**	−0.4 (104)**	0.6 (212)**	0.3 (131)**
($g\,week^{-1}$)		0.9 (204)**	−0.4 (6)	0.8 (96)**	−0.4 (96)**	0.8 (204)**	0.4 (121)**
		0.8 (204)**	0.8 (6)	0.8 (90)**	−0.5 (90)**	0.8 (204)**	0.2 (90)
Infiltration			0.6 (14)*	0.9 (104)**	−0.4 (104)**	0.7 (218)**	0.3 (131)**
($kg\,m^2\,d^{-1}$)			0.0 (6)	0.8 (96)**	−0.5 (96)**	0.8 (204)**	0.4 (121)**
			0.3 (6)	0.9 (90)**	−0.6 (90)**	0.6 (204)**	0.0 (90)
Accu.				– (4)	– (4)	−0.6 (14)*	−0.3 (14)
(% <2 mm)				– (2)	– (2)	0.1 (6)	−0.6 (6)
				– (2)	– (2)	−0.2 (6)	−0.1 (6)
Bedload					−0.4 (104)**	0.9 (104)**	0.2 (57)
($kg\,m^2\,d^{-1}$)					−0.4 (96)**	0.7 (96)**	0.2 (39)
					−0.6 (90)**	0.8 (90)**	0.0 (35)
Bedload						−0.5 (104)**	0.1 (57)
(% < 2mm)						−0.2 (96)*	0.1 (39)
						−0.6 (90)**	−0.2 (35)
Water level							0.1 (131)
(cm)							0.4 (121)**
							0.2 (90)

* $p < 0.05$. ** $p < 0.01$.

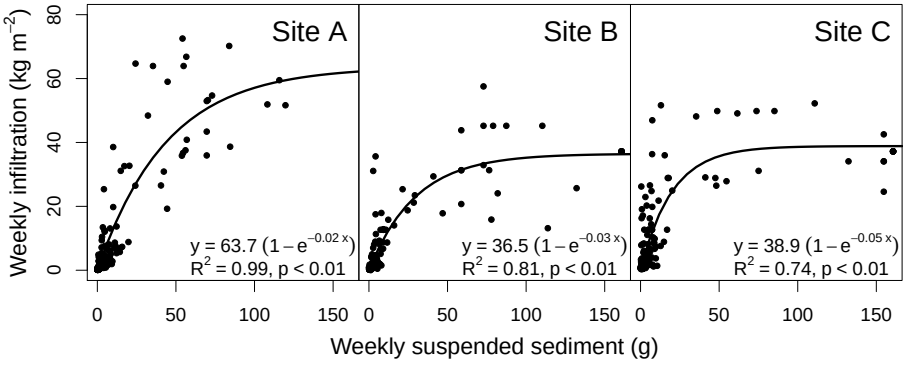

Fig. 8. Weekly sediment infiltration in relation to the total weekly SS load assessed with SS samplers at the three sites. R^2 and p of the nonlinear regressions were calculated after Zuur et al. (2009).

sediment infiltration baskets are filled very quickly at high water level if not emptied in short enough intervals.

Results suggest a linear relationship of the data with smaller sediment infiltration rates (0 to $2\,kg\,m^{-2}\,d^{-1}$; Fig. 9, right). According to Bond (2002), sediment infiltration is governed primarily by sediment supply or transport rates until the point when interstitial spaces become clogged with fine sediments. The data from this study support this statement qualitatively (see Sect. 3.2 and the highly significant correlation between both water level and SS load with sediment infiltration rates as well as with bedload rates, Table 8). The sediment infiltration rates are, however, almost

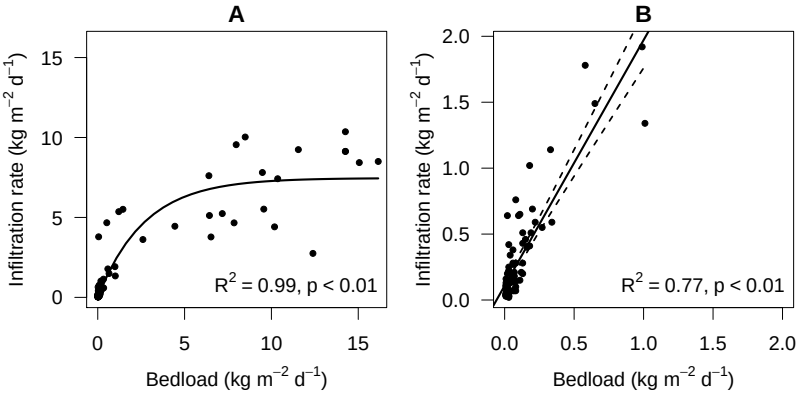

Fig. 9. Relationship between infiltration rate of fine sediment measured with sediment baskets and bedload measured with bedload traps at site A. **(A)**: all data with a nonlinear regression line, R^2 and p were calculated after Zuur et al. (2009); **(B)**: linear regression for data with infiltration rates smaller 2 kg m^{-2} d^{-1}, dashed lines are the 95 % confidence intervals.

twice the bedload until the mentioned level at a sediment infiltration rate of 2 kg m^{-2} d^{-1} (Fig. 9, right). This is probably attributable to an instrumental bias of the bedload samplers. According to Bond (2002), trapping efficiency of the bedload traps is lower for the silt and fine sand (<0.25 mm) fractions (only 20–40 % at some sites). During low sediment infiltration rates, this fraction accounted for most of the infiltrated sediments (Fig. 7). In contrasts to the sediment infiltration baskets, where infiltrated sediments are caught in a matrix of coarse sediment, fines can be easier washed out of bedload traps. A closer assessment of the differences in trapping efficiency of the sediment infiltration baskets and the bedload traps can only be accomplished under well defined conditions in a stream channel.

Sediment caught by bedload traps is mainly dependent on the water level and SS load. Because of the solid wall of the traps, the vertical hydraulic gradient has no influence on this process (Table 8). In contrast, less fine sediment infiltration was expected with a positive vertical hydraulic gradient (= upwelling) in the sediment infiltration baskets. This has been shown in previous studies (Brunke, 1999; Seydell et al., 2009). This relationship, however, was not observed in this study: at sites A and B, fine sediment infiltration was slightly higher in upwelling zones compared to downwelling zones (Table 8). This is likely attributable to the high variability of hydrological exchange processes (e.g. Brunke and Gonser, 1997). The vertical hydraulic gradient measurements represent only the specific hydraulic conditions at a certain time while sediment infiltration was measured over a week with possible changing vertical hydraulic gradients.

Multiple regression analyses for sediment infiltration as response variable with SS load (measured by SS sampler), SSC_{NTU}, bedload, water level and vertical hydraulic gradient as explanatory variables were conducted. SS load as single explanatory variable was the best predictor for sediment infiltration at all sites. Due to the equilibration or saturation of sediment infiltration, the sediment infiltration rate is best described by a nonlinear regression model (Fig. 8). As such, the strong correlation between sediment infiltration and the occurrence of fine sediment in the water column found in other studies (Acornley and Sear, 1999; Zimmermann and Lapointe, 2005) could be confirmed.

3.5.3 Sediment accumulation baskets

Only a small number of accumulation baskets resisted the flood; consequently, only a small data set across the two field seasons was available (site A: $n = 14$, site B: $n = 6$, site C: $n = 6$). This makes statistical analyses difficult and only general conclusions concerning the net fine sediment accumulation are possible (see Sect. 3.3). Fine sediment accumulation decreased from upstream to downstream, i.e., from site A to site C. In contrast, SS concentration and load and bedload increased from upstream to downstream, while fine sediment infiltration did not differ significantly between the sites. This is probably attributable to the higher resuspension and scouring of fines downstream due to higher water level, resulting in higher SS concentration and bedload, but smaller net sediment infiltration and accumulation.

Spearman rank analyses between sediment accumulation and other methods are only feasible at site A, which has the largest dataset. These analyses indicate a positive correlation between accumulated fine sediment and mean SS load ($\rho = 0.8$, $p < 0.01$) as well as mean infiltration of fines ($\rho = 0.6$, $p < 0.05$; Table 8).

Higher mean water levels above the accumulation baskets lead to resuspension of fine sediment, resulting in a negative Spearman rank correlation between water level and sediment accumulation ($\rho = -0.6$, $p < 0.05$; Table 8). A smaller amount of fine sediment accumulation in plots with higher water level and flow velocity compared to plots with lower water level was reported previously (e.g. Acornley and Sear, 1999; Levasseur et al., 2006). Finally, multiple linear

regression analyses with SS load and vertical hydraulic gradient (VHG) as explanatory variables indicate less sediment accumulation in upwelling zones than in downwelling zones:

$$\text{Accumulation} = 14.2 + 0.6 \times \text{SS} - 21.5 \times \text{VHG}, \quad R^2 = 0.7,\ p < 0.05 \tag{2}$$

The results of Seydell et al. (2009) support these findings. They even noted that subsurface flow patterns have a larger influence on sediment deposition than the suspended sediment concentration in the river. Due to cross correlations between the mentioned dependent variables (Table 8), other multiple regressions are not possible.

4 Conclusions

We compared different methods to capture temporal and spatial dynamics of suspended sediment (SS), fine sediment infiltration and accumulation. These methods were correlated and tested for their suitability for a river in the Swiss Plateau. A comparison to other studies as well as a cross-comparison between methods indicated a general agreement between the methods. All methods are, however, connected with some instrumental and sampling errors, which can not always be distinguished from spatial heterogeneity in the river. This calls for laboratory tests to assess the instrumental biases under controlled hydraulic and sediment conditions.

Methods to capture SS (OBS sensors and SS samplers) indicate big spatial and temporal differences in SS. OBS data have a higher temporal resolution. SS samplers can provide important information on the composition of SS and a better spatial distribution can be achieved because of their relatively low construction cost. Due to the dense sampling network the correlation between SS load collected with SS samplers and sediment infiltration rate was better than between sediment infiltration rate and SSC_{NTU} assessed by a single OBS sensor per site.

Sediment infiltration baskets are a quasi standardized method to obtain spatial and temporal differences of fine sediment infiltration but do not represent natural conditions. Sediment <4 mm are removed at regular intervals, causing an overestimation of the real capacity of sediment infiltration in an undisturbed riverbed. Sediment infiltration is mainly governed by water level and SS concentration. A major hydrological event can result in a total siltation of the sediment infiltration baskets. With higher water levels, scouring increases, resulting in a equilibrium between SS input and scouring. Bedload traps have a larger volume than sediment infiltration baskets, but they are associated with other problems as the difficulty to dig them flush into the riverbed. Furthermore, they are susceptible to scouring at high flows due to their large contact surface. We conclude that sediment infiltration baskets are better suited for a highly dynamic canalized river of the Swiss Plateau.

Sediment accumulation baskets do not assess the temporal behaviour of fine sediment infiltration but the accumulation over a certain time period. The loss of baskets at high flow generated the biggest problem associated with the accumulation baskets. They can not be renewed as their purpose is the assessment of accumulation during the entire field period. Additionally, they overestimate the fine sediment proportion. Differences in the effective size of the pore spaces, the gap between the inner and the outer sediment baskets or the solid bottom of the baskets are possible reasons for this overestimation. Less fine sediment accumulates in upwelling zones and with a higher mean water level due to scouring. Even though SS concentration and load and sediment assessed with the bedload traps increased from up- to downstream, less fine sediment accumulated downstream. This was probably due to more scouring downstream.

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References

Acornley, R. M. and Sear, D. A.: Sediment transport and siltation of brown trout (Salmo trutta L.) spawning gravels in chalk streams, Hydrol. Process., 13, 447–458, 1999.

Asselman, N. E. M., Middelkoop, H., and van Dijk, P. M.: The impact of changes in climate and land use on soil erosion, transport and deposition of suspended sediment in the River Rhine, Hydrol. Process., 17, 3225–3244, doi:10.1002/hyp.1384, 2003.

Baxter, C., Hauer, F. R., and Woessner, W. W.: Measuring groundwater-stream water exchange: New techniques for installing minipiezometers and estimating hydraulic conductivity, T. Am. Fish. Soc., 132, 493–502, 2003.

Bilotta, G. S. and Brazier, R. E.: Understanding the influence of suspended solids on water quality and aquatic biota, Water Res., 42, 2849–2861, doi:10.1016/j.watres.2008.03.018, 2008.

Bond, N. R.: A simple device for estimating rates of fine sediment transport along the bed of shallow streams, Hydrobiologia, 468, 155–161, 2002.

Brunke, M.: Colmation and depth filtration within streambeds: Retention of particles in hyporheic interstices, Int. Rev. Hydrobiol., 84, 99–117, 1999.

Brunke, M. and Gonser, T.: The ecological significance of exchange processes between rivers and groundwater, Freshw. Biol., 37, 1–33, 1997.

Bucher, R.: Feinsedimente in schweizerischen Fliessgewässern, Fischnetz-Publikation. Projekt "Netzwerk Fischrückgang Schweiz", Teilprojekt 01/07, EAWAG, Dübendorf, 2002.

Downing, J.: Twenty-five years with OBS sensors: The good, the bad, and the ugly, Cont. Shelf Res., 26, 2299–2318, doi:10.1016/j.csr.2006.07.018, 2006.

Duvert, C., Gratiot, N., Evrard, O., Navratil, O., Nemery, J., and Prat, C. and Esteves, M.: Drivers of erosion and suspended sediment transport in three headwater catchments of the Mexican Central Highlands, Geomorphology, 123, 243–256, doi:10.1016/j.geomorph.2010.07.016, 2010.

EBP-WSB-Agrofutura: Ganzheitliche Gewässerplanung im Einzugsgebiet Wiggertal, Bau- und Umwelt und Wirtschaftsdepartement des Kantons Luzern und Baudepartement des Kantons Aargau, 2005.

Fehr, R.: Einfache Bestimmung der Korngrössenverteilung von Geschiebematerial mit Hilfe der Linienzahlanalyse, Schweizer Ingenieur und Architekt, 38, 1104–1109, 1987.

Greig, S. M., Sear, D. A., and Carling, P. A.: The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management, Sci. Total Environ., 344, 241–258, doi:10.1016/j.scitotenv.2005.02.010, 2005.

Heywood, M. J. T. and Walling, D. E.: The sedimentation of salmonid spawning gravels in the Hampshire Avon catchment, UK: implications for the dissolved oxygen content of intragravel water and embryo survival, Hydrol. Process., 21, 770–788, doi:10.1002/hyp.6266, 2007.

Horowitz, A. J., Rinella, F. A., Lamothe, P., Miller, T. L., Edwards, T. K., Roche, R. L., and Rickert, D. A.: Variations in suspended sediment and associated trace-element concentrations in selected riverine cross-sections, Environ. Sci. Technol., 24, 1313–1320, doi:10.1021/es00079a003, 1990.

Hütte, M. and Niederhauser, P.: Ökomorphologie Stufe F. Methoden zur Untersuchung und Beurteilung der Fliessgwässer in der Schweiz, Bundesamt für Umwelt, Wald und Landschaft, Mitteilungen zum Gewässerschutz, 27, 1998.

IUSS: World reference base for soil resources, Tech. Rep., FAO, 2006.

Julien, H. and Bergeron, N.: Effect of fine sediment infiltration during the incubation period on Atlantic salmon (Salmo salar) embryo survival, Hydrobiologia, 563, 61–71, 2006.

Levasseur, M., Bergeron, N., Lapointe, M., and Bérubé, F.: Effects of silt and very fine sand dynamics in Atlantic salmon (Salmo salar) redds on embryo hatching success, Can. J. Fish. Aquat. Sci., 63, 1450–1459, 2006.

Lisle, T. E.: Sediment transport and resulting deposition in spawning gravels, North Coastal California, Water Resour. Res., 25, 1303–1319, 1989.

Lisle, T. E. and Lewis, J.: Effects of sediment transport on survival of salmonid embryos in a natural stream – a simulation approach, Can. J. Fish. Aquat. Sci., 49, 2337–2344, 1992.

Minella, J. P. G., Merten, G. H., Reichert, J. M., and Clarke, R. T.: Estimating suspended sediment concentrations from turbidity measurements and the calibration problem, Hydrol. Process., 22, 1819–1830, doi:10.1002/hyp.6763, 2008.

Navratil, O., Esteves, M., Legout, C., Gratiot, N., Nemery, J., Willmore, S., and Grangeon, T.: Global uncertainty analysis of suspended sediment monitoring using turbidimeter in a small mountainous river catchment, J. Hydrol., 398, 246–259, doi:10.1016/j.jhydrol.2010.12.025, 2011.

Newcombe, C. and Jensen, J.: Channel suspended sediment and fisheries: A synthesis for quantitative assessment of risk and impact, N. Am. J. Fish. Manage., 16, 693–727, 1996.

Owens, P., Batalla, R., Collins, A., Gomez, B., Hicks, D., Horowitz, A., Kondolf, G., Marden, M., Page, M., Peacock, D., Petticrew, E., Salomons, W., and Trustrum, N.: Fine-grained sediment in river systems: Environmental significance and management issues, River Res. Appl., 21, 693–717, 2005.

Packman, J., Comings, K., and Booth, D.: Using turbidity to determine total suspended solids in urbanizing streams in the puget lowlands, in: Managing change in water resources and the environment, Can. Wat. Resour. Association Annual Meeting, 158–165, Vancouver, 1999.

Phillips, J. M., Russell, M. A., and Walling, D. E.: Time-integrated sampling of fluvial suspended sediment: a simple methodology for small catchments, Hydrol. Process., 14, 2589–2602, 2000.

Schälchli, U.: Basic equations for siltation of riverbeds, J. Hydraul. Eng.-Asce, 121, 274–287, 1995.

Scheurer, K., Alewell, C., Bänninger, D., and Burkhardt-Holm, P.: Climate and land-use changes affecting river sediment and brown trout in alpine countries – a review, Environ. Sci. Pollut. R., 16, 232–242, doi:10.1007/s11356-008-0075-3, 2009.

Sear, D. A.: Fine sediment infiltration into gravel spawning beds within a regulated river experiencing floods - ecological implications for salmonids, Regul. Rivers-Res. Manage., 8, 373–390, 1993.

Sear, D. A., Frostick, L., Rollinson, G., and Lisle, T.: The significance and mechanics of fine-sediment infiltration and accumulation in gravel spawning beds, Am. Fish. Soc. Symposium, 65, 149–173, 2008.

Seydell, I., Ibisch, R., and Zanke, U.: Intrusion of suspended sediments into gravel riverbeds: influence of bed topography studied by means of field and laboratory experiments, Advanc. Limnol., 61, 67–85, 2009.

Soulsby, C., Youngson, A., Moir, H., and Malcolm, I.: Fine sediment influence on salmonid spawning habitat in a lowland agricultural stream: a preliminary assessment, Sci. Total Environ., 265, 295–307, 2001.

Sponagel, H., Grottenthaler, W., Hartmann, K.-J., Hartwich, R., Janetzko, P., Joisten, H., Kuhn, D., Sabel, K.-J., and Traidl, R.: Bodenkundliche Kartieranleitung, Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover, 2005.

Spreafico, M., Lehmann, C., Jakob, A., and Grasso, A.: Feststoffbeobachtung in der Schweiz, Tech. Rep., Bundesamt für Wasser und Geologie/Swiss Federal Office for Water and Geology, 2005.

Stucki, P.: Methoden zur Untersuchung und Beurteilung der Fliessgewässer. Makrozoobenthos Stufe F, Bundesamt für Umwelt, Bern, Umwelt-Vollzug, 1027, 2010.

Young, M., Hubert, W., and Wesche, T.: Biases associated with four stream substrate samplers, Can. J. Fish. Aquat. Sci., 48, 1882–1886, 1991.

Zimmermann, A. E. and Lapointe, M.: Sediments infiltraion traps: their use to monitor salmonid spawning habitat in headwater tributaries of the Cascapedia River, Quebec, Hydrol. Process., 19, 4161–4177, doi:10.1002/hyp.5879, 2005.

Zimmermann, A., Coulombe-Pontbriand, M., and Lapointe, M.: Biases of submerged bulk and freeze-core samples, Earth Surf. Proc. Land., 30, 1405–1417, doi:10.1002/esp.1202, 2005.

Zuur, F. A., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M.: Mixed effects models and extensions in ecology with R, Springr Science + Business Media, New York, 2009.

Chapter 5

Effects of river morphology, hydraulic gradients, and sediment deposition on water exchange and oxygen dynamics in salmonid redds

This chapter is submitted as a full paper to *Science of the Total Environment*:

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Abstract

Native salmonid populations are reported to decline worldwide. Fine sediment, decreasing gravel permeability and oxygen supply to incubating salmonid embryos is often considered as main contributing factor. However, oxygen supply also depends on hydraulic conditions driving water flow through salmonid redds. Altogether, a more generalized perspective is needed to better understand the constraints for successful salmonid incubation in the many heavily modified fluvial ecosystems of the northern hemisphere. To this end, the relative importance of hydraulic gradients, riverbed and redd morphology as well as fine sediment deposition for the temporal and spatial dynamics of dissolved oxygen (DO) concentrations and water exchange in artificial brown trout redds in a heavily modified river of the Swiss Plateau was studied. Half of the redds in the two downstream sites were excavated or buried during high flow events in early winter, while redd loss at the upstream site was substantially lower (8%). This pattern was most likely related to increasing flood heights from up- to downstream. Specific water infiltration rates (q) and DO concentrations in the redds were highly dynamic and driven on multiple temporal and spatial scales: Temporally, the high permeability of the redd gravel and the typical pit-tail structure of redds directly after redd construction, leading to high dissolved oxygen, disappeared within a month, when fine sediments had infiltrated and the redd structure was leveled out. On the scale of hours to days DO concentrations and q increased during high flows, but decreased during the falling limb of the water level, most likely related to exfiltration of oxygen depleted ground- or hyporheic water. DO concentrations also decreased under prolonged base flow conditions, when increased infiltration of silt and clay particles clogged the riverbed and reduced q. Spatially, artificial log steps affected fine sediment accumulation, q and interstitial DO in the redds. Altogether, the results demonstrate that multiple factors have to be considered for successful river management in salmonid streams, including riverbed structure and local and regional hydrogeological conditions.

Keywords: sediment infiltration, sediment accumulation, specific infiltration rate, Swiss Plateau, river modification, brown trout

Introduction

Native salmonid populations are reported to decline in Switzerland (Burkhardt-Holm and Scheurer, 2007) as well as in the United Kingdom (Youngson et al., 2002) and North America (Brown et al., 1994; Huntington et al., 1996). Habitat degradation is considered a major threat for native salmonids (e.g., Brown et al., 1994; Burkhardt-Holm and Scheurer, 2007; Gilvear et al., 2002; Hicks et al., 1991). In this regard, fine sediment (< 2 mm) deposition has been often discussed as one contributing factor (e.g., Jensen et al., 2009 and studies cited therein). Deposited fine sediment can decrease redd gravel permeability and interstitial flow (e.g., Brunke, 1999; Schälchli, 1995), which hinders oxygen supply to incubating salmonid embryos, thereby affecting their survival (Greig et al., 2007a; Greig et al., 2005; Heywood and Walling, 2007). Oxygen availability for the incubating embryo depends on several further aspects such as the relative contributions of oxygenated river water infiltration and exfiltration of oxygen depleted ground- or interstitial water in the redd (Malcolm et al., 2006; Malcolm et al., 2009) or the oxygen demand of organic material (Greig et al., 2007b). Although these factors vary extensively, both temporally and spatially (Brunke and Gonser, 1997; Greig et al., 2007b; Malcolm et al., 2006), only few studies resolved these processes on appropriate temporal and spatial scales.

Modeling approaches on the redd scale indicate that hyporheic velocities and dissolved oxygen (DO) concentrations within the egg pocket are enhanced due to the spawning activity, which led to reduced fine sediment and thus higher hydraulic conductivity (Tonina and Buffington, 2009; Zimmermann and Lapointe, 2005). Redd scale hyporheic exchange, measured on a centimeters to meter scale, can also be induced by the pit-tail structure of salmonid redds (Figure 1A,Tonina and Buffington, 2009). However, this initial structure cannot be expected to remain intact during high flow events (Ottaway et al., 1981) and hence hydraulic conditions on the redd scale likely change during the incubation season. Moreover, recent research clearly indicates the need for a multi-scale approach (Baxter and Hauer, 2000; Zimmermann and Lapointe, 2005), not only considering the redd scale in the magnitude of centimeters to meter (local scale, Figure 1A) but also river structures in the meter scale (intermediate scale, Figure 1B) as well as the transient character of surface water and groundwater flow regimes in the magnitude of tenth of meters to kilometers (regional scale, Figure 1C) when investigating the dynamics of abiotic conditions in salmonid redds. Hydraulic processes driven on all these scales can be expected to affect hyporheic exchange

in a particular redd, and hence oxygen supply to the incubating embryos (Baxter and Hauer, 2000; Malcolm et al., 2008).

In Western Europe and North America many rivers with viable salmonid populations are heavily modified, i.e. channelized and with lateral stabilizations and artificial steps introduced for slope reduction (Brookes, 1988; Gilvear et al., 2002; Wohl, 2006). In channelized rivers the lack of geomorphic features can substantially reduce hyporheic exchange (Malcolm et al., 2010), whereas hydraulic gradients related to artificial steps can markedly increase hyporheic exchange (Endreny et al., 2011). The latter usually generate predictable flow-paths, with increased river water downwelling above steps and upwelling of hyporheic water below steps (Figure 1B, e.g., Gooseff et al., 2006; Huber et al., 2013; Kasahara and Hill, 2006). Accordingly, artificial steps can increase hyporheic exchange in modified rivers (Kasahara and Hill, 2006; Sawyer et al., 2011) and hence could also affect water flow and oxygen dynamics in salmonid redds. Despite this, the effects of artificial steps on the abiotic conditions in salmonid redds have, to our knowledge, not been investigated so far. This knowledge, integrating between the sciences of hydrology, geomorphology and freshwater ecology, would provide important input for process-based river management in the many heavily modified salmonid streams of the northern hemisphere (e.g. Gilvear et al., 2002; Newson et al., 2012).

To this end, the current study evaluates the relative contribution of fine sediment, hydraulic gradients, river morphology, and regional geomorphology to specific water infiltration and oxygen dynamics in artificial brown trout redds in a heavily modified headwater river of the Swiss Plateau (Enziwigger, Canton Lucerne, Switzerland), which also maintains a viable brown trout population (Schager et al., 2007).

The objective of this study was to provide a detailed investigation of the factors affecting the abiotic redd environment in a heavily modified river including (*i*.) an investigation of fine sediment deposition, hydraulic conditions (i.e. specific infiltration q, vertical and horizontal hydraulic gradients, and water level) and their effects on oxygen dynamics in the redds, (*ii*.) an assessment of the morphological change of the riverbed and of the characteristic pit and tail structure of the redds and (*iii*.) a comparison of the measured data with the results of a groundwater flow model, which was set up for one of the three experimental sites (cf. Huber et al., 2013). In contrast to most previously published studies, data were collected with high spatial and temporal resolution (i.e. weekly or continuously) to explicitly characterize the

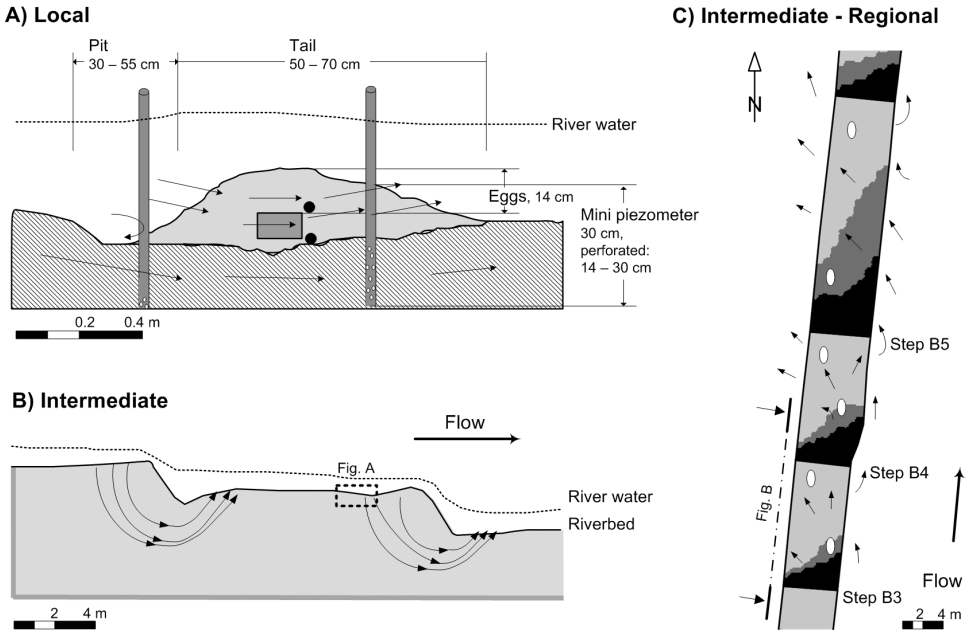

Figure 1 - Schematic view of **(A)** longitudinal section of an artificial redd (modified after Greig et al., 2007b) including the location of the mini piezometers, the egg pockets (square) and temperature probes (bullet points) with the local scale flow pattern, **(B)** the hyporheic flow on an intermediate scale induced by riverbed steps according to the model calculations of Huber et al. (2013), and **(C)** the intermediate and regional scale water exchange processes (top view). Modeled data from Huber et al. (2013). Black: only exfiltration, grey: ex- and infiltration, light grey: only infiltration. Arrows indicate the main direction of the interstitial- and groundwater flow, ovals represent the positions of the redds (for naming see Figure 2).

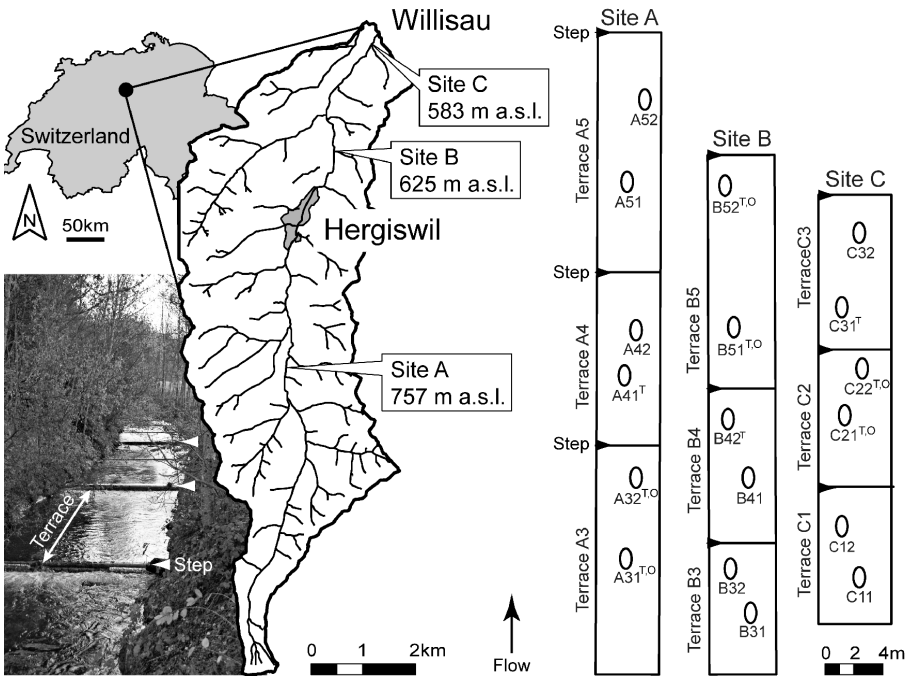

Figure 2 - Location of the Enziwigger watershed in Switzerland. The photograph shows the step and terrace structure at study site B. The watershed map of the river Enziwigger and the towns Willisau and Hergiswil (Canton of Lucerne, Switzerland) shows the locations of the three field sites A, B and C, while the schematic on the right illustrates the location of the redds within each field site. Here, superscripts indicate redds with continuous temperature (T) and oxygen (O) measurements.

temporal and spatial dynamics of the measured parameters. Together with the study of Michel et al. (submitted), which investigated the parameters affecting brown trout embryo survival in the redds, our results provide an integrated perspective on the factors affecting salmonid incubation success in comparable anthropogenically modified river environments.

Materials and methods

Study site and general setup

The river Enziwigger is a small channelized river located near Willisau, Canton of Lucerne, Switzerland with a total watershed area of about 31 km^2 (Figure 2). Mean discharge, measured in Willisau (Figure 2) by the Cantonal authorities (Nov. 2007 – Nov. 2008) was 2.1 $m^3 s^{-1}$, minimum and maximum discharge were 1.1 $m^3 s^{-1}$ and 10.1 $m^3 s^{-1}$, respectively. During the 20th century the Enziwigger was straightened, channelized and cross-channel log steps were installed as slope breakers to prevent deep channel erosion and bed-scouring during flood events (Figure 2). Like for most rivers in the Swiss Plateau its morphology is strongly modified: Only 5% is close to natural or natural, 21% is little affected and 74% is strongly affected or even artificial (classified with the Swiss modular stepwise procedure for ecomorphology after Hütte and Niederhauser, 1998; Stucki, 2010).Despite these strong modifications, its biological condition, classified with the macrozoobenthos module of the Swiss modular stepwise procedure (Stucki, 2010), is considered good (EBP-WSB-Agrofutura, 2005). The only fish species in the Enziwigger is the brown trout *Salmo trutta*, which maintains a viable population (EBP-WSB-Agrofutura, 2005; Schager et al., 2007). The flow regime of the Enziwigger is neither affected by hydro-power facilities nor by effluents from a waste water treatment plant.

Measurements were conducted at three experimental sites along the river named A, B and C (from up- to downstream; Figure 2) at altitudes from 757 to 583 m above sea level. The riverbed at all sites is stabilized with artificial log steps, which strongly affect hyporheic exchange on an intermediate scale with river water infiltration upstream of the steps and exfiltration of hyporheic water downstream of the steps (Figure 1B). At site A the bedrock beneath the riverbed lies in a depth of a few decimeters and the hydrogeologic settings are assumed to be dominated by lateral inflow or the exfiltration of ground- and/or hyporheic water. Piezometer measurements at sites B and C and groundwater flow modeling at site B

indicate on a regional scale a hydraulic gradient from the river to the main valley aquifer on the left side of the river and consequently a domination of river water infiltration (Figure 1C, Huber et al., 2013). The influence of river flow stage and transient hillside groundwater flow have a minor impact on these intermediate and regional flow patterns (Huber et al., 2013). Each site was equipped with six artificial salmonid redds in places were natural brown trout redds had been mapped in November 2008. In the Enziwigger, these locations are mostly consistent over years (P. Amrein, Fish and Wildlife Service, Canton of Lucerne, pers. com.). Data were collected during two spawning seasons (season 1 (S1): November 2009 to end of March 2010; season 2 (S2): November 2010 to end of March 2011) in 18 artificial redds per year (n = 36 redds in total). Redds are labeled after the site (A, B, C), the terraces between two steps (1-5), the redd location within a terrace (1 and 2) and the season (S1 and S2). Redd A41_S1, e.g., stands for the first redd in the fourth investigated terrace at site A during the first season (Figure 2). A detailed description of the river characteristics and field locations is given in Schindler Wildhaber et al. (2012b).

Sediment collection and analyses

Each redd was equipped with two sediment baskets to assess weekly fine sediment infiltration and net fine sediment accumulation during the entire field season (cf. Acornley and Sear, 1999; Greig et al., 2005; Heywood and Walling, 2007). One of them was emptied at weekly intervals to measure the weekly infiltration rates (= infiltration basket). The second set of sediment baskets was emptied at the end of the incubation season to measure net accumulation of fine sediment during the incubation period (= accumulation basket, Sear et al., 2008). At each site, the sediment basket data was complemented with four to seven freeze core samples to characterize the sediment stratification of the undisturbed river gravel. For a detailed description of the used baskets, the freeze core technique and their handling see Schindler Wildhaber et al. (2012b).

Grain size distribution was measured in a subsample of the freeze cores' fine sediment fraction of the layers 0–10 cm, 10–20 cm and 20–30 cm and in two subsamples of the fine sediment collected in accumulation baskets. Representative subsamples were obtained by a sample divider (Retsch, Haan, Germany). Additionally, grain size distribution of weekly infiltrated fine sediment samples (n = 80) was determined. The rest of the infiltrated fine sediment was pooled for each redd and a subsample was analyzed to obtain a mean grain size distribution of the infiltrated fine sediment. Grain size fractions were named according to the

German soil taxonomy standard (DIN EN ISO 14688-1): Sand: 0.063–2mm, silt: 0.002–0.063 mm and clay: < 0.002 mm (Sponagel et al., 2005).

Porosity (*n*) was calculated for each site on the basis of sediment grain size distributions from freeze core samples by the formula

$$n = 48.6 \cdot C_u^{-0.2} \quad (1)$$

Where $C_u = d_{60} / d_{10}$ (diameter of grain size at the 60th and 10th percentile of the cumulative sample mass) (Schälchli, 1995).

The *fredle index* of the accumulation baskets and of the freeze core samples was calculated by the formula

$$fredle\ index = \frac{d_g}{S_o} \quad (2)$$

Where d_g is the geometric mean grain size and S_o is the sorting coefficient derived by taking the square root of the quotient of the grain size at the 75th percentile divided by that at the 25th percentile (Lotspeich and Everest, 1981). The *fredle index* is a central tendency quality index of the redd gravel composition, which gets smaller with smaller permeability of the sediment.

Oxygen

Continuous oxygen measurements were conducted with Aanderaa oxygen optodes 3835 (Aanderaa data instruments, Bergen, Norway) buried at the same depth as the incubating brown trout embryos (approx. 14 cm, cf. Michel et al., submitted). Oxygen contents in mg l^{-1} as well as saturation (%) were measured every ten seconds and mean values were logged in ten minute intervals. One optode per site was installed during season 1 (redds A32, B51 and C21) and two during season 2 (Figure 2). Moreover, oxygen concentrations in each redd were measured manually every second week in mini piezometers located in the pit and tail of each redd (Figure 1A) with the PreSens oxygen dipping probe mini-sensor (PreSens Precision Sensing GmbH, Regensburg, Germany). Each manual oxygen measurement was conducted twice: once in the “old” interstitial water and once in the refluent “new” water approximately 30 minutes after the “old water” had been extracted.

Riverbed and redd morphology

Riverbed morphology was mapped in a 0.5 m horizontal resolution in season 1 shortly after redd construction (October 26th 2009) and on December 27th after several high flow events.

These data were used to assess morphological changes induced by high flow events on the river segment scale. The water depth above the deepest point of the pit and the highest point of the tail was measured weekly to quantify temporal changes of the typical redd structure.

Hydraulic investigations

Flow-stage at each site was measured every 15 seconds with pressure transmitter probes (STS, Sensor Technik Sirnach, Switzerland) and average values were logged at 10 minutes intervals during both field seasons.

Water levels above pit and tail of each redd were recorded weekly to assess its heterogeneity within sites. Vertical hydraulic gradients (VHG) in the redds were measured weekly after Baxter et al (2003) in mini piezometers installed in the pit and tail of each redd (Figure 1; for details see Schindler Wildhaber et al., 2012b). The VHG is a dimensionless parameter calculated by the formula

$$\mathrm{VHG} = \frac{\Delta h}{\Delta l} \tag{3}$$

where Δh is the difference in head between the water level in the piezometer and the level of the stream surface and Δl is the depth from the streambed surface to the first holes in the piezometer (Baxter et al., 2003). Positive values indicate an energy gradient potentially sufficient to produce upwelling and negative values indicate an energy gradient potentially sufficient to produce downwelling. In the following, it is referred to the VHG values as upwelling or downwelling processes, although they are actually only a measure of up- and downwelling potential (Baxter and Hauer, 2000). The differences in VHGs between the pit and the tail piezometer of each redd was defined as the horizontal hydraulic gradient (HHG), which is an indicator for hydraulic gradients driving water flow through the redd.

To obtain the temporal and spatial change of specific infiltration rates (q) in the redds, the one-dimensional heat pulse method was used (e.g., Hatch et al., 2006). For this, stream water temperature and temperatures at two different depths just above and below the incubating brown trout embryos (approx. 12 and 20 cm, respectively) were recorded every minute using thermocouple temperature probes (Campbell Scientific 105 E). Three redds per site were equipped with one or two temperature probes (Figure 2). In redds equipped with two temperature probes, q could be calculated for the upper part (q_u, 0 to about 12 cm), the bottom part (q_b, about 12 cm to about 20 cm) and the total part (q_t, 0 to about 20 cm). In redds with one temperature probe, q could only be assessed in the upper part. The diurnal amplitude

variations in temperature in the different depths and the diurnal phase variations were used to calculate *q*, but only the results of the former method were used for further interpretations because of their higher stability. The used method allowed calculating two specific infiltration rate values per day.

The diurnal sinusoidal alternation was filtered out of the temperature data by a discrete bandpass filter (FIR-filter with hamming-Window, 5001 filter coefficients, cut-off frequency $0.8 \cdot f_{Day}$ / $1.5 \cdot f_{Day}$,). All field temperature data were sampled with a frequency of one measurement per minute. When field sample periods exceeded one minute, e.g., due to technical problems, linear interpolation was used to fill up gaps up to 10 minutes. Data gaps exceeding 10 minutes were marked as missing and not further evaluated. Data-points with time offset between the daily minima or maxima peaks of the corresponding sinusoidal temperature curves at the different depths exceeding 20% of a day period (i.e. 288 minutes) were also removed from further processing. The resulting temperature amplitude ratio (A_r) was used to estimate *q*.

The specific infiltration rate *q* and the vertical flow velocity (v_f) are extracted according to equation 4 (Ingebritsen et al., 2006) and equation 5 (Hatch et al., 2006, slightly transposed).

$$q = v_f \cdot n \tag{4}$$

$$v_f = \left(\frac{\rho \cdot c}{\rho_f \cdot c_f} \right) \cdot v \tag{5}$$

For parameter definition and values see Table 1. The vertical fluid velocity (v) can be determined by the amplitude ratio (A_r), identified as v_{Ar}. The values were gaines by a numerical solver from the equations 6 and 7 (Hatch et al., 2006)

$$\frac{2\kappa_e}{\Delta z} \ln\left(A_r + \sqrt{\frac{\alpha(v_{Ar}) + v_{Ar}^2}{2}}\right) - v_{Ar} = 0 \tag{6}$$

where

$$\alpha(v) = \sqrt{v^4 + (8\pi \cdot f_{Day} \cdot \kappa_e)^2} \tag{7}$$

The effective thermal diffusivity (κ_e) is estimated according to Hatch et al. (2006) by the equation

$$\kappa_e = \frac{\sigma}{\rho \cdot c} + \beta \cdot \left| v_f \right| \tag{8}$$

where the components of the first term are gained from:

$$\sigma = n \cdot \sigma_f + (1-n) \cdot \sigma_s \tag{9}$$

$$\rho = n \cdot \rho_f + (1-n) \cdot \rho_s \tag{10}$$

$$c = \frac{n\rho_f c_f + (1-n)\rho_s c_s}{n\rho_f + (1-n)\rho_s} \tag{11}$$

The second term of the equation 8 was excluded from the calculations as its contribution to the value of κ_e is negligible with the thermal dispersivity (β) = $1 \cdot 10^{-3}$ as proposed by Hatch et al. (2006) and Keery et al. (2007) but it would strongly increase the complexity of the analysis (Keery et al., 2007).

Heat is mainly transferred through riverbed sediments by advection and conduction. Heat advection describes the heat transfer related to water flow through the sediment, while heat conduction describes the molecular transport of thermal energy (e.g., Constantz, 2008). The relative contribution of advection and conduction to heat transfer can be quantified with the dimensionless Peclet number (*Pe*) (Silliman et al., 1995):

$$Pe = \frac{v_f \cdot n \cdot l}{D} \tag{12}$$

where l is the characteristic length, set as 0.01 m due to the range of the setup. The thermal diffusivity D is given by:

$$D = \frac{K_e}{c_s \cdot \rho_s} \tag{13}$$

where K_e is the thermal conductivity of the saturated sediment (Table 1). If *Pe* is smaller than approximately $2 \cdot 10^{-4}$, advection component of the solution has little impact for fluxes and conductive heat transport dominates (Silliman et al., 1995).

Median Peclet numbers were between approximately 0.01 and 0.1, indicating that heat is not only transported by molecular transport of thermal energy (conduction) but also by water flow (advection) (Silliman et al., 1995).

Table 1 - Physical parameters used for calculating specific infiltration rates *q* in alphabetic order (1. Roman letters, 2. Greek letters).

Symbol	Values	Unit	Parameter
A		°C	Amplitude of thermal oscillation
A_r		-	Temperature (T) amplitude ratio (upper / lower T amplitude)
c		J kg^{-1} °C^{-1}	Specific heat of sediment-fluid system
c_f	4208	J kg^{-1} °C^{-1}	Specific heat of fluid (water at 4°C) (Lemmon et al., 2012)
c_s	775	J kg^{-1} °C^{-1}	Specific heat of sediments, average between values of Schön (1996) (cited by Rau et al.) and Revil (2000) (cited by Keery et al., 2007)
f_{Day}	$11.5 \cdot 10^{-6}$	s^{-1}	Frequency of a day period (24h)
K_e	1	J m^{-1}s^{-1}K^{-1}	Thermal conductivity of the saturated sediment, Carslaw and Jaeger (1959) (cited by Silliman et al., 1995)
n	0.23	-	Porosity, assessed from freeze core samples
q		m s^{-1}	Specific infiltration rate
v		m s^{-1}	Velocity of thermal front
v_{Ar}		m s^{-1}	Velocity of thermal front derived from the amplitude ratio A_r
v_f		m s^{-1}	Vertical fluid velocity, positive number = down welling (Goto et al., 2005)
β	$1 \cdot 10^{-3}$	m	Thermal dispersivity (cited by Hatch et al., 2006)
$\Delta\phi$		s	Temperature amplitude phase shift
κ_e		m^2 s^{-1}	Effective thermal diffusivity
ρ		kg m^{-3}	Density of saturated sediment
ρ_f	1000	kg m^{-3}	Density of fluid (water at 4°C; Kuchling, 1976)
ρ_s	2650	kg m^{-3}	Density of sediment (e.g., Kuntze et al., 1994)
σ	1.50	W m^{-1} K^{-1}	Thermal conductivity of saturated sediment (Constantz, 2008)
σ_f	0.60	W m^{-1} K^{-1}	Thermal conductivity of fluid (water; Ingebritsen et al., 2006)

Results and discussion

Spatiotemporal changes in riverbed and redd morphology

The riverbed morphology of the Enziwigger changed substantially during high flow events, despite the steps to prevent deep scouring. This was especially true for the two downstream sites B and C (Figure 2), where flood events in December 2009 triggered river gravel accumulation or scouring up to 0.9 m (Figure 3). These data represent only the final outcome of the bed scouring processes and hence actual gravel displacement during the event peaks might have been even higher. All of site B was strongly affected by scouring, while at site C the gravel bed scoured predominately in the pools below steps and accumulated towards the right bank of the river. Sediment displacements varied from terrace to terrace within a site. For example, changes of the riverbed morphology below step 3 at site C were much smaller than at the other two examined steps (Figure 3, right). This was probably due to the slightly wider riverbed (5.0–5.5 m at step C3, 4.5–5 m at step C2 and 4.5 m at step C1) causing lower water levels and hence less shear stress. Sediment transport in rivers is a discontinuous process and sediment often moves in pulses (Klingemann and Emmett, 1982) affected by bed-form and associated sediment sorting (Cudden and Hoey, 2003) or by debris flows (Hoffman and Gabet, 2007). Hence, bed scouring and gravel deposition is partly stochastic, at least on an intermediate scale within individual river sections. Along the entire river (i.e. regional scale) an increase in gravel displacement was evident at sites B and C as compared to site A (visual interpretation). In total, half of the redds in sites B and C were lost (Figure 3), while only 8% of the redds were lost at the most upstream site A. This pattern is most likely related to increased shear stress in sites B and C attributable to higher water levels and only marginally smaller slopes (Schindler Wildhaber et al., 2012b), as also indicated by increasing bedloads and suspended sediment loads from up- to downstream (Schindler Wildhaber et al., 2012b). In support of this notion the probability of redd excavation increased with the water level above the redd (glm, $p < 0.05$).

Winter flood events in some Swiss rivers and also in rivers worldwide tend to increase over the last decade (Birsan et al., 2005; IPPC, 2007; Middelkoop et al., 2001; Scheurer et al., 2009; Thodsen, 2007). In the Enziwigger high-flow events in early winter are unusual, but have been suggested to increase during the last decades (P. Amrein, Fish and Wildlife Service, Canton Lucerne, Switzerland, pers. comm.). Accordingly, the high redd loss reported here raises concerns how the observed and predicted increases of winter floods affect

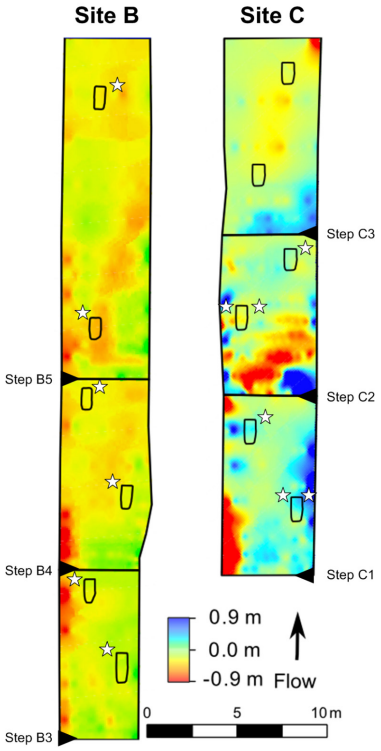

Figure 3 - Differences between the riverbed topography measured in October and December 2009 at the two downstream sites B and C. Negative values indicate gravel bed erosion, positive values sediment deposition. Black ovals are the positions of the artificial redds. Redds lost during season 1 are marked by a star on the left side, while redds lost during season 2 are marked by a star on the right side.

salmonid recruitment in some populations (cf. Goode et al., 2013; Lapointe et al., 2000), in rivers like the Enziwigger were small egg-burial depths (0–9 cm; Riedl and Peter, 2013) make salmonid redds more susceptible to scouring.

In addition to redd loss, high-flow events also strongly affected the morphology of the remaining redds. Initially, the mean difference between the depth of the tail and pit of newly built redds was 9.4 ± 2.8 cm (Figure 4A). After one month and some high flow events, most redds were basically leveled out (Figure 4A) and high amounts of fine sediments have infiltrated (Schindler Wildhaber et al., 2012b). Both likely affected the water exchange in redds, either by reducing horizontal pumping flow or by decreasing redd gravel permeability (e.g., Greig et al., 2005; Schälchli, 1995). This clearly suggests that the often discussed positive effect of the highly permeable redd substratum and the pit-tail structure on water exchange in salmonid redds (e.g., Tonina and Buffington, 2009) were rapidly obliterated by gravel displacements and fine sediment infiltration. This observations agree with Ottaway et al. (1981) who documented a flattening of brown trout redds already after the first high water event subsequent to spawning. The concept of enhanced downwelling of oxygenated water due to the redd morphology is still widely discussed (e.g., Greig et al., 2007b; Tonina and

Buffington, 2009; Zimmermann and Lapointe, 2005). Our data indicate that redd morphology could contribute to local redd scale exchange processes, but only during the first few weeks after redd building (see below). Once the pit-tail structure has been leveled out, exchange processes on intermediate or regional scales likely gain importance for the oxygen supply to developing embryos. Processes driven on these scales therefore need to be considered in management plans to ensure salmonid embryo survival in modified river environments.

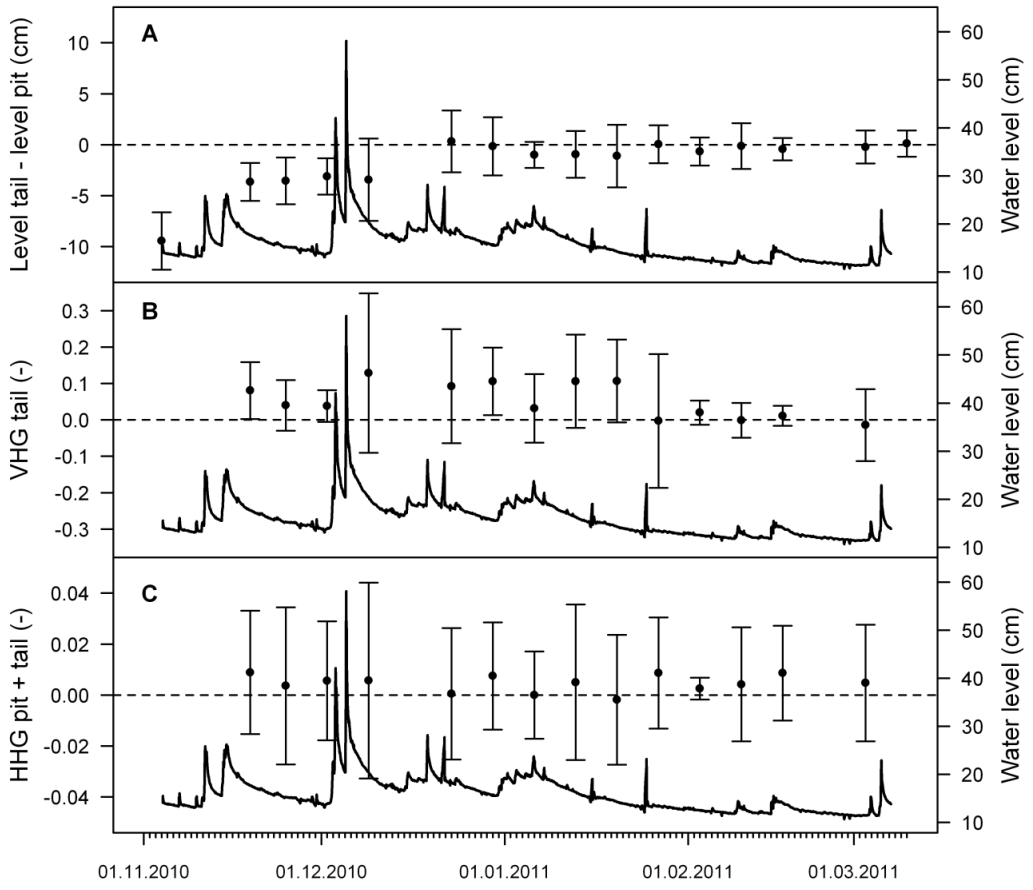

Figure 4 - In each panel, black line shows the flow stage at site B. Symbols within panels denote **(A)** the mean ± standard deviation (sd) of the riverbed level differences between tail and pit **(B)** mean ± sd of the vertical hydraulic gradients (VHG) in the tail of the redds, and **(C)** mean ± sd of the horizontal hydraulic gradients (HHG) between pit and tail of the redd. Values were calculated from all 18 redds during the season 2. A positive hydraulic gradient indicates upwelling, a negative gradient downwelling.

Hydraulic dynamics in the redds

Spatial patterns of the hydraulic dynamics

Vertical hydraulic gradients (VHGs) measured in mini piezometers did not parallel the modeled intermediate scale down- and upwelling patterns induced by the steps (Huber et al., 2013, Figure 1B). Most redds were not located in the main up- and downwelling zones predicted by the model, but in areas where downwelling, upwelling and horizontal advection alternates (Figure 1C). Hence, VHGs were too small to be accurately measured with the applied mini piezometer approach. In redd A32_S1, which provides an exception being located only 0.65 m above a step, considerable downwelling potential was measured (-0.07 +/- 0.06). When this redd was located 1.65 m upstream of the step in season 2 (redd A32_S2; Figure 2), VHGs changed between up- and downwelling conditions with a mean close to zero (0.03 ± 0.08). These observations agree with model predictions. In general, vertical hydraulic gradients on the redd scale can be expected to show substantial temporal variation related to water level fluctuations but also changes in riverbed morphology (see above).

Calculated specific infiltration rates q generally confirmed the predictions from the groundwater flow modeling. Mean q_t increased with smaller distance to the next downstream step indicating increased downwelling above steps (Table 2). In redds located further upstream, where the model predicted horizontal advection zones or exfiltration (Figure 1C), smaller and also negative q_t values were found. Also, weekly fine sediment deposition increased with shorter distance to the downstream step (Spearman rank correlation, fine sediment: $\rho = -0.45$, $p < 0.05$, silt: $\rho = -0.52$, $p < 0.05$, clay: $\rho = -0.57$, $p < 0.01$), likely related to increased river water infiltration above steps. Increased weekly fine sediment infiltration had no negative effect on specific water infiltration in redds (Table 2). In contrast to the weekly sediment infiltration, net fine sediment accumulation did not increase with shorter distance to the step (all $p > 0.12$). Fine sediment accumulation not only depends on fine sediment infiltration, but also on the water level, since higher water levels lead to resuspension of fine sediment (Schindler Wildhaber et al., 2012b). The specific infiltration rate q decreased significantly in redds with higher fine sediment accumulation and increased with a higher maximal water level above the redd (Table 2).

Hydraulic exchange processes can vary within single redds on a scale of only tenth of centimeters. In most redds q was lower in 12–20 cm depth as compared to the upper 12 cm of redd gravel (t-test, $p < 0.01$; Figure 5). Freeze core samples of undisturbed gravel in the study

area confirmed a significantly lower silt and clay level in the upper part (0–10 cm) compared to deeper part (i.e. 10–20 cm and 20–30 cm, cf. Schindler Wildhaber et al., 2012b). This "bottom up" filling of riverbed gravel and salmonid redds is common when particle sizes are small enough to pass through gravel interstices (Greig et al., 2007b). A comparable increase in fine sediment content paralleled by a decreased hydraulic conductivity was also found in the studies of Brunke (1999) and Sear (1993). Accordingly, the decrease of q with depth reported here suggests higher fine sediment content around our brown trout eggs (i.e. at 12–20 cm depth) compared to the entire redd gravel. A similar distinction between the upper and lower part of the redd gravel was made by Meyer (2003), who further reported that the fine sediment content in the egg-pocket was a better predictor for salmonid embryo survival than fine sediment content in the entire gravel column. Our study might corroborate this conclusion, and further indicates that this phenomenon could also cause a decreased water exchange around the eggs.

Table 2 - Spearman rank correlations between median specific infiltration rate q in the upper part (0–12 cm; q_u) and the total part (0–20 cm, q_t) and the total amount accumulated fine sediment (< 2 mm), the accumulated silt and clay fraction, the sum of weekly infiltrated fine sediment, the *fredle index* of the accumulation baskets, the maximal water level above the redd and the distance of the redd to the upstream and downstream step. Sample size (n) is given in parentheses.

Parameter	**q_u (m s^{-1})**	**q_t (m s^{-1})**
Fine sediment accu. (%)	**-0.79, p = 0.03 (8)**	**-0.89, p = 0.03 (6)**
Silt and clay accu. (%)	-0.52, p = 0.20 (8)	-0.49, p = 0.36 (6)
Fine sediment infiltration (g)	-1.9, p = 0.58 (8)	-0.18, p = 0.57 (6)
Fredle index (-)	0.71, p = 0.06 (8)	0.77, p = 0.09 (6)
Water max. (cm)	**0.60, p = 0.03** (13)	**0.66, p = 0.03** (10)
Distance upstream step (cm)	0.15, p = 0.62[a] (13)	0.61, p = 0.06[a] (10)
Distance downstream step (cm)	-0.17, p = 0.58[a] (13)	**-0.68, p = 0.03[a]** (10)

[a] Mean q of February and March to get a mean q value of the undisturbed river gravel.

At redd A32_S1 the specific infiltration *q* was higher in the bottom part than the upper part of the redd (Figure 5, see Figure 2 for location of the redd), which was probably related to the lower mean water level above this redd (9.8 ± 2.0 cm), triggering high fine sediment deposition and only limited scouring (Schindler Wildhaber et al., 2012b). Redd A32_S1 was also the only redd that was temporally covered with ice, which could have caused decreased water flow over the redd. In addition, VHG measurements indicated substantial downwelling potential in this redd (VHG = -0.07 ± 0.06, see above). Both factors possibly increased the fine sediment input in the entire gravel column of this redd (Brunke, 1999; Schindler Wildhaber et al., 2012b; Seydell et al., 2009) and hence decreased specific water infiltration *q* also in the upper part. A low *q* was also found in the upper part of redd A31_S1 (Figure 5), which also had a very low mean water level (2.5 ± 1.7 cm). During the second field season, the locations of the two redds were changed to build them on spots with deeper mean water levels (A31_S2: 15.1 ± 3.5 cm, A32_S2: 12.4 ± 3.5 cm). Now less fine sediment accumulated (Schindler Wildhaber et al., 2012b) and higher specific infiltration rates *q* were found (Figure 5). Further, these patterns of *q* were closely paralleled by the oxygen dynamics in these redds (see below).

Temporal pattern of the hydraulic dynamics

Slightly positive VHGs, indicating upwelling processes, were measured in the tail of most redds at the beginning of the incubation period (Figure 4B). In contrast, horizontal hydraulic gradients (HHG) between pit and tail did not indicate increased horizontal pumping flows between pit and tail (Figure 4C). The classical redd scale flow pattern, with downwelling in the pit and upwelling in the tail causing pumping flow through the redd (Tonina and Buffington, 2009), was therefore not confirmed by the HHG measurements. One reason for this might be the influence of the riverbed morphology or the water levels on the vertical and horizontal hydraulic gradients, as indicated by significant correlations between VHGs and the water level (ρ = 0.4–0.6, p <0.05 in 8 of the 18 redds, Figure 6). In the remaining ten redds similar trends could be observed, albeit being non-significant (data not shown). During base flow, VHGs were mostly negative or around zero, indicating downwelling or horizontal advection flow, which agrees with model predictions (Huber et al., 2013). Upwelling or lateral flow dominated for VHG values measured at higher water levels (Figure 6). Upwelling can be triggered by different mechanisms: on an intermediate scale it can be increased below steps (Huber et al., 2013), while on a regional scale upwelling can occur when increasing riparian groundwater levels are paralleled by decreasing stream water levels. The latter is

common during the recession limb of flood events (Geist et al., 2008; Malcolm et al., 2006; 2003; Soulsby et al., 2009). In the Enziwigger, VHG measurements during the rising limb or flood events were not possible because of dangerous physical conditions. Most of the higher water levels in Figure 6 therefore represent data points measured during the recession limb of flood events. Given this, it is plausible that these positive hydraulic gradients (Figure 6) indicate recharge of groundwater on a regional scale causing upwelling conditions in the redds.

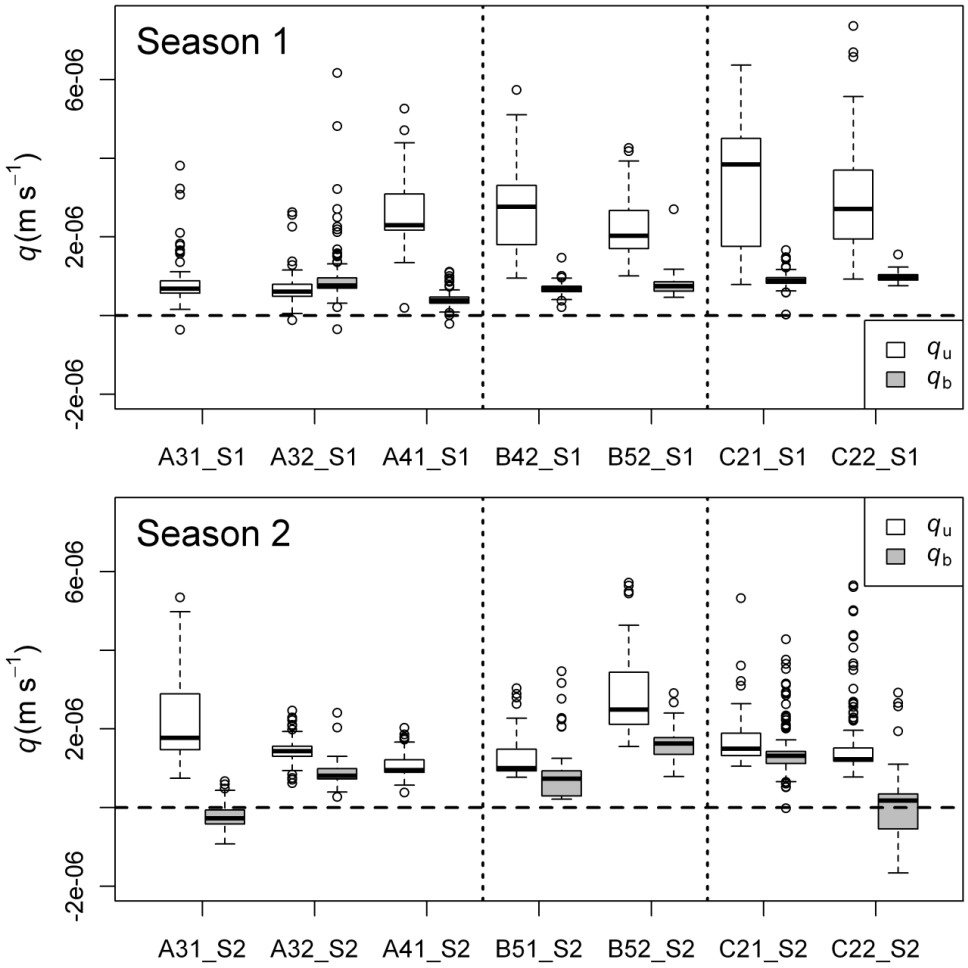

Figure 5 - Specific water infiltration rates in the upper (q_u) and the bottom part (q_b) of the redds during season 1 (top) and season 2 (bottom). Negative values indicate upwelling, positive values indicate downwelling. For each redd the horizontal line indicates the median, the box interquartile range (i.e. center 50% of the data), whiskers mark maximum and minimum values, and points denote values exceeding 1.5 times the interquartile range. Among seasons, redds were built in the same location (Figure 2) with the exception of redds A31 and A32

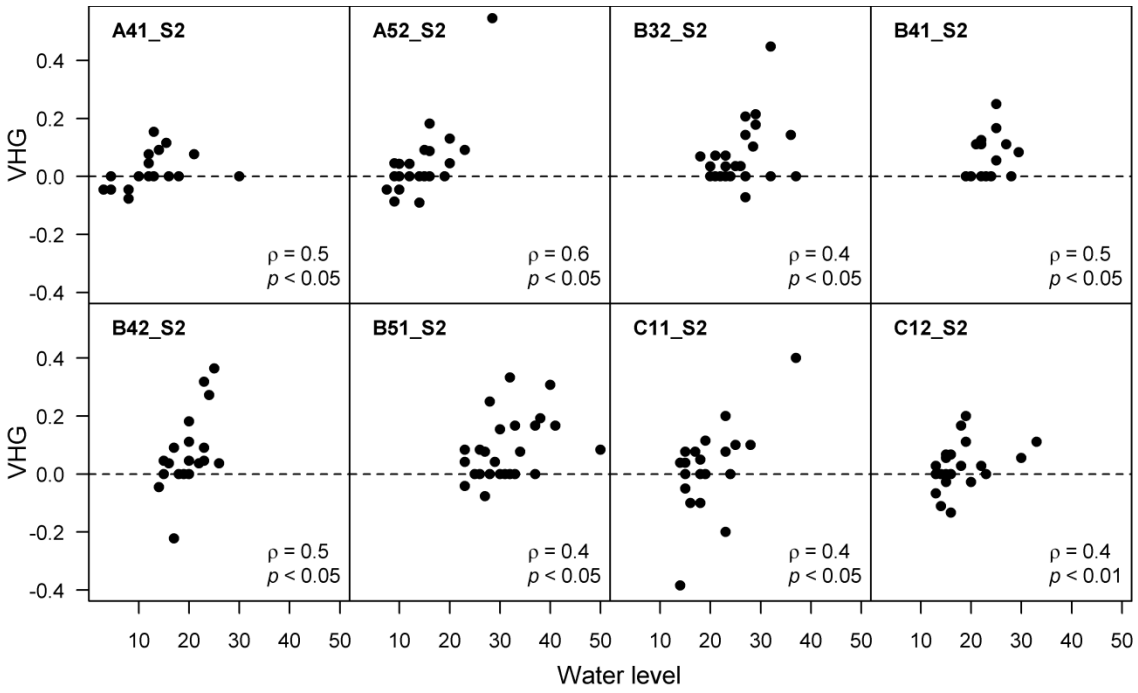

Figure 6 - Relationship between water level and vertical hydraulic gradient (VHG) for individual redds from the season 2. Within each panel Spearman correlation coefficient rho (ρ) and the *p*-value are given. Positive VHG indicates upwelling, negative VHG indicates downwelling. The location of each redd is given in Figure 2.

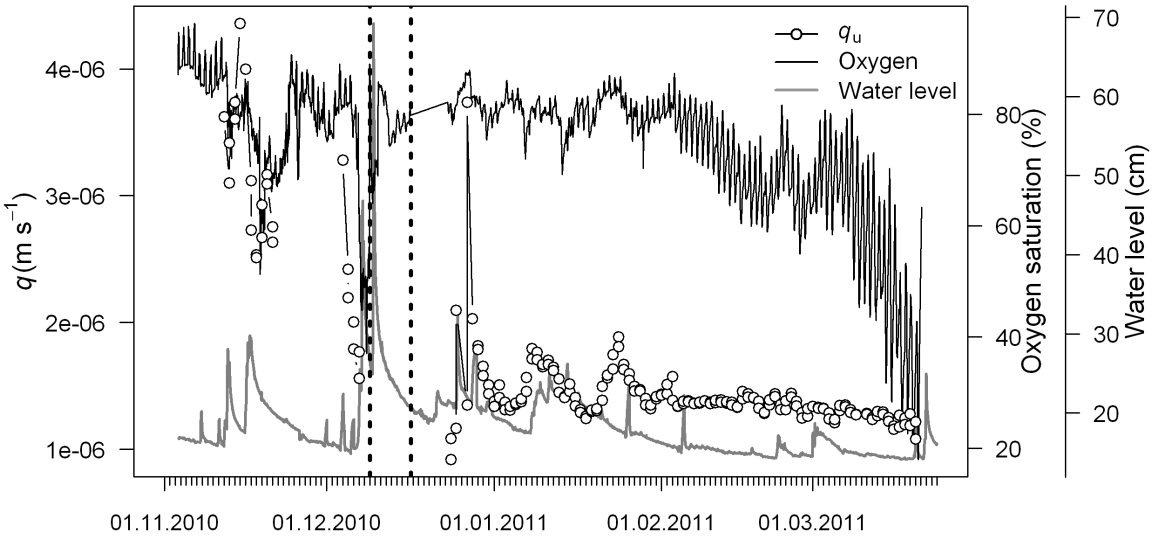

Figure 7 - Example of the temporal dynamics of the specific infiltration in the upper part of the redd gravel (q_u), the oxygen concentration and the water level. Shown are data from redd C21_S2 (cf Figure 2). A period when oxygen and temperature probes were dug out is marked with vertical dashed lines.

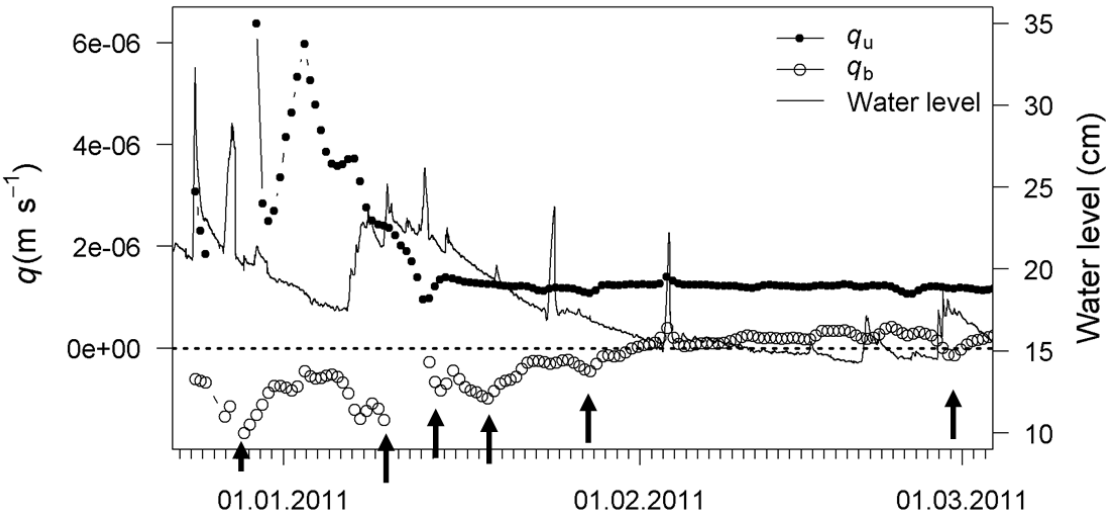

Figure 8 - Temporal changes of the specific infiltration rate *q* in the upper and the bottom part of the redd C22_S2 (cf. Figure 2for location of the redd). Negative values indicate upwelling, positive values indicate downwelling. The arrows point to periods with increased upwelling during the falling limb of high flow events.

Hyporheic flow paths in rivers can be very complex in a 3D view, also changing with discharge and morphology (Tonina and Buffington, 2007). These complex temporal dynamics can best be seen in the specific water infiltration rates. Initially, *q* values in most redds were consistently in the range of 6–7 · 10^{-6} m s^{-1}. Within a month *q* decreased remarkably, likely due to the infiltration of fine sediments and changes in redd morphology, finally reaching a stable minimum around 1–2 · 10^{-6} m s^{-1} for the rest of the incubation season (Figure 7, Figure 5). However, on the scale of hours and days *q* was still responsive to high flow events, generally increasing during high-flow events and returning to baseline levels afterwards (Figure 7). This most likely reflects increased water infiltration driven by local changes in groundwater heads and probably also increasing gravel permeability related to remobilization of fine sediment (e.g., Brunke, 1999; Keery et al., 2007; Schälchli, 1995).

Temporally negative *q* values were found in the bottom part (approx. 12–20 cm) of two redds (A31_S2 and C22_S2), indicating upwelling. In parallel consistent downwelling conditions were measured in the upper part (Figure 5). This pattern probably reflects the interference of exchange processes on an intermediate scale with groundwater upwelling driven on a regional scale. This can be clearly seen in redd C22 (Figure 8), which was built just above a step,

where downwelling can be expected to predominate (Huber et al., 2013). At the same time it was located on the right side of the Enziwigger, close to a small tributary river probably driving exfiltration of groundwater at this redds location (Figure 2). Given this specific location, we suspect that river water infiltrated in the upper part of this redd (positive q), while groundwater exfiltrated into the lower part (negative q). As discussed in the previous paragraph, groundwater exfiltration in this redd increased during the recession limb of high flow events (Figure 8).

Specific water infiltration rates q calculated with the intermediate groundwater flow model for specific redd locations in downwelling zones ranged between $8.5 \cdot 10^{-7}$ and $1.5 \cdot 10^{-5}$ m s^{-1} (season 1, Huber et al., 2013). Measured daily q_t values at redd B42_S1 correlated significantly with the modeled values (Pearson's $r = 0.3$, $p < 0.05$). In contrast, at redd B52_S1, no significant correlation was found. The groundwater flow model was set up using hydraulic heads of the river and groundwater as well as the local and regional topography. The actual q in the redds also depends on the hydraulic conductivity, affected by fine sediment deposition and hydraulic gradients on the redd scale. These differences could have contributed to the lack of correlation in the latter redd, since water exchange rates on the redd scale can vary strongly, either depending on reach scale bedform character and barriers (Baxter and Hauer, 2000) or differences in hydraulic conductivities (Brunke and Gonser, 1997). Altogether, these results indicate that groundwater flow modeling as applied in Huber et al. (2013) provides good predictions of exchange processes on the regional and intermediate scale, but might be more limited in predicting exchange processes on the scale of individual salmonid redds.

Altogether, these findings illustrate that water exchange processes in salmonid redds are complex and driven on multiple scales. Consequently, fine sediment effects on salmonid embryo survival can be expected to differ depending on the redd location relative to river morphology and structure but also on regional aspects, such as river interactions with the valley aquifer.

Oxygen

Manual vs. continuous oxygen measurements

Manual oxygen measurements, conducted on bi-weekly intervals during season 2 in mini-piezometers, indicated a high interstitial dissolved oxygen (DO) concentration in the redds (10.1 ± 2.2 mg l^{-1} / 75.7 ± 15.7%). After pumping out the interstitial water, the measured average DO concentration increased significantly to 11.6 ± 1.8 mg l^{-1} (85.9 ± 12.4%, *t*-test, $p < 0.05$). This was possibly related to river water with higher mean oxygen concentration (13.3 ± 0.8 mg l^{-1} / 98.2 ± 4.5%) infiltrating and replacing the extracted interstitial water in the piezometers (Heywood and Walling, 2007). DO concentrations measured manually in mini piezometers were generally higher than measured by permanent oxygen probes. As a result, DO concentrations measured manually only poorly correlated with DO concentrations from permanent oxygen measurements. Accordingly, manual oxygen measurements appeared to be no accurate predictors for the oxygen concentration in the redds. Oxygen concentrations in salmonid redds can vary substantially with time (Heywood and Walling, 2007, this study), and hence even weekly or bi-weekly measuring intervals have a high risk to miss these dynamics, underestimating extreme values (Malcolm et al., 2006). In conclusion, our results warrant that manual interstitial oxygen measurements in piezometers have a risk to over- or underestimate the actual amount of oxygen present in salmonid redds and might provide a poor descriptor for oxygen dynamics during the incubation season. Given these limitations and methodological bias of the manual DO data, further interpretations are based only on the continuous DO measurements.

Spatial oxygen dynamics

DO concentrations from continuous measurements in redds documented a high variability on small spatial scales (i.e. between redds), but also a general increase from up- to downstream (Table 3). Interstitial oxygen concentrations at site A (redd A32_S1) were especially low during season 1 with DO concentrations below 3 mg l^{-1}, a critical threshold for salmonid embryo survival (Michel et al., submitted), during 44 of total 135 days of egg incubation time. The low DO concentrations in this redd could be caused by the observed low specific water infiltration rate *q* (see above). When this redd was moved to a spot with a higher water level during season 2 (redd A32_S2) not a single day below 3 mg l^{-1} occurred (Table 3). In contrast, redd A31_S2, which was built about six meters upstream of A32_S2, had 14 days with DO concentrations below 3 mg l^{-1}, probably related to upwelling of DO depleted hyporheic water, as discussed in below (Figure 5). Days below 3 mg l^{-1} were far less frequent at sites B and C

(Table 3). These observed low oxygen concentrations at site A could be related to the artificial log steps, breaking down the river slope, inhibiting natural river gravel movements and thus triggering high fine sediment accumulation at sites with low water levels. At the downstream sites, water levels and shear stress were generally higher, leading to a flushing of infiltrated fine sediment, resulting in less fine sediment accumulation at the downstream sites (Schindler Wildhaber et al., 2012b).

Table 3 - Mean oxygen concentrations calculated from continuous measurement with permanent oxygen probes in one redd per site during season 1 (S1) and two redds per site during season 2 (S2). Given are mean ± standard deviations, minimum (min) and number of days with mean oxygen concentrations below 3 mg l^{-1}.

Site	Mean ± sd O_2 (mg l^{-1})			Min O_2		Days O_2 < 3 mg l^{-1}	
	S1	S2	Mean (S1 + S2)	S1	S2	S1	S2
A	4.6 ± 3.3	8.4 ± 3.4	6.6 ± 3.8	0.0	0.1	44	14 | 0
B	9.6 ± 2.1	10.0 ± 2.3	9.8 ± 2.2	3.2	0.0	0	4 | 2
C	9.6 ± 1.8	10.3 ± 1.3	10.0 ± 1.6	0.6	3.8	1	1 | 0

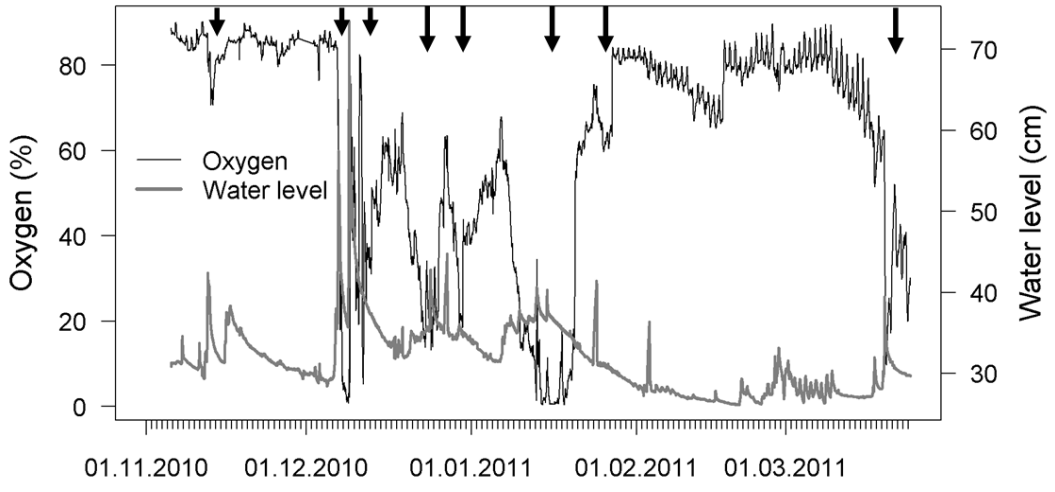

Figure 9 - Example for temporal oxygen concentration and water level dynamics (redd A31_S2, cf. Figure 2 for location of the redd). Arrows mark the decrease of oxygen concentrations during the falling limb high flow events.

Only a small number of accumulation baskets in redds with permanent oxygen measurements resisted floods, resulting in a very small data set across the two field seasons (Schindler Wildhaber et al., 2012b). In total, four redds with permanent oxygen data and accumulation baskets remained intact. Hence, statistical analyses with mean DO concentration depending on total accumulated fine sediment were limited. However, these four data points were surprisingly even spread and showed a perfect linear relationship that suggests a linear decrease of DO concentration with increasing fine sediment accumulation ($R^2 = 0.85$, $p = 0.05$). In absolute values the redd with a high fine sediment accumulation (redd A32_S1: 25.5% of the basket), showed a low mean DO concentration (4.7 ± 3.3 mg l^{-1}; 34.6 ± 24.8%). In the redd with low fine sediment accumulation (redd A32_S2: 13.1%), mean DO concentration was high (10.8 ± 1.1 mg l^{-1}; 80.5 ± 6.6 %). Finally, the two redds with medium sediment accumulation (B51_2: 19.4% and A31_2: 20.9%) also showed medium DO concentrations of 8.8 ± 2.5 and 8.4 ± 3.3 mg l^{-1} (68.3 ± 18.4 and 62.9 ± 24.7%), respectively. This finding parallels previous studies demonstrating a negative relationship between interstitial fine sediment content and oxygen concentration (e.g. Heywood and Walling, 2007).

Temporal oxygen dynamics

On the scale of hours and days, DO concentrations decreased during the falling limb of high flow events. This pattern was most pronounced in redds where temporal water exfiltration was measured, e.g., redd A31_S2 (Figure 9, see also above), most likely related to intermittent exfiltration of depleted ground- or hyporheic water through the redd. The same has been reported in other studies (Malcolm et al., 2010; Malcolm et al., 2006; Soulsby et al., 2009). Decreasing DO concentrations during the falling limb of high flow events were also found in locations where no exfiltration was measured (e.g., redd C21_S2, Figure 7). The fact that interstitial DO returned to normal levels shortly after the rising limb of high flow events suggest that this pattern is also related to exfiltration of depleted ground- or hyporheic water rather than increased fine sediment deposition, which would have likely caused more prolonged effects (Figure 7).

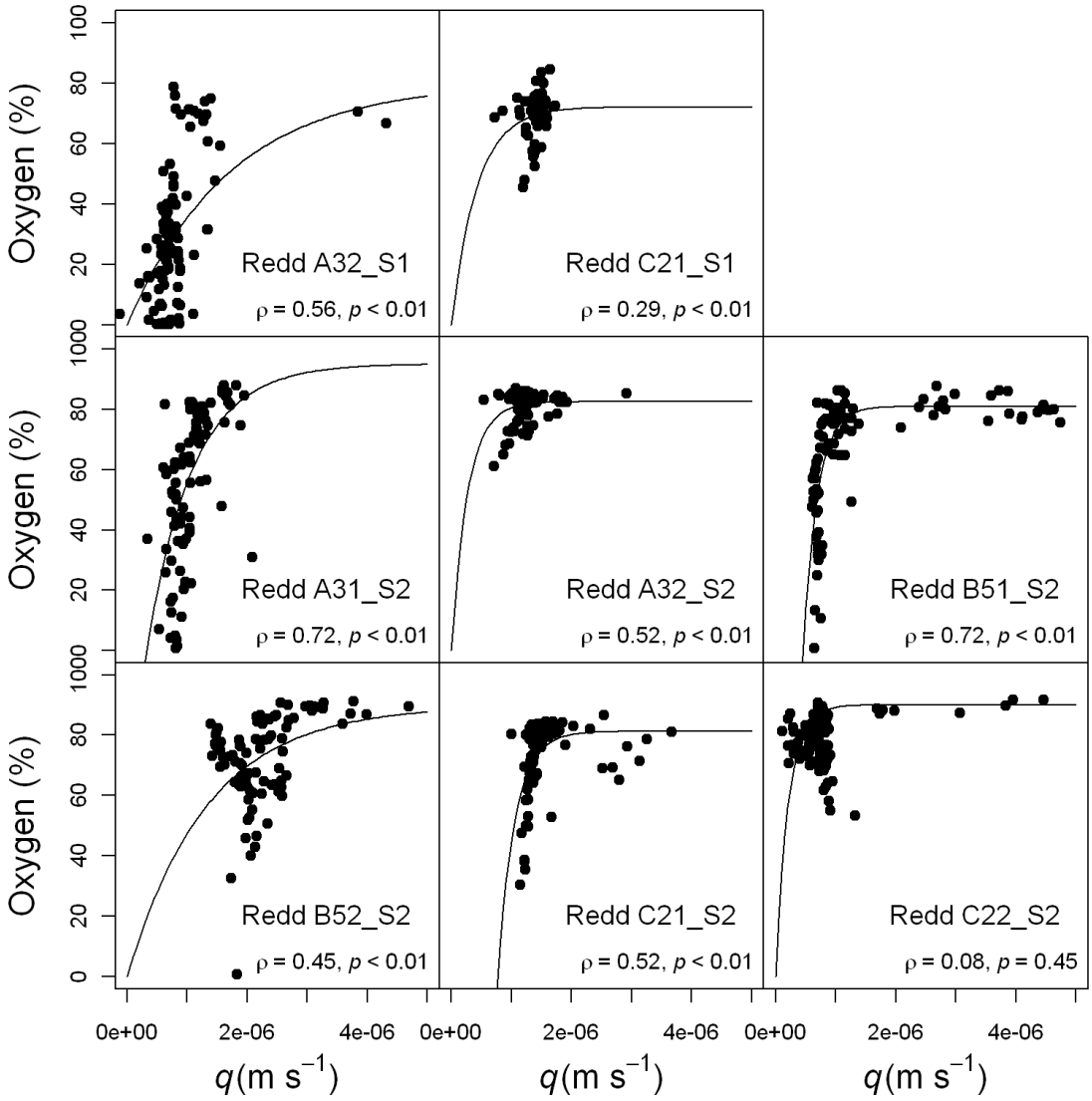

Figure 10 - Relationships between the mean daily oxygen concentrations in redds and the specific infiltration rates *q*. Lines show non-linear regression line. Within each panel Spearman correlation coefficient rho (*ρ*) and the *p*-value are given. See Figure 2 for location of the redds.

In addition to these changes in interstitial DO on the scale of hours and days, two general trends were evident over the entire incubation period. The first trend could be observed at the beginning of the incubation season: Shortly after redd construction, DO was generally high and paralleled by high specific water infiltration rates *q* (between 10 and 12 mg l^{-1}, about 80–90% oxygen saturation; for an example see Figure 7). Within a few weeks interstitial DO decreased in parallel with *q*, as also reflected in significant correlations between these two parameters (Figure 10). The different form of this relationship among redds (Figure 10) could be related to local conditions at the redd location that also affect interstitial DO (e.g., organic

content, groundwater influence). The second trend could be observed at the end of the incubation season, when several redds showed a distinct decrease in interstitial oxygen during spring, i.e. just before hatching (Figure 7, Figure 9). This decrease was usually preceded by prolonged periods of base flow, when smaller sediment particles infiltrated in the redds (Schindler Wildhaber et al., 2012b). These silt and clay sized particles can effectively induce siltation of the riverbed, thereby decreasing hydraulic conductivity (Schälchli, 1995). Moreover, the organic matter concentration of the infiltrated fine sediment increased during base flow conditions (Schindler Wildhaber et al., 2012a). Together with rising water temperatures during spring, the latter could have further decreased the oxygen concentration in the redds (Greig et al., 2007b). This decrease towards the time of hatching, when oxygen demand of the developing embryos is at maximum (Greig et al., 2007b), has most likely also affected embryo survival in some redds (Michel et al., submitted).

Conclusion

Scouring of redds during high flow events provided a high risk (50%) for brown trout embryo survival in the two downstream sites B and C, while at the most upstream site A redd loss was substantially lower (8%). However, at the upstream site, DO concentrations were generally lower, making the redd environment less favorable for the embryo survival. These low oxygen concentrations at site A could be due to the artificial log steps, breaking down the river slope, inhibiting natural river gravel movements and thus triggering high fine sediment accumulation at sites with low water levels. The increased downstream scouring was mostly related to the increasing water levels and hence bed shear stress due to the channelized riverbed. Given the predicted and observed increases in winter precipitation events (e.g., Scheurer et al., 2009), this observation raises concerns that redd loss might increase in the downstream reaches of channelized rivers like the Enziwigger. The relevance of this finding for salmonid population dynamics remains to be evaluated.

Our data demonstrate that specific water infiltration rates (q) and DO concentrations in salmonid redds are highly dynamic and driven on multiple scales. Clearly, q and interstitial DO in salmonid redds are affected by conditions at the redd location, such as the amount of deposited fine sediment, organic content and redd morphology. However, local factors in the magnitude of centimeters to meters are regularly superimposed by processes driven on intermediate (meter scale) and regional scales (magnitude of tenth of meters to kilometers). On an intermediate scale the introduced steps can affect patterns of fine sediment

accumulation, specific water infiltration and hence interstitial DO. On the catchment scale the relocation of the river to the right valley side caused hydraulic conditions favoring river water infiltration in redds located towards the left bank of the river. In this regard, our results document, to our knowledge, for the first time an effect of artificial steps on water exchange and oxygen supply in salmonid redds. Given the complexity of all these processes, multiple predictors have to be considered to predict salmonid embryo survival, including riverbed structure, local hydrogeological conditions and the hydrological setting of the river in the valley aquifer. An investigation how these factors might affect salmonid embryo survival is part of an accompanying publication (Michel et al., submitted).

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References

Acornley RM, Sear DA. Sediment transport and siltation of brown trout (Salmo trutta L.) spawning gravels in chalk streams. Hydrological Processes 1999; 13: 447-458.

Baxter C, Hauer FR, Woessner WW. Measuring groundwater-stream water exchange: New techniques for installing minipiezometers and estimating hydraulic conductivity. T. Am. Fish. Soc. 2003; 132: 493-502.

Baxter CV, Hauer FR. Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 2000; 57: 1470-1481.

Birsan MV, Molnar P, Burlando P, Pfaundler M. Streamflow trends in Switzerland. Journal of Hydrology 2005; 314: 312-329.

Brookes A. Channelized Rivers: Perspectives for Environmental Management. Chichester: Wiley, 1988.

Brown LR, Moyle PB, Yoshiyama RM. Historical Decline and Current Status of Coho Salmon in California. North American Journal of fisheries management 1994; 14: 237-261.

Brunke M. Colmation and depth filtration within streambeds: Retention of particles in hyporheic interstices. Int. Rev. Hydrobiol. 1999; 84: 99-117.

Brunke M, Gonser T. The ecological significance of exchange processes between rivers and groundwater. Freshwater Biology 1997; 37: 1-33.

Burkhardt-Holm P, Scheurer K. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquatic Sciences 2007; 69: 51-70.

Carslaw HS, Jaeger JC. Conduction of Heat in Solids. New York: Oxford University, 1959.

Constantz J. Heat as a tracer to determine streambed water exchanges. Water Resources Research 2008; 44.

Cudden JR, Hoey TB. The causes of bedload pulses in a gravel channel: The implications of bedload grain-size distributions. Earth Surface Processes and Landforms 2003; 28: 1411-1428.

EBP-WSB-Agrofutura. Ganzheitliche Gewaesserplanung im Einzugsgebiet Wiggertal. Bau- und Umwelt und Wirtschaftsdepartement des Kantons Luzern und Baudepartement des Kantons Aargau 2005.

Endreny T, Lautz L, Siegel DI. Hyporheic flow path response to hydraulic jumps at river steps: Flume and hydrodynamic models. Water Resources Research 2011; 47.

Geist DR, Arntzen EV, Murray CJ, McGrath KE, Bott YJ, Hanrahan TP. Influence of river level on temperature and hydraulic gradients in chum and fall Chinook salmon spawning areas downstream of Bonneville Dam, Columbia river. North American Journal of Fisheries Management 2008; 28: 30-41.

Gilvear DJ, Heal KV, Stephen A. Hydrology and the ecological quality of Scottish river ecosystems. Science of the Total Environment 2002; 294: 131-159.

Goode JR, Buffington JM, Tonina D, Isaak DJ, Thurow RF, Wenger S, et al. Potential effects of climate change on streambed scour and risks to salmonid survival in snow-dominated mountain basins. Hydrological Processes 2013; 27: 750-765.

Gooseff MN, Anderson JK, Wondzell S, LaNier J, Haggerty R. A modeling study of hyporheic exchange pattern and the sequence, size, and spacing of stream bedforms in mountain stream networks, Oregon, USA (Retraction of vol 19, pg 2915, 2005). Hydrological Processes 2006; 20: 2441-+.

Goto M, Matsukara N, Hagiwara Y. Heat transfer characteristics of warm water flow with cool immiscible droplets in a vertical pipe. Experimental Thermal and Fluid Science 2005; 29: 371-381.
Greig S, Sear D, Carling P. A field-based assessment of oxygen supply to incubating Atlantic salmon (*Salmo salar*) embryos. Hydrological Processes 2007a; 21: 3087-3100.
Greig SM, Sear DA, Carling PA. The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management. Science of the Total Environment 2005; 344: 241-258.
Greig SM, Sear DA, Carling PA. A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. Hydrological Processes 2007b; 21: 323-334.
Hatch CE, Fisher AT, Revenaugh JS, Constantz J, Ruehl C. Quantifying surface water-groundwater interactions using time series analysis of streambed thermal records: Method development. Water Resources Research 2006; 42.
Heywood MJT, Walling DE. The sedimentation of salmonid spawning gravels in the Hampshire Avon catchment, UK: implications for the dissolved oxygen content of intragravel water and embryo survival. Hydrological Processes 2007; 21: 770-788.
Hicks BJ, Hall JD, Bisson PA, Sedell JR. Responses of salmonids to habitat changes. In: Meehan WR, editor. Influences of forest and rangeland management on salmonid fishes and their habitats. 19, 1991, pp. 483-518.
Hoffman DE, Gabet EJ. Effects of sediment pulses on channel morphology in a gravel-bed river. Geological Society of America Bulletin 2007; 119: 116-125.
Huber E, Huggenberger P, Epting J, Schindler Wildhaber Y. Zeitliche und räumliche Skalen der Fluss-Grundwasser Interaktion: Ein multidimensionaler Untersuchungsansatz - Spatiotemporal scales of river-groundwater interaction: A multidimensional investigation approach. Grundwasser 2013.
Huette M, Niederhauser P. Oekomorphologie Stufe F. Methoden zur Untersuchung und Beurteilung der Fliessgwaesser in der Schweiz. Bundesamt für Umwelt, Wald und Landschaft, Mitteilungen zum Gewaesserschutz 1998; 27.
Huntington C, Nehlsen W, Bowers J. A survey of healthy native stocks of anadromous salmonids in the Pacific Northwest and California. Fisheries 1996; 21: 6-14.
Ingebritsen S, Sanford W, Neuzil C. Groundwater in Geologic Processes. New York: Cambridge University Press, 2006.
IPPC. Climate Change 2007: The Physical Science Basis, Summary for Policymakers. Intergovernmental panel on climate change. 2007.
Jensen DW, Steel EA, Fullerton AH, Pess GR. Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. Reviews in Fisheries Science 2009; 17: 348-359.
Kasahara T, Hill AR. Hyporheic exchange flows induced by constructed riffles and steps in lowland streams in southern Ontario, Canada. Hydrological Processes 2006; 20: 4287-4305.
Keery J, Binley A, Crook N, Smith JWN. Temporal and spatial variability of groundwater-surface water fluxes: Development and application of an analytical method using temperature time series. Journal of Hydrology 2007; 336: 1-16.
Klingemann PC, Emmett WW. Gravel bedload transport processes. In: Hey RD, Bathurst JC, Thorne CR, editors. Gravel-bed Rivers. Wiley, Chichester, 1982, pp. 145-169.
Kuchling H. Taschenbuch der Physik. Thun: Verlag Harri Deutsch, 1976.
Kuntze H, Roeschmann G, Schwerdtfeger G. Bodenkunde. Stuttgart: Ulmer UTB für Wissenschaft, 1994.

Lapointe M, Eaton B, Driscoll S, Latulippe C. Modelling the probability of salmonid egg pocket scour due to floods. Canadian Journal of Fisheries and Aquatic Sciences 2000; 57: 1120-1130.

Lemmon EW, McLinden MO, Friend DG. "Thermophysical Properties of Fluid Systems" in NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Eds. P.J. Linstrom and W.G. Mallard, National Institute of Standards and Technology, Gaithersburg MD, 20899, http://webbook.nist.gov, retrieved February 1, 2012.

Lotspeich FD, Everest FH. A new method for reporting and interpreting textural composition of spawning gravel. In: Service USF, editor. Pacific Northwest Research Note PNW-369, 1981, pp. 12p.

Malcolm IA, Greig SM, Youngson AF, Soulsby C. Hyporheic influences on salmon embryo survival and performance. In: Sear D, DeVries P, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. 65. American Fisheries Society, Bethesda, USA, 2008, pp. 225-248.

Malcolm IA, Middlemas CA, Soulsby C, Middlemas SJ, Youngson AF. Hyporheic zone processes in a canalised agricultural stream: implications for salmonid embryo survival. Fundamental and Applied Limnology 2010; 176: 319-336.

Malcolm IA, Soulsby C, Youngson AF. High-frequency logging technologies reveal state-dependent hyporheic process dynamics: implications for hydroecological studies. Hydrological Processes 2006; 20: 615-622.

Malcolm IA, Soulsby C, Youngson AF, Tetzlaff D. Fine scale variablility of hyporheic hydrochemistry in salmon spawning gravels with contrasting groundwater-surface water interactions. Hydrogeology Journal 2009; 17: 161-174.

Malcolm IA, Youngson AF, Soulsby C. Survival of salmonid eggs in a degraded gravel-bed stream: Effects of groundwater-surfacewater interactions. River Research and Applications 2003; 19: 303-316.

Meyer CB. The importance of measuring biotic and abiotic factors in the lower egg pocket to predict coho salmon egg survival. Journal of Fish Biology 2003; 62: 534-548.

Michel C, Schindler Wildhaber Y, Epting J, Thorpe KL, Huggenberger P, P B-H, et al. Relative importance of fine-sediment, hydraulic gradients and river morphology for brown trout embryo survival in a heavily modified river. submitted.

Middelkoop H, Daamen K, Gellens D, Grabs W, Kwadijk JCJ, Lang H, et al. Impact of climate change on hydrological regimes and water resources management in the Rhine basin. Climatic Change 2001; 49: 105-128.

Newson M, Sear D, Soulsby C. Incorporating hydromorphology in strategic approaches to managing flows for salmonids. Fisheries Management and Ecology 2012; 19: 490-499.

Ottaway EM, Carling PA, Clarke A, Reader NA. Observations on the structure of brown trout, *Salmo trutta Linnaeus*, redd. Journal of Fish Biology 1981; 19: 593–607.

Rau GC, Andersen MS, McCallum AM, Acworth RI. Analytical methods that use natural heat as a tracer to quantify surface water-groundwater exchange, evaluated using field temperature records. Hydrogeology Journal; 18: 1093-1110.

Revil A. Thermal conductivity of unconsolidated sediments with geophysical applications. Journal of Geophysical Research-Solid Earth 2000; 105: 16749-16768.

Riedl C, Peter A. Timing of brown trout spawning in Alpine rivers with special consideration of egg burial depth. Ecology of Freshwater Fish 2013: 1-14.

Sawyer AH, Cardenas MB, Buttles J. Hyporheic exchange due to channel-spanning logs. Water Resources Research 2011; 47.

Schager E, Peter A, Burkhardt-Holm P. Status of young-of-the-year brown trout (*Salmo trutta* fario) in Swiss streams: factors influencing YOY trout recruitment. Aquatic Sciences 2007; 69: 41-50.

Schälchli U. Basic equations for siltation of riverbeds. J. Hydraul. Eng.-Asce 1995; 121: 274-287.

Scheurer K, Alewell C, Banninger D, Burkhardt-Holm P. Climate and land-use changes affecting river sediment and brown trout in alpine countries - a review. Environmental Science and Pollution Research 2009; 16: 232-242.

Schindler Wildhaber Y, Liechti R, Alewell C. Organic matter dynamics and stable isotope signature as tracers of the sources of suspended sediment. Biogeosciences 2012a; 9: 1985-1996.

Schindler Wildhaber Y, Michel C, Burkhardt-Holm P, Bänninger D, Alewell C. Measurement of spatial and temporal fine sediment dynamics in a small river. Hydrol. Earth Syst. Sci. 2012b; 16: 1501-1515.

Schön J. Physical properties of rocks: fundamentals and principles of petrophysics - handbook of geophysical exploration. New York: Pergamon, 1996.

Sear DA. Fine sediment infiltration into gravel spawning beds within a regulated river experiencing floods - ecological implications for salmonids. Regulated Rivers-Research & Management 1993; 8: 373-390.

Sear DA, Frostick LB, Rollinson G, Lisle TE. The significance and mechanics of fine-sediment infiltration and accumulation in gravel spawning beds. Am. Fish. Soc. Symposium 2008; 65: 149-173.

Seydell I, Ibisch RB, Zanke UCE. Intrusion of suspended sediments into gravel riverbeds: influence of bed topography studied by means of field and laboratory experiments. Advanc.Limnol. 2009; 61: 67-85.

Silliman SE, Ramirez J, McCabe RL. Quantifying downflow through creek sediments using temperature time-series - One-dimensional solution incorporating measured surface-temperature. Journal of Hydrology 1995; 167: 99-119.

Soulsby C, Malcolm IA, Tetzlaff D, Youngson AF. Seasonal and inter-annual variability in hyporheic water quality revealed by continous monitoring in a salmon spawning stream. River research and applications 2009; DOI: 10.1002/rra.1241.

Sponagel H, Grottenthaler W, Hartmann K-J, Hartwich R, Janetzko P, Joisten H, et al. Bodenkundliche Kartieranleitung. Hannover: Bundesanstalt fuer Geowissenschaften und Rohstoffe, 2005.

Stucki P. Methoden zur Untersuchung und Beurteilung der Fliessgwaesser. Makrozoobenthos Stufe F. Bundesamt fuer Umwelt, Bern. Umwelt-Vollzug 2010; 1027.

Thodsen H. The influence of climate change on stream flow in Danish rivers. Journal of Hydrology 2007; 333: 226-238.

Tonina D, Buffington JM. Hyporheic exchange in gravel bed rivers with pool-riffle morphology: Laboratory experiments and three-dimensional modeling. Water Resources Research 2007; 43: 16.

Tonina D, Buffington JM. A three-dimensional model for analyzing the effects of salmon redds on hyporheic exchange and egg pocket habitat. Canadian Journal of Fisheries and Aquatic Sciences 2009; 66: 2157-2173.

Wohl E. Human impacts to mountain streams. Geomorphology 2006; 79: 217-248.

Youngson AF, MacLean JC, Fryer RJ. Rod catch trends for early-running MSW salmon in Scottish rivers (1952-1997): divergence among stock components. Ices Journal of Marine Science 2002; 59: 836-849.

Zimmermann AE, Lapointe M. Integranular flow verlocity through salmonid redds: sensitivity to fines infiltration from low intensity sediment transport events. River research and applications 2005; 21: 865-881.

Chapter 6

Relative importance of fine sediment, hydraulic gradients and river morphology for brown trout embryo survival in a heavily modified river

This chapter is submitted as a full paper to *Science of the Total Environment*:

Michel C*, Schindler Wildhaber Y*, Epting J, Thorpe KL, Huggenberger P, Alewell C, and Burkhardt-Holm P. Relative importance of fine sediment, hydraulic gradients and river morphology for brown trout embryo survival in a heavily modified river. * Shared first authorship

Abstract

Worldwide declines of native salmonid populations are linked to habitat degradation. Increased fine sediment (< 2 mm) accumulation, decreasing oxygen supply to embryos, can be one contributing factor. Oxygen supply also depends on gravel permeability and hydraulic gradients, which affect water exchange in redds. Therefore, a generalized approach is necessary to understand constraints for salmonid embryo survival in the many modified river environments. We investigated brown trout embryo survival to hatch (STH) together with ten physicochemical, hydraulic and morphological parameters in a heavily modified stream. The most important predictors for embryo survival were identified by multivariate statistical modelling. Up to 50% brown trout embryos survived with interstitial oxygen exceeding 3 mg L^{-1}; embryos endured up to six days ≤ 1 mg L^{-1}. Oxygen depletion had a stronger effect on late stage embryo survival. Partial least squares regression identified the horizontal hydraulic gradient, fredle index, distance to artificial log steps upstream, and amount of accumulated fine sediment as influential predictors for embryo STH. Among these, 70.9 % of the variation in STH could be explained by a logistic regression model containing redd distance to the next upstream step (26.4%, $p = 0.004$), fredle index (27.2%, $p = 0.003$), and horizontal hydraulic gradient (10.1%, $p = 0.04$). The amount accumulated fine sediment ($p = 0.75$), field seasons ($p = 0.93$) and field sites ($p = 0.66$) were non-significant. These results suggest that salmonid STH is sensitive to redd gravel permeability, measured as fredle index, and negatively affected by accumulated fine sediment. This effect was counterbalanced by hydraulic gradients related to artificial log steps, which enhanced hyporheic exchange. We provide evidence that in some heavily modified rivers artificial steps could support salmonid STH. Finally, a conceptual summary is delineated to illustrate the interaction of investigated parameters and how they could affect brown trout STH in comparable heavily modified rivers.

Keywords: *hyporheic exchange, salmonid fish, river restoration, artificial steps, habitat degradation, human impact, Swiss plateau*

Introduction

Native salmonid populations are reported to be in decline in Switzerland (Burkhardt-Holm et al., 2005), the United Kingdom (Youngson et al., 2002), and North-America (Brown et al., 1994; Huntington et al., 1996). Among others, habitat degradation is considered a major contributing factor (e.g. Hicks et al., 1991; Brown et al., 1994; Gilvear et al., 2002; Burkhardt-Holm and Scheurer, 2007) with numerous studies focusing on fine sediment as a single factor for decreased salmonid embryo survival (e.g. Jensen et al., 2009 and studies cited therein). However, recent research substantiates that a more generalized perspective is necessary to understand the constraints for salmonid incubation success in degraded and modified river environments (Malcolm et al., 2010; Newson et al., 2012).

During spawning, salmonid fish deposit their eggs in distinct gravel nests, called redds, where the embryos incubate up to several months (Crisp, 2000). Embryo survival during this intra-gravel stage depends strongly on oxygen supply to the developing embryos, which itself depends on the water-flow through the egg pocket and the interstitial oxygen concentration (Malcolm et al., 2008). Deposited fine sediment can decrease redd gravel permeability and hence hinder oxygen supply (Greig et al., 2007a; Heywood and Walling, 2007). However, water-flow through salmonid redds also depends on river morphology and hydraulic gradients driving interstitial flow (Soulsby et al., 2001; Malcolm et al., 2006; Malcolm et al., 2010; Schindler Wildhaber et al., submitted). Laboratory studies indicate that hydraulic gradients can to some degree counterbalance the negative effect of fine sediment on interstitial flow and salmonid incubation success (Lapointe et al., 2004). The same could be expected in rivers where hyporheic exchange is strongly influenced by gravel permeability and hydraulic boundary conditions, which also depend on local geomorphic features (Brunke and Gonser, 1997; Baxter and Hauer, 2000; Kasahara and Wondzell, 2003). Altogether, it can be expected that several parameters simultaneously affect water-flow and quality in salmonid redds, and hence embryo survival (Greig et al., 2007b).

In channelized rivers the lack of geomorphic features can substantially decrease hyporheic water exchange (Malcolm et al., 2010), whereas hydraulic gradients related to artificial in-stream structures (e.g. steps, gabions, weirs) can increase hyporheic exchange (Hester and Doyle, 2008). These river modifications, present in many anthropogenically modified fluvial ecosystems of the northern hemisphere (Brookes, 1988; Gilvear et al., 2002; Wohl, 2006), could therefore also affect salmonid incubation success. Despite this, no field study so far has

simultaneously investigated the relative importance of fine sediment, hydraulic gradients and structural modifications for salmonid embryo survival. Such a study could help to better understand the factors contributing to successful embryo incubation in heavily modified rivers, thereby also providing new perspectives for management decisions to maintain viable salmonid populations.

The current study investigates the relative importance of ten factors that could affect brown trout embryo survival to hatch (STH) in a small and heavily modified headwater stream of the Swiss Plateau (Enziwigger, Canton Lucerne, Switzerland; Figure 1). During land reclamation in the 20th century, the Enziwigger was straightened, channelized, and cross-channel log steps were installed to prevent bed scouring during flood events (Figure 1). As a consequence hyporheic exchange within terraces (i.e. intermediate scale) is markedly influenced by the steps (Huber et al., in press). Despite its strong modifications, it maintains a viable brown trout *Salmo trutta* population (Schager et al., 2007). Schindler Wildhaber et al. (submitted) demonstrated that specific water infiltration and oxygen concentrations in the investigated artificial brown trout redds were affected by the amount accumulated fine sediment, as well as local and regional hydraulic gradients related to the river morphology. The current analysis therefore not only focused on fine sediment deposition, but a suite of factors that could simultaneously affect brown trout embryo STH in the Enziwigger. Particularly, we included hydraulic gradients and the modified river structure to provide a holistic perspective on salmonid embryo survival in a strongly modified river environment.

Materials & Methods

Field location

The Enziwigger is a straightened and channelized stream located in central Switzerland (Canton of Lucerne) and has a total watershed area of about 31 km^2 (Figure 1). Mean discharge, measured in Willisau by the Cantonal authorities (Nov. 2007 – Nov. 2008) was 2.1 m^3 s^{-1}, minimum and maximum discharge were 1.1 m^3 s^{-1} and 10.1 m^3 s^{-1}, respectively. Like most rivers in the Swiss Plateau, the morphology of the Enziwigger is strongly modified: 5% is natural or close to natural, 21% is little affected and 74% is strongly affected or artificial (EBP-WSB-Agrofutura, 2005). Artificial in-stream structures include lateral stabilizations and steps to prevent deep channel erosion and bed-scouring during flood events (Figure 1). Despite the strong morphological modifications, the biological condition is considered good (EBP-WSB-Agrofutura, 2005). The only fish species in the Enziwigger is the brown trout,

which maintains a viable population (Schager et al., 2007). No stocking is carried out and the river is neither affected by hydropower nor by effluents of wastewater treatment plants.

Experimental design

In winter 2009/10 (season 1) and winter 2010/11 (season 2) artificial brown trout redds were set up at three sites along the river (Figure 1). The most upstream site A is located in an area surrounded by forest and pasture. Sites B and C are in areas dominated by pasture and arable land. All sites contain artificial steps and terraces, which create a repetitive step-pool-glide morphology (Figure 1). In each season six artificial redds were built per site, resulting in a total of 36 investigated redds. Redds were located in areas where natural brown trout redds are regularly observed (pers. obs.; P. Amrein, Fish and Wildlife Service, Canton Lucerne). Within terraces redds were built in glide sections, always one towards the downstream step and one more towards the upstream step (Figure 1). In the following, when referring to a particular redd and season, the redd name (e.g. C31) is extended by _S1 (for season 1) or _S2 (for season 2), respectively.

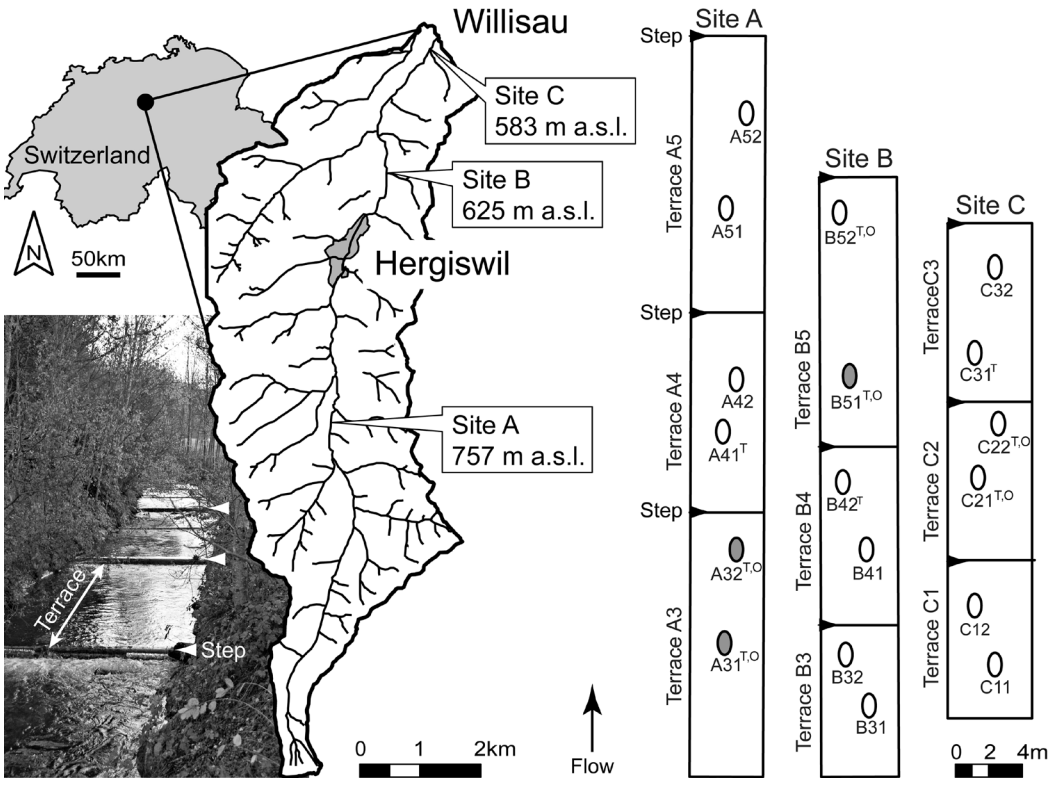

Figure 1 – Location of the Enziwigger watershed in Switzerland. The photograph shows the step and terrace structure at study site B. The watershed map of the river Enziwigger shows the towns Willisau and Hergiswil (Canton of Lucerne, Switzerland) and the location of the three field sites A, B and C. The schematic on the right illustrates the location of the redds within each field site. Here, superscripts indicate redds with continuous temperature (T) and oxygen (O) measurements, redds with continuous oxygen and embryo survival data discussed in the text are grey.

Brown trout embryo survival to hatch (STH) was assessed with egg-capsules (e.g. Scrivener, 1988; Dumas and Marty, 2006) inserted in the redd gravel at a depth commonly observed for riverine brown trout (approx.12–15 cm; DeVries, 1997). Twenty capsules per redd were planted, each with ten fertilized brown trout eggs (n = 200 eggs per redd). Fertilized eggs were obtained during routine collection of spawners by the cantonal authorities in early November and fertilization was conducted at the fish holding facility of the cantonal fish warden in Willisau (P. Amrein, Fish and Wildlife Service, Canton Luzern). For the experiment, eggs stripped from ripe females (season 1: n = 16, length = 25.1 ± 1.8 cm and season 2: n = 10, length = 27.9 ± 2.6 cm; mean ± sd) were pooled and artificially inseminated with pooled milt (season 1: n = 8 males, length = 29.5 ± 1.8 cm and season 2: n = 7, length = 26.1 ± 1.3 cm; mean ± sd). Fish of this size represent first time spawners in the Enziwigger (Schager et al., 2007). After water hardening, eggs were transferred into capsules and transported to field sites in buckets filled with spring water originating from the Enziwigger watershed. The water temperature was slowly adjusted to that of the river, and capsules were planted in redds within less than six hours post fertilization. As control, fertilized eggs (n = 300) were placed directly after water hardening in an egg incubator at the holding facility. To control for the effect of transportation, a further 300 eggs were transported to the field locations, returned to the holding facility, and placed in the egg incubator. The egg incubator was supplied with spring water originating from the Enziwigger watershed. The STH in the incubator was used to determine the natural mortality of the egg batches within each season.

Some egg capsules were lost during bed-scouring events. Embryo STH was therefore determined from different numbers of capsules per redd: The mean number of capsules sampled per redd was 13 (min = 10, max = 14) in season 1 and 17 (min = 9, max = 20) in season 2. Altogether, STH was determined from a mean of 138 (min = 90, max = 200) fertilized brown trout eggs per redd. Prior to data analyses, survival data within each season was normalized to the respective transportation control, which is a common method to account for differences in egg quality between years (cf. Rubin and Glimsäter, 1996).

Data analysis

All data were analyzed with the software R v2.12.0 (R Development Core Team, 2011) using multivariate methods. In total, ten parameters were included as explanatory variables (Table 1). To identify the most important predictors for brown trout STH among these ten explanatory variables we first used partial least squares regression to screen for the influential

variables (PLS regression; Eriksson et al., 2006). PLS regression was used first because it provides a robust approach to evaluate the relative influence of multiple predictor variables on a response variable in co-linear and small-sized data-sets (Wold et al., 2001). Its good performance has been also demonstrated for comparable ecological data-sets (Carrascal et al., 2009). PLS regression was conducted with the function plsr (pls package; Mevik and Wehrens, 2007). The response variable (proportion survival to hatch; STH) was arcsine transformed prior to the analysis (Quinn and Keough, 2002). Once the model was fit, the variable influence on projection score (VIP; calculated in reference to Chong and Jun, 2005) was used to evaluate the influence of each predictor variable on embryo STH. Variables with a VIP > 1 were considered influential (cf. Eriksson et al., 2006).

Table 1 – Explanatory variables included in multivariate analysis. For each variable a short description and references (Ref.) providing detailed information about its meaning and how variables were measuredare given.

No	Variable	Description	Ref.
1	Infiltrated fine sediment	Mean amount of weekly deposited fine sediment in the redd	1, 2
2	Accumulated fine sediment	Net amount of fine sediment deposited over the entire incubation season	1, 2
3	Fredle index	Central tendency measure of sediment composition that relates positively with gravel permeability	2,3,4
4	Water level	Mean water level over the redd;calculated from manual measurements	2
5	Vertical hydraulic gradient	A measure for the vertical hydraulic gradient driving up- and downwelling through the redd, calculated from manual measurements	2,5
6	Horizontal hydraulic gradient	A measure for the hydraulic gradient driving horizontal pumping flow through the redd; calculated from manual measurements	2
7	Interstitial oxygen	Mean interstitial oxygen concentration in the redd;calculated from bi-weekly manual measurements	2
8	Total organic carbon	The net amount of organic carbon deposited in the redd during the incubation season	6
9	Distance to upstream step	Distance of the redd to the step located upstream in the terrace	2,7
10	Distance to downstream step	Distance of the redd to the step located downstream in the terrace	2,7

References: 1 - Schindler Wildhaber et al. (2012b), 2 - Schindler Wildhaber et al. (submitted), 3 - Lotspeich and Everest (1981), 4 - Barnard (1992), 5 - Baxter et al. (2003), 6 - Schindler Wildhaber et al. (2012a), 7 - Huber et al. (in press)

Following this screening, the individual relationship between the identified influential predictor variables (VIP > 1) and embryo STH was quantified by multivariate logistic regression (quasi-binomial error structure, logit link function; Faraday, 2006). Prior to this analysis, the Pearson product-moment correlation coefficient was used to quantify the amount of co-variation among influential variables. Variance inflation factors (VIF; function corvif, AED package) were applied to test if the amount of co-variation among influential explanatory variables prohibits using them in a single multivariate regression model (Zuur et al., 2010). To account for effects of our sampling design, both field seasons and field sites, as well as their interaction, were included as categorical explanatory variables in the initial full model. Significant predictor variables were then identified via step-wise model simplification using pair-wise model comparisons (Faraday, 2006). As recommended for logistic regression models with a quasi-binomial error structure *F*-tests were applied in pair-wise comparisons (function: drop1; Faraday, 2006). Significance was accepted at $p \leq 0.05$.

A limited number of redds were equipped with continuous oxygen measurement probes (Figure 1; cf. Schindler Wildhaber et al., submitted). Unfortunately, in most of these redds the egg capsules were lost during bed-scouring events (see below). Hence, these data could not be included in the multivariate analysis, but will be discussed qualitatively.

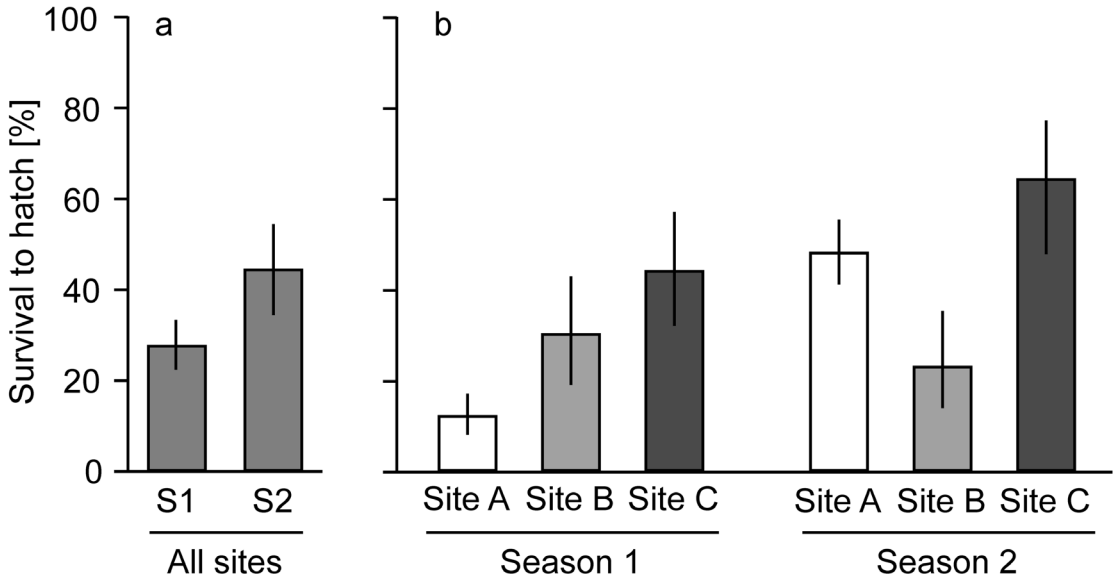

Figure 2 – Graph (a) shows the mean embryo survival to hatch for all study sites during seasons 1 (S1) and 2 (S2). Graph (b) shows the mean embryo survival to hatch in the individual field sites (A, B and C) for seasons 1 and 2. Bars represent mean ± SE.

Results

Embryo survival among years and sites

Mean brown trout STH in the control/transportation control was 73%/76% and 72%/60% in seasons 1 and 2, respectively. The corrected mean ± SEM brown trout STH in the Enziwigger was 34 ± 4%, and ranged from a minimum of zero survival in a redd at site A (redd A31_S1) to a maximum of 74% at the downstream site C (redd C31_S2). The mean STH within seasons showed fluctuations between field sites (Figure 2b). Compared to the other explanatory variables these differences were non-significant in the multivariate logistic regression analysis (see below).

Oxygen concentration in redds and embryo survival

In several redds egg capsules were lost during bed-scouring events, including most redds with continuous oxygen measurements (cf. Schindler Wildhaber et al., submitted). Altogether, four redds with continuous oxygen measurements and embryo survival data remained intact, and this data will be discussed here. Oxygen concentrations during the incubation season are compared to a reference value of 3 mg L^{-1}, because brown trout embryos have been documented to survive well with this oxygen level (Roussel, 2007; 8.2 ± 0.6 °C water temperature). In **redd A32_S1** interstitial oxygen was initially high, but dropped sharply in early December occasionally reaching complete depletion (Figure 3a). Altogether, interstitial oxygen was below 3 mg L^{-1} on 44 days in this redd, and below 1 mg L^{-1} for 22 of these days. The mean ± sd oxygen concentration was 4.6 ± 3.3 mg L^{-1}, and embryo STH was 10%. In **redd A32_S2** oxygen concentrations were mostly above 8 mg L^{-1}, and no day below 3 mg L^{-1} occurred (Figure 3b). The mean ± sd oxygen concentration was 10.8 ± 1.8 mg L^{-1} and embryo STH was 49%. In **redd A31_S2,** which was built about six meters upstream of A32_S2, interstitial oxygen was more variable throughout the incubation season and altogether 14 days with less than 3 mg L^{-1} occurred (Figure 3c). Moreover, embryos in this redd experienced six days with less than 1mg L^{-1} oxygen in mid-January. The mean ± sd oxygen concentration was 8.4 ± 3.4 mg L^{-1}, and embryo STH was 47%. In **redd B51_S2** oxygen concentrations were mostly above 5 mg L^{-1} until early March, but then oxygen levels dropped below 3 mg L^{-1} until hatch, in this period also four days with less than 1 mg L^{-1} occurred (Figure 3d). The mean ± sd oxygen concentration was 8.9 ± 2.5 mg L^{-1}, and embryo STH was 35%. For a discussion of the continuous oxygen data from all investigated redds see Schindler Wildhaber et al. (submitted).

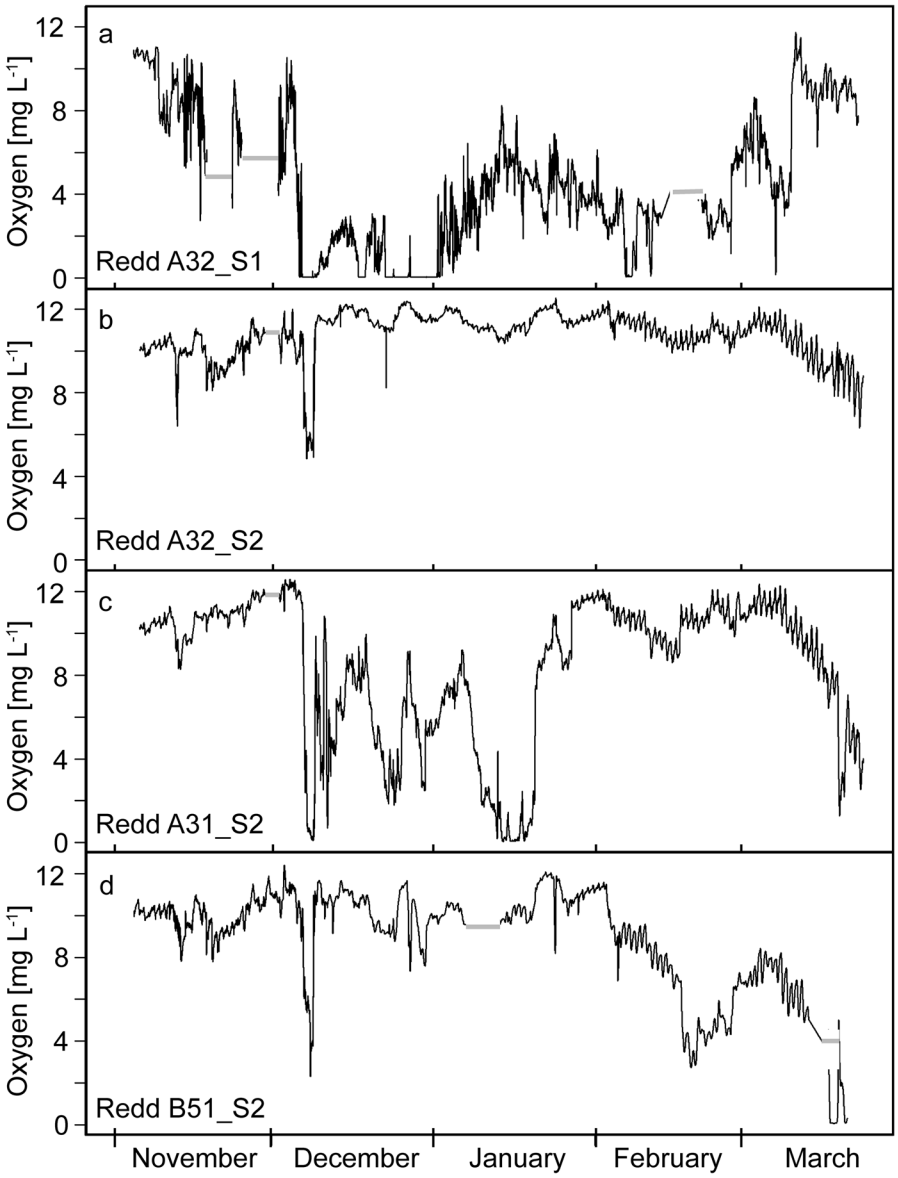

Figure 3 – Interstitial oxygen concentration in the four redds where continuous measurements and embryo survival data were available. Horizontal gray bars mark periods of missing data.

Table 2 – Results of the multivariate logistic regression analysis. Given are the explained deviance (D_{exp}), parameter estimates (Estimate) and their standard error (SE), as well as their significance levels (*p*) from the optimal logistic regression model. Dist. step up = distance to upstream step, HHG = Horizontal hydraulic gradient, see also Table 1.

Variable	D_{exp}	Estimate	SE	*p*
Intercept		-1.09	0.42	0.021
Fredle index	27.2%	0.15	0.04	0.003
Dist. step up	26.4%	-0.15	0.04	0.004
HHG	10.1%	10.19	4.57	0.045
Total	70.9%			

Relative importance of factors affecting embryo survival

The PLS regression identified the horizontal hydraulic gradient, the fredle index, the distance to the upstream step, and the amount accumulated fine sediment as influential predictors for embryo STH in the Enziwigger (VIP > 1; Figure 4). The PLS regression model containing these four influential parameters explained 71.4% of the variance in embryo STH.

The only significant correlation among influential predictor variables could be observed between the fredle index and the amount of accumulated fine sediment (Figure S1a). Variance inflation factors permitted using all influential variables in a single multivariate regression model (all VIFs ≤ 2.57; Zuur et al., 2010). Among influential predictor variables step-wise logistic regression analysis identified the fredle index, the distance to the upstream step, and the horizontal hydraulic gradient as significant explanatory variables (Table 2). The logistic regression model containing these three explanatory variables explained 70.9% of the total deviance in embryo STH (Table 2). The fredle index and the distance to the next upstream step explained equal proportions of 27.2% and 26.4%, respectively. The horizontal hydraulic gradient was just significant explaining 10.1% (Table 2), but this result was mostly related to two influential data-points (Figure 5c) from a single redd consistently located below a step in site C (redd position C31, Figure 1). Altogether, embryo STH decreased with distance to the next upstream step (Figure 5a) and increased with the fredle index (Figure 5b), and the horizontal hydraulic gradient (Figure 5c). The amount of accumulated fine sediment (p = 0.75), field seasons (p = 0.93) and field sites (p = 0.66) did not contribute significantly.

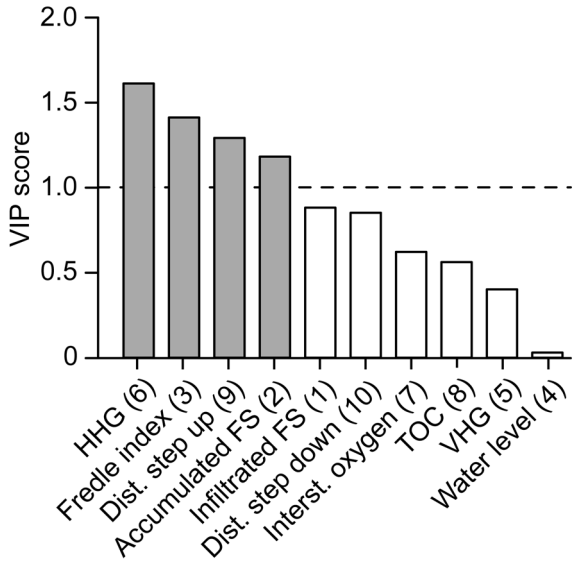

Figure 4 – Variable influence on prediction (VIP) scores for all explanatory variables evaluated in the partial least squares regression analysis. Influential predictor variables (VIP > 1, horizontal dashed line), also included in the multivariate logistic regression analysis, are marked in grey. Abbreviations: horizontal hydraulic gradient (HHG), total organic carbon (TOC), vertical hydraulic gradient (VHG), and fine sediment (FS); number in brackets gives the identifier of the variable in Table 1.

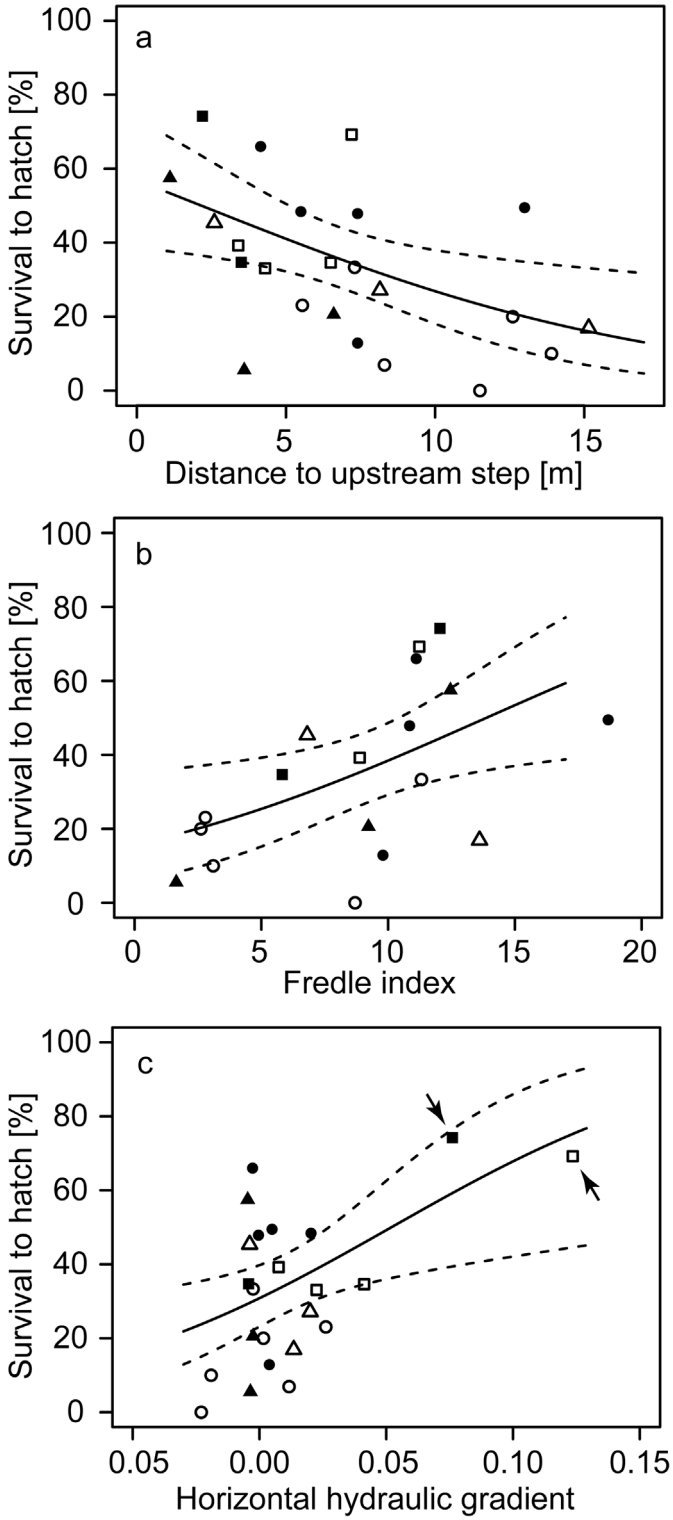

Figure 5 – Relationship between brown trout survival to hatch and significant predictor variables identified in the multivariate logistic regression (Table 2). Lines are mean regression line ± 95% point-wise confidence intervals as predicted from the fitted generalized linear model. Symbol filling denotes seasons (2009/10 = open, 2010/11 = filled) and shape denotes field sites (● = site A, ▲ = site B, and ■ = site C). Arrows in graph c mark influential data-points mentioned in the results section.

Integrating over the whole project of the last 4 years (this study and Schindler Wildhaber et al., submitted) a process-based conceptual summary of the interaction of investigated parameters and how they affect brown trout embryo STH in comparable heavily modified river environments can be delineated (Figure 6): Flow dynamics in the Enziwigger affect gravel permeability e.g. by causing sediment re-suspension, and by affecting hydraulic gradients. Hydraulic gradients and gravel permeability jointly drive water exchange in the redds . Hydraulic gradients further affect fine sediment accumulation, with increased accumulation related to down-welling. The interstitial oxygen concentration in the redds is affected by the degree of hyporheic water exchange itself but also the relative contribution of ground- and surface water. The interstitial oxygen concentration as well as the frequency and duration of periods of low oxygen then affect salmonid embryo survival.

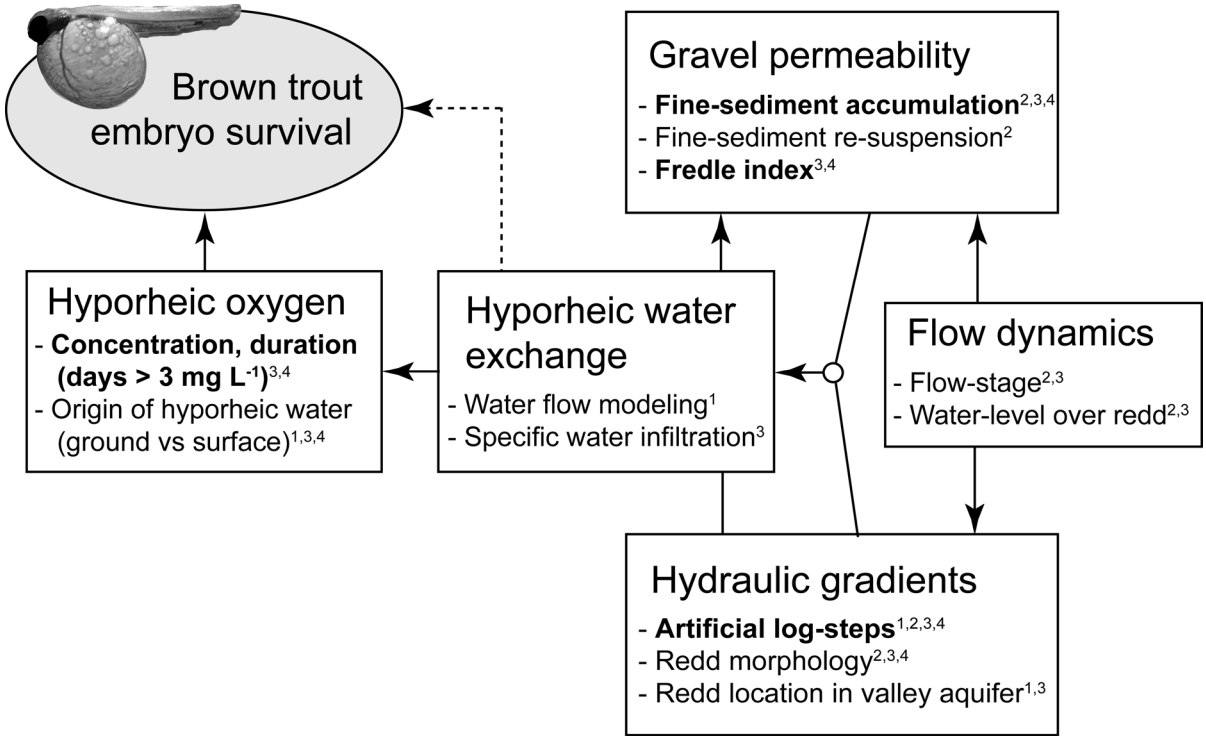

Figure 6 – Conceptual summary illustration of the interaction of investigated parameters and how they affect brown trout embryo survival in our heavily modified study river. In each box a general descriptive term is given on top, with parameters investigated in our project listed below: [1]Huber et al. (in press), [2]Schindler Wildhaber et al. (2012b), [3]Schindler Wildhaber et al. (submitted), and [4]this study (bold parameters directly affected embryo survival). Arrows indicate that parameter affects the response parameter directly, and the open circle indicates that parameters jointly affect the response parameter. Dashed arrow indicates that water exchange might also affect embryo directly survival e.g. by removing metabolic waste.

Discussion

A generalized approach, integrating the sciences of geomorphology, hydrology and freshwater ecology, is necessary to better understand the constraints for salmonid incubation success in the many degraded and modified fluvial ecosystems that provide spawning habitat for salmonids (Gilvear et al., 2002; Malcolm et al., 2010; Newson et al., 2012). To this end we evaluated the relative importance of ten factors potentially affecting brown trout embryo survival in a heavily modified headwater stream of the Swiss Plateau.

Embryo survival among years and sites

The corrected embryo STH for the Enziwigger (mean = 34%, min = 0%, max = 74%) was comparable with a field study investigating brown trout embryo STH using artificial redds in streams in Sweden (Rubin and Glimsäter, 1996; mean = 32%, min = 0%, max = 89%) but lower than values reported for a study in France (Dumas et al., 2007; mean = 53%, min = 45%, max = 88%). Survival to hatch in our controls was lower than for egg batches from larger brown trout (Rubin, 1995; Rubin and Glimsäter, 1996; Dumas et al., 2007), but in the range of similar sized spawners (Roussel, 2007). Brown trout available for our experiment were most likely first time spawners, which can produce eggs with lower quality and reduced survival (Bromage et al., 1992; Brooks et al., 1997). Also differences in genetic composition of the spawners, which can affect embryo survival in salmonids, may have contributed (Brooks et al., 1997; Nagler et al., 2000). Finally, smaller brown trout can be expected to produce smaller eggs (Roff, 1992), which are reported to be more sensitive to oxygen depletion (Einum et al., 2002). We cannot conclude, which of these factors contributed to the lower survival observed in our experiment, but as control embryo survival was consistent among both field seasons our conclusions about the factors affecting brown trout embryo STH in the Enziwigger remain valid.

The differences in embryo survival between years and sites observed here (Figure 2a, b) are common in natural river systems (Meyer, 2003; Bagliniere et al., 2005; Dumas et al., 2007). Bagliniere et al. (2005) reported up to five-fold inter-annual variation in brown trout embryo survival in two tributaries of the River Oir, France. Dumas et al. (2007), using similar egg-capsules to ours, documented up to two-fold differences in brown trout STH between sampling sites in other French streams. These fluctuations were attributed to changes in interstitial water quality or spawning gravel composition (e.g. gravel permeability, organic

content, or amount fine sediment). Indeed, our multivariate analysis indicated that gravel permeability, measured as Fredle index, and to a lesser degree accumulated fine sediment affected brown trout STH in the Enziwigger. In contrast, total organic carbon (TOC) content did not have a marked influence, most likely because values observed in the Enziwigger are far lower than in other river systems (Schindler Wildhaber et al., 2012a).

Oxygen concentration in redds and embryo survival

Sufficient interstitial oxygen is crucial for salmonid embryo survival during their intra-gravel stage (Greig et al., 2007b; Malcolm et al., 2008). The importance of sufficient interstitial oxygen for brown trout embryo survival can be seen in redd A32_S1, where oxygen concentrations were below 3 mg L^{-1} on 44 days and of these below 1 mg L^{-1} on 22 days between December and early January. These periods of very low oxygen concentrations likely caused the low embryo survival of 10% in season 1 – especially since embryo STH was markedly increased at this redd location in season 2 (redd A32_S2), when no day with less than 3 mg L^{-1} occurred. Moreover, embryo STH in redd A31_S2 (47% STH) was comparably to the STH in redd A32_S2 despite six days with less than 1 mg L^{-1} in mid-January. Our continuous oxygen data therefore suggests that up to 50% of brown trout embryos may survive in natural redds when oxygen concentrations exceed 3 mg L^{-1} during most of the incubation season, which agrees with laboratory studies (Einum et al., 2002; Roussel, 2007), but contrasts critical values inferred from manual measurements in other field studies (see below).

It is also rarely considered in field studies that the oxygen demand of salmonid embryos changes with developmental stage, being at maximum around hatch (Greig et al., 2007b). In Atlantic salmon *Salmo salar* critical values of 0.53 to 2.17 mg L^{-1} have been reported for early eggs, which increased towards 4.06 to 7.00 mg L^{-1} close to hatch (Crisp, 2000). We observed 12% higher embryo survival in redd A31_S2 compared to redd B51_S2. The major difference between these redds was not the mean oxygen concentration, which was even 0.5 mg L^{-1} higher in redd B51_S2, but the timing of phases of oxygen depletion: In redd A31_S2 embryos experienced eight days below 3 mg L^{-1} in mid-January, but towards hatch oxygen concentrations were consistently above 3 mg L^{-1}. In contrast, interstitial oxygen in redd B52_S2 was consistently above 3 mg L^{-1} until early-March, but then, just around hatch, the oxygen level dropped below 3 mg L^{-1}, reaching almost complete depletion for four days. In the light of these data, we suggest that under many environmental conditions the timing and

duration of low oxygen, rather than a mean oxygen concentration, is relevant for salmonid embryo survival.

Mean critical oxygen concentrations for brown trout reported in field studies, calculated from manual point-wise measurements, range between 6.9 and 9.9 mg L^{-1} (reviewed in Malcolm et al., 2008). In our study STH was still noticeable when oxygen concentrations exceeded 3 mg L^{-1} during most of the incubation season. Mean oxygen concentrations, calculated from bi-weekly point-wise manual measurements, had also no explanatory power in our multivariate analyses, most likely because there were no accurate measures of the oxygen concentration present in our redds (cf. Schindler Wildhaber et al., submitted). Given the high variability of oxygen concentrations in salmonid redds, it is unlikely that manual measurements can reflect the actual oxygen concentration affecting salmonid embryos (see also Malcolm et al., 2006; Malcolm et al., 2010). While repeatedly low values might indicate critical hyporheic conditions for embryo incubation, a few high values will not necessarily indicate good spawning gravel quality. Most embryo mortality could occur during critical phases of severe oxygen depletion that point measurements can miss. Taken together, reported mean critical oxygen concentrations of 6.9 to 9.9 mg L^{-1} calculated from manual measurements (reviewed in Malcolm et al., 2008) possibly overestimate oxygen concentrations needed for successful brown trout embryo incubation.

An average of 70% STH has been demonstrated for brown trout embryos reared under hypoxic (3.0 mg L^{-1}) conditions, which was only 15% lower than under normoxic (10.3 mg L^{-1}) conditions (Roussel, 2007). In another study, 57% of brown trout embryos exposed to 2.3 mg L^{-1} oxygen from the eyed-stage onward survived to hatch, which was on average 37% lower than with 14 mg L^{-1} oxygen (Einum et al., 2002). Together with our data we therefore suggest that brown trout embryos can survive when oxygen concentrations above 3 mg L^{-1} predominate, a value comparable to the lower critical level of 4.06 mg L^{-1} reported for Atlantic salmon close to hatch (Crisp, 2000). Moreover, they can likely endure up to a week with less than 1 mg L^{-1}, especially during early embryo development. Salmonid embryos regularly adjust their developmental and metabolic rates to cope with phases of low oxygen (Geist et al., 2006; Miller et al., 2008), which can affect growth, muscle development and heart rates (Matschak et al., 1997; Matschak et al., 1998; Czerkies et al., 2002; Roussel, 2007). We suspect that brown trout embryos in our study also adjusted their metabolic rates to

cope with phases of low oxygen, although this and potential consequences later in life remain to be investigated.

Relative importance of factors affecting embryo survival

Fine sediment in salmonid redds can negatively affect embryo survival by decreasing redd gravel permeability, interstitial flow and hence oxygen supply (Greig et al., 2007b; Jensen et al., 2009).We measured three parameters to assess different aspects of fine sediment effects on brown trout embryo STH: *i*.) The infiltration rate, a measure of the weekly fine sediment deposition, identifying redds located in areas with repeated high fine sediment deposition. *ii*.) fine sediment accumulation measuring the net fine sediment deposition over the entire incubation period, identifying redds with high fine sediment content and *iii*.) the fredle index, a granular metric, integrating the geometric mean and the dispersion of particle sizes and thus representing a redd gravel quality index related to gravel permeability (Lotspeich and Everest, 1981; Barnard, 1992). In our analysis, the fredle index was a slightly better predictor for brown trout STH than the amount of accumulated fine sediment. The latter was only identified as influential in the partial least squares regression, but was non-significant in the multivariate logistic regression model. Granular metrics (e.g. geometric mean diameter (d_g), fredle index) as predictors of embryo survival have been discussed to be site specific, and their explanatory power might differ between river systems (Malcolm et al., 2008). In some rivers, they have been suggested as weak predictors for salmonid embryo STH (Greig et al., 2005). In others, a positive relationship between the fredle index and embryo STH was found, mostly in redds with sufficient oxygen and hydraulic gradients to drive interstitial flow (Sowden and Power, 1985). Thus, they might only be good predictors for pre-emergent salmonid embryo survival in rivers where most hyporheic water originates sufficiently oxygenated surface water (Sowden and Power, 1985; Malcolm et al., 2008). Our results agree with this notion, since most hyporheic water in the Enziwigger originates from well oxygenated surface water with short residence times and less from long residence groundwater, which could be oxygen depleted (Huber et al., in press). Altogether, our results corroborate that increased fine sediment can decrease salmonid embryo survival *via* its negative effect on gravel permeability

Laboratory studies indicate that the negative effect of fine sediment on gravel permeability can be counterbalanced by hydraulic gradients driving sufficient interstitial flow (Lapointe et al., 2004). This is the most likely explanation for the positive effect of the artificial step

structures on brown trout embryo survival documented here. In support of this, the steps had a marked effect on hydraulic exchange within the terraces (Huber et al., in press), as well as water exchange and abiotic conditions in our redds (Schindler Wildhaber et al., submitted). Comparable studies that specifically investigated the effect of artificial in-stream structures on salmonid embryo survival are sparse. The publication of Klassen and Northcote (1988) is, to our knowledge, the only study that specifically investigated the effect of gravel filled cross-channel wire cages (i.e. weir gabions) on Pink salmon *Oncorhynchus gorbuscha* embryo survival. The authors compared the mean embryo survival between gabion sites and reference locations, without considering the redd position relative to the structures. This might explain why they did not find any effect, because the positive effect of cross-channel in-stream structures on hyporheic exchange strongly depends on the distance to the structure (Endreny et al., 2011). Hence, our analysis, which explicitly considered the redd location relative to the steps, provides first evidence that artificial in-stream structures could affect salmonid embryo survival, at least in channelized rivers with good hyporheic water quality.

Steps in general, either natural or artificial, have a marked influence on hyporheic water exchange in streams (Kasahara and Wondzell, 2003; Gooseff et al., 2006; Tonina and Buffington, 2007). Usually down-welling occurs just above the step, whereas upwelling of hyporheic water dominates downstream of the step (Kasahara and Hill, 2006; Endreny et al., 2011). In the Enziwigger a similar exchange pattern was confirmed by direct measurements of hydraulic heads, conductivity, and temperature in river and groundwater as well as by groundwater flow modeling (Huber et al., in press) and specific water infiltration rates in our redds (Schindler Wildhaber et al., submitted). Accordingly, brown trout embryo STH increased in redds located closer to or in the upwelling zone below steps. Increased embryo survival in upwelling sites was also observed for sockeye salmon *Oncorhynchus nerka* (Garrett et al., 1998) and for brook trout *Salvelinus fontinalis* as well as brown trout in the Midwest and eastern regions of the USA (Waters, 1995). We see two possible explanations: i) Either, the upwelling water decreased fine sediment accumulation (e.g. Seydell et al., 2009) and thus increased gravel permeability, or upwelling water per se provided a more stable incubation environment (see below). In our study, gravel permeability and fine sediment accumulation were only related to the distance to the downstream step (Schindler Wildhaber et al., 2012b), but not the distance to the upstream step. Therefore, it appears more likely to us that the upwelling water provided a more stable incubation environment, which can support salmonid embryo survival in rivers where the hyporheic water quality is good (Hansen, 1975;

Bjorn and Reiser, 1991). As outlined above this is most likely the case in the Enziwigger, since most hyporheic water represents short-residence surface water of good quality (Schindler Wildhaber et al., 2012a; Huber et al., in press).

Conclusion

Our results suggest that up to 50% brown trout embryo survival can occur in natural redds when oxygen concentrations exceed 3 mg L^{-1}, and even with up to six days below 1 mg L^{-1}. Likewise, our results suggest that brown trout embryos might be more sensitive to oxygen depletion ($\leq$ 1 mg L^{-1}) close to hatch. Often, manual measurements are unlikely to provide accurate measures for the actual oxygen concentration affecting salmonid embryos in natural redds. Successful river management in salmonid streams should therefore focus on factors that affect the dynamics of oxygen supply to incubating salmonid embryos. In this regard, we provide evidence that in modified river environments hydraulic gradients related to artificial in-stream structures could sometimes counterbalance the negative effect of fine sediment on gravel permeability, and hence benefit salmonid embryo survival. In support of this, the distance to the log-steps also affected specific water infiltration rates and oxygen concentrations in our redds (Schindler Wildhaber et al., submitted). It is likely that this result can also be transferred to other surface-water dominated river systems with good water quality, however, whether this result can be transferred to river systems with other hydraulic boundary conditions, for example groundwater-dominated rivers, remains to be evaluated. Altogether, the results presented here and in Schindler Wildhaber et al. (submitted) clearly indicate that finding a single best predictor for salmonid embryo survival is not feasible and might be at worst misleading. Instead, future river management should apply a process-based understanding, integrating the sciences of hydrology, geomorphology and aquatic ecology, to understand and shape the factors that ultimately determine successful embryo incubation in the many heavily modified salmonid streams of the northern hemisphere.

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Supplementary information

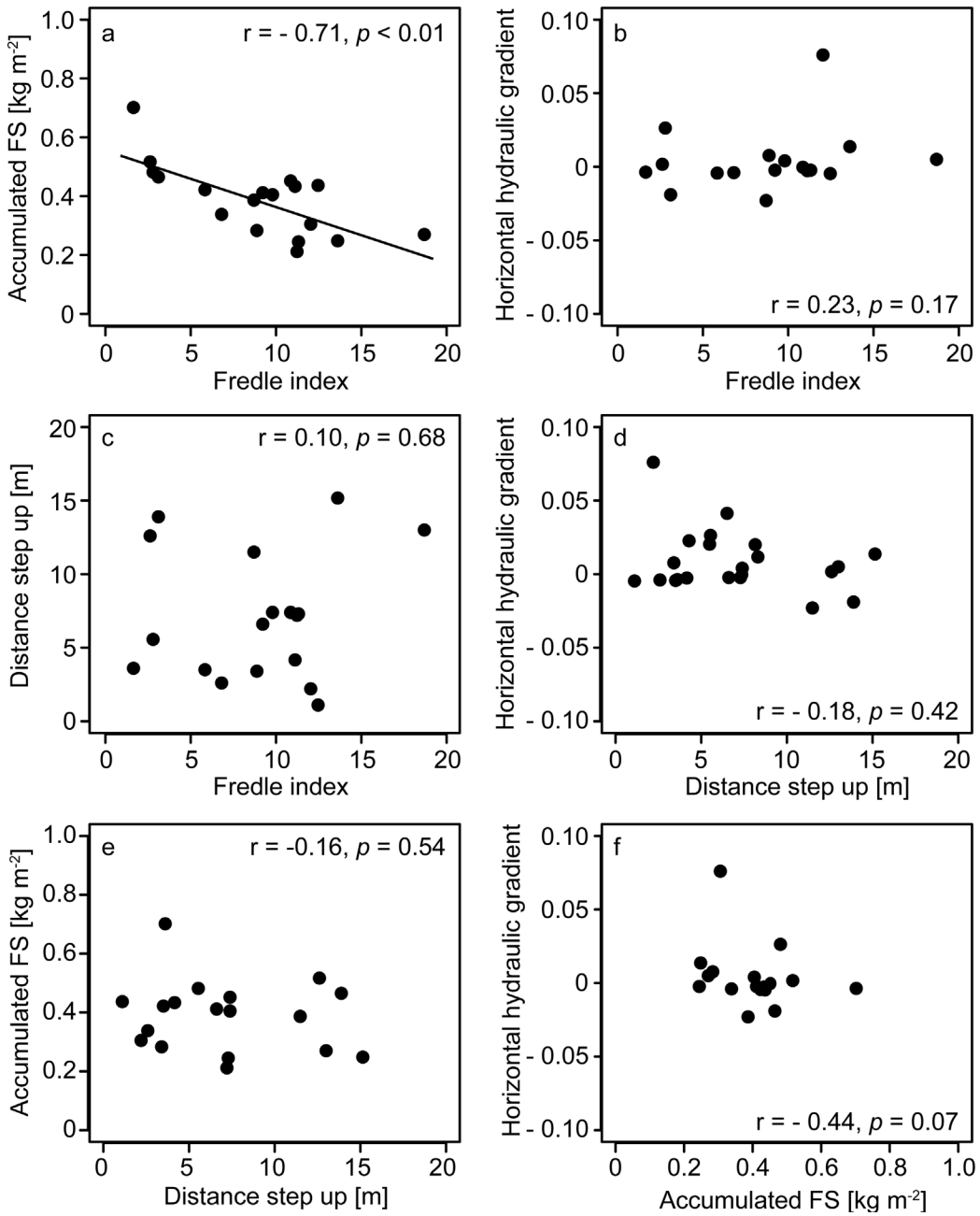

Figure S1 – Pair-wise relationship between all influential predictor variables identified in partial least squares regression analysis (VIP > 1, Figure 4). Within each graph the Pearson product-moment correlation coefficient (*r*) and the *p*-value (*p*) are given. Black line represents best fit line. Abbreviations are FS = fine sediment, Distance step up = Distance to next upstream step.

References

Bagliniere JL, Marchand F, Vauclin V. Interannual changes in recruitment of the Atlantic salmon (*Salmo salar*) population in the River Oir (Lower Normandy, France): relationships with spawners and in-stream habitat. Ices Journal of Marine Science 2005; 62: 695-707.

Barnard K. Physical and chemical conditions in Coho salmon (*Oncorhynchus kisutch*) spawning habitat in freshwater creek, Northern California. MSc. Humboldt State University, Arcata CA, 1992, pp. 81.

Baxter C, Hauer FR, Woessner WW. Measuring groundwater-stream water exchange: New techniques for installing minipiezometers and estimating hydraulic conductivity. Transactions of the American Fisheries Society 2003; 132: 493-502.

Baxter CV, Hauer FR. Geomorphology, hyporheic exchange, and selection of spawning habitat by bull trout (*Salvelinus confluentus*). Canadian Journal of Fisheries and Aquatic Sciences 2000; 57: 1470-1481.

Bjorn T, Reiser D. Habitat requirements of salmonids in streams. In: Meehan W, editor. Influences of forest and rangeland management on salmonid fishes and their habitats, Special Publication. 19. American Fisheries Society, Bethesda, 1991.

Bromage N, Jones J, Randall C, Thrush M, Davies B, Springate J, et al. Broodstock Management, Fecundity, Egg Quality and the Timing of Egg-Production in the Rainbow-Trout (Oncorhynchus-Mykiss). Aquaculture 1992; 100: 141-166.

Brookes A. Channelized Rivers: Perspectives for Environmental Management. Chichester: Wiley, 1988.

Brooks S, Tyler CR, Sumpter JP. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 1997; 7: 387-416.

Brown LR, Moyle PB, Yoshiyama RM. Historical Decline and Current Status of Coho Salmon in California. North American Journal of fisheries management 1994; 14: 237-261.

Brunke M, Gonser T. The ecological significance of exchange processes between rivers and groundwater. Freshwater Biology 1997; 37: 1-33.

Burkhardt-Holm P, Giger W, Guttinger H, Ochsenbein U, Peter A, Scheurer K, et al. Where have all the fish gone? Environmental Science & Technology 2005; 39: 441a-447a.

Burkhardt-Holm P, Scheurer K. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquatic Sciences 2007; 69: 51-70.

Carrascal LM, Galvan I, Gordo O. Partial least squares regression as an alternative to current regression methods used in ecology. Oikos 2009; 118: 681-690.

Chong IG, Jun CH. Performance of some variable selection methods when multicollinearity is present. Chemometrics and Intelligent Laboratory Systems 2005; 78: 103-112.

Crisp DT. Trout and Salmon: Ecology, conservation and rehabilitation: Fishing News Books, Blackwell Sciece 2000.

Czerkies P, Kordalski K, Golas T, Krysinski D, Luczynski M. Oxygen requirements of whitefish and vendace (Coregoninae) embryos at final stages of their development. Aquaculture 2002; 211: 375-385.

DeVries P. Riverine salmonid egg burial depths: review of published data and implications for scour studies. Canadian Journal of Fisheries and Aquatic Sciences 1997; 54: 1685-1698.

Dumas J, Bassenave JG, Jarry M, Barriere L, Glise S. Effects of fish farm effluents on egg-to-fry development and survival of brown trout in artificial redds. Journal of Fish Biology 2007; 70: 1734-1758.

Dumas J, Marty S. A new method to evaluate egg-to-fry survival in salmonids, trials with Atlantic salmon. Journal of Fish Biology 2006; 68: 284-304.
EBP-WSB-Agrofutura. Ganzheitliche Gewaesserplanung im Einzugsgebiet Wiggertal. Bau- und Umwelt und Wirtschaftsdepartement des Kantons Luzern und Baudepartement des Kantons Aargau 2005.
Einum S, Hendry AP, Fleming IA. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proceedings of the Royal Society B-Biological Sciences 2002; 269: 2325-2330.
Endreny T, Lautz L, Siegel DI. Hyporheic flow path response to hydraulic jumps at river steps: Flume and hydrodynamic models. Water Resources Research 2011; 47.
Eriksson L, Johansson E, Kettaneh-Wold J, Trygg C, Wikström C, Wold S. Multi- and megavariate data analysis – principles and applications: Umetrics AB, 2006.
Faraday JD. Extending the linear model with R. Boca Raton: Chapman and Hall, 2006.
Garrett JW, Bennett DH, Frost FO, Thurow RF. Enhanced incubation success for kokanee spawning in groundwater upwelling sites in a small Idaho stream. North American Journal of fisheries management 1998; 18: 925-930.
Geist DR, Abernethy CS, Hand KD, Cullinan VI, Chandler JA, Groves PA. Survival, development, and growth of fall chinook salmon embryos, alevins, and fry exposed to variable thermal and dissolved oxygen regimes. Transactions of the American Fisheries Society 2006; 135: 1462-1477.
Gilvear DJ, Heal KV, Stephen A. Hydrology and the ecological quality of Scottish river ecosystems. Science of the Total Environment 2002; 294: 131-159.
Gooseff MN, Anderson JK, Wondzell S, LaNier J, Haggerty R. A modeling study of hyporheic exchange pattern and the sequence, size, and spacing of stream bedforms in mountain stream networks, Oregon, USA (Retraction of vol 19, pg 2915, 2005). Hydrological Processes 2006; 20: 2441-+.
Greig S, Sear D, Carling P. A field-based assessment of oxygen supply to incubating Atlantic salmon (*Salmo salar*) embryos. Hydrological Processes 2007a; 21: 3087-3100.
Greig SM, Sear DA, Carling PA. The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management. Science of the Total Environment 2005; 344: 241-258.
Greig SM, Sear DA, Carling PA. A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. Hydrological Processes 2007b; 21: 323-334.
Hansen EA. Some effects of groundwater on brown trout redds. Transactions of the American Fisheries Society 1975; 104: 100-110.
Hester ET, Doyle MW. In-stream geomorphic structures as drivers of hyporheic exchange. Water Resources Research 2008; 44.
Heywood MJT, Walling DE. The sedimentation of salmonid spawning gravels in the Hampshire Avon catchment, UK: implications for the dissolved oxygen content of intragravel water and embryo survival. Hydrological Processes 2007; 21: 770-788.
Hicks BJ, Hall JD, Bisson PA, Sedell JR. Responses of salmonids to habitat changes. In: Meehan WR, editor. Influences of forest and rangeland management on salmonid fishes and their habitats. 19, 1991, pp. 483-518.
Huber E, Huggenberger P, Epting J, Schindler Wildhaber Y. Zeitliche und räumliche Skalen der Fluss-Grundwasser-Interaktion: Ein multidimensionaler hydrogeologischer Untersuchungsansatz. Grundwasser in press: DOI 10.1007/s00767-013-0220-x.
Huntington C, Nehlsen W, Bowers J. A survey of healthy native stocks of anadromous salmonids in the Pacific Northwest and California. Fisheries 1996; 21: 6-14.

Jensen DW, Steel EA, Fullerton AH, Pess GR. Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. Reviews in Fisheries Science 2009; 17: 348-359.

Kasahara T, Hill AR. Hyporheic exchange flows induced by constructed riffles and steps in lowland streams in southern Ontario, Canada. Hydrological Processes 2006; 20: 4287-4305.

Kasahara T, Wondzell SM. Geomorphic controls on hyporheic exchange flow in mountain streams. Water Resources Research 2003; 39.

Klassen HD, Northcote TG. Use of gabion weirs to improve spawning habitat for pink salmon in a small logged watershed. North American Journal of fisheries management 1988; 8: 36-44.

Lapointe M, Bergeron N, Berube F, Pouliot M, Johnston P. Interactive effects of substrate sand and silt contents, redd-scale hydraulic gradients, and interstitial velocities on egg-to-emergence survival of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 2004; 61: 2271-2277.

Lotspeich FD, Everest FH. A new method for reporting and interpreting textural composition of spawning gravel. In: Service USF, editor. Pacific Northwest Research Note PNW-369, 1981, pp. 12p.

Malcolm IA, Greig SM, Youngson AF, Soulsby C. Hyporheic influences on salmon embryo survival and performance. In: Sear D, DeVries P, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. 65. American Fisheries Society, Bethesda, USA, 2008, pp. 225-248.

Malcolm IA, Middlemas CA, Soulsby C, Middlemas SJ, Youngson AF. Hyporheic zone processes in a canalised agricultural stream: implications for salmonid embryo survival. Fundamental and Applied Limnology 2010; 176: 319-336.

Malcolm IA, Soulsby C, Youngson AF. High-frequency logging technologies reveal state-dependent hyporheic process dynamics: implications for hydroecological studies. Hydrological Processes 2006; 20: 615-622.

Matschak TW, Stickland NC, Mason PS, Crook AR. Oxygen availability and temperature affect embryonic muscle development in Atlantic salmon (*Salmo salar* L). Differentiation 1997; 61: 229-235.

Matschak TW, Tyler DD, Stickland NC. Metabolic enzyme activities in Atlantic salmon (*Salmo salar* L.) embryos respond more to chronic changes in oxygen availability than to environmental temperature. Fish Physiology and Biochemistry 1998; 18: 115-123.

Mevik BH, Wehrens R. The pls package: Principal component and partial least squares regression in R. Journal of Statistical Software 2007; 18: 23.

Meyer CB. Importance of measuring biotic and abiotic factors in the lower egg pocket to predict salmon egg survival Journal of Fish Biology 2003; 62: 534-548.

Miller SC, Reeb SE, Wright PA, Gillis TE. Oxygen concentration in the water boundary layer next to rainbow trout (*Oncorhynchus mykiss*) embryos is influenced by hypoxia exposure time, metabolic rate, and water flow. Canadian Journal of Fisheries and Aquatic Sciences 2008; 65: 2170-2177.

Nagler JJ, Parsons JE, Cloud JG. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 2000; 184: 177-183.

Newson M, Sear D, Soulsby C. Incorporating hydromorphology in strategic approaches to managing flows for salmonids. Fisheries Management and Ecology 2012; 19: 490-499.

Quinn GP, Keough MJ. Experimental design and data analysis for biologists: Cambridge University Press, 2002.

R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2011.
Roff DA. The evolution of life histories, theory and analysis. New York: Chapman & Hall, 1992.
Roussel JM. Carry-over effects in brown trout (*Salmo trutta*): hypoxia on embryos impairs predator avoidance by alevins in experimental cihannels. Canadian Journal of Fisheries and Aquatic Sciences 2007; 64: 786-792.
Rubin JF. Estimating the success of natural spawning of salmonids in streams. Journal of Fish Biology 1995; 46: 603-622.
Rubin JF, Glimsäter C. Egg-to-fry survival of the sea trout in some streams of Gotland. Journal of Fish Biology 1996; 48: 585-606.
Schager E, Peter A, Burkhardt-Holm P. Status of young-of-the-year brown trout (*Salmo trutta* fario) in Swiss streams: factors influencing YOY trout recruitment. Aquatic Sciences 2007; 69: 41-50.
Schindler Wildhaber Y, Liechti R, Alewell C. Organic matter dynamics and stable isotope signature as tracers of the sources of suspended sediment. Biogeosciences 2012a; 9: 1985-1996.
Schindler Wildhaber Y, Michel C, Burkhardt-Holm P, Baenninger D, Alewell C. Measurement of spatial and temporal fine sediment dynamics in a small river. Hydrology and Earth System Scences 2012b; 16: 1501-1515.
Schindler Wildhaber Y, Michel C, Epting J, Wildhaber R, Huber E, Huggenberger P, et al. Effects of river morphology, hydraulic gradients, and sediment deposition on water exchange and oxygen dynamics in salmonid redds. submitted.
Scrivener JC. Two devices to assess incubation survival and emergence of salmonid fry in an estuary streambed. North American Journal of fisheries management 1988; 8: 248-258.
Seydell I, Ibisch RB, Zanke UCE. Intrusion of suspended sediments into gravel riverbeds: influence of bed topography studied by means of field and laboratory experiments. Advanc.Limnol. 2009; 61: 67-85.
Soulsby C, Malcolm IA, Youngson AF. Hydrochemistry of the hyporheic zone in salmon spawning gravels: A preliminary assessment in a degraded agricultural stream. Regulated Rivers-Research & Management 2001; 17: 651-665.
Sowden TK, Power G. Prediction of rainbow trout embryo survival in relation to groundwater seepage and particle size of spawning Substrates. Transactions of the American Fisheries Society 1985; 114: 804-812.
Tonina D, Buffington JM. Hyporheic exchange in gravel bed rivers with pool-riffle morphology: Laboratory experiments and three-dimensional modeling. Water Resources Research 2007; 43: 16.
Waters TF. Sediment in streams. Sources, biological effects, and control. Bethesda, MD: American Fisheries Society, 1995.
Wohl E. Human impacts to mountain streams. Geomorphology 2006; 79: 217-248.
Wold S, Sjostrom M, Eriksson L. PLS-regression: a basic tool of chemometrics. Chemometrics and Intelligent Laboratory Systems 2001; 58: 109-130.
Youngson AF, MacLean JC, Fryer RJ. Rod catch trends for early-running MSW salmon in Scottish rivers (1952-1997): divergence among stock components. Ices Journal of Marine Science 2002; 59: 836-849.
Zuur AF, Ieno EN, Elphick CS. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 2010; 1: 3-14.

Chapter 7

Final remarks and outlook

"... more complete, rather than simply more, case studies are needed."

(Stuart and Losos, in press)

This is a quote borrowed from a recent publication on character displacement, which is evolutionary divergence driven by interspecies competition. Though it has been published in another context, I believe it describes well what is needed in fine sediment research. For my thesis I conducted research on how suspended fine sediment affects juvenile salmonid fish (**Part 1**), and how fine sediment deposition affects salmonid embryo survival in a heavily modified river environment (**Part 2**). Altogether, my results corroborate that fine sediment can affect salmonid fish at both investigated life stages (e.g. Newcombe and Jensen, 1996). In addition, my experiments contribute new insights on how fine sediment affects salmonid fish.

Part 1 – Effects on juvenile salmonid fish

Our *in vivo* experiment (**Chapter 2**) was designed to investigate physical damage as well as related systemic and apical effects caused by suspended mineral particle pulses. Its results indicate that small-sized (< 30 μm) pure mineral particles do not necessarily impair gill function, but they affected the rainbow trout via turbidity. The mica particle pulses caused an initial stress response, but the fish had adapted by day 8 of the exposure. Over 24 days they decreased condition and prompted a physiological response suggestive of metabolic changes, which both indicate that the particle pulses caused food deprivation. This response pattern suggests that the particulate pulses initially acted as a perceived stressor, while over longer time periods they affected the metabolic homeostasis. Also, over 24 days the lowest particle concentration caused lipid peroxidation in the gill. This effect did not impair gill function over this time-period, but it might affect gill function when the exposure becomes more chronic. Hence, cytotoxic effects of clay particles in salmonid gill epithelia clearly need further research (**Chapter 3**). In summary, depending on exposure duration the mechanism by which pulses of small sized mineral particles affect salmonid fish might differ. It is important to distinguish whether mineral particles affect salmonid fish via turbidity or by physical damage.

Without gill damage and a strongly disturbed homeostasis I would expect salmonid fish, firstly, to recover more quickly, and secondly, to adapt differently to the sediment pulses. Under natural conditions the fish might have moved away from the sediment plumes immediately (e.g. Crosa et al., 2010), which they were not able to do in my laboratory exposure. Thus, the observed response pattern might not be directly transferable to natural conditions. However, my experiment indicates that when salmonid fish cannot avoid sediment plumes they can adapt. Related physiological adaptations could be reflected in both the hypothalamo-pitiuary-interrenal axis (Wendelaar Bonga, 1997; Wingfield et al., 2011). Therefore, our results call for further experiments to quantify the behavioral and physiological adaptations and to understand how the fish adapted to the particulate pulses. This knowledge could ultimately help evaluate to what extent repeated suspended fine sediment transport events affect salmonid fish, and also to plan their intensity and timing if possible, e.g. during dredging or reservoir flushing (Harvey and Lisle, 1998; Crosa et al., 2010).

Our *in vitro* experiment (**Chapter 3**) demonstrated that natural mineral particles can cause cytotoxic effects in gill epithelial cells. Clay particles were generally more cytotoxic than framework silicates. In addition, the kind of cytotoxic effect differed between the clay particles studied: mica caused oxidative stress and a dilated endoplasmic reticulum, while kaolin caused cell membrane damage. These results also suggest that natural clay particles could exert cytotoxic effects comparable to some synthetic nanoparticles in gill epithelial cells (cf. **Chapter 3**). Care should be taken when applying these results to *in vivo* conditions. Under natural conditions the gill epithelium is protected by a mucus layer, which can hinder particle contact with the epithelial cells. Moreover, in live fish, the gill epithelium is exposed to mineral particles suspended in a constant water flow. Only some particles might settle on gill lamellae, and fish can also remove these by coughing (Berg and Northcote, 1985). Another important aspect is that mineral particles properties and hence cytotoxicity may differ when dispersed in water compared to cell culture medium. Thus, exposure under *in vivo* conditions differs from our *in vitro* conditions. In our *in vitro* system, the particles settled directly on the cells and this might have amplified effects. In this regard, I see two important follow-up experiments: First, the magnitude of particle uptake in RTgill-W1 should be quantified. Second, it should be clarified whether the observed cytotoxic effects were related to settling of the particles on the cells, or if they developed after the particles were taken up. If the effects manifested after particle uptake, comparable cytotoxic effects might occur in gill cells or macrophages that incorporated particles *in vivo*. Finally, one could expose RTgill-W1

cells to fine sediment particles suspended in water, which might be a more environmentally relevant exposure (Dayeh et al., 2002).

Considering our results in Part 1 I believe that we need more research to understand which aspect(s) of the suspended load in aquatic ecosystems actually affects salmonid fish. In the *in vivo* exposure with pure mineral particles (**Chapter 2**) no indication of an impaired gill function, severe physical damage or a strongly disturbed homeostasis could be found. This result also suggests to me that, at least up to 24 days of exposure, the mineral particles *per se* might sometimes not be the primary cause of damage (see also Lake and Hinch, 1999). Effects might rather depend on surface properties of the particle itself (**Chapter 3**) or of toxic compounds, biofilm or pathogens on the particle surface (Gerbersdorf et al., 2011; Häfeli et al., 2011). For example, particle-bound anthropogenic pollutants have been demonstrated to add to toxicological effects of re-suspended fine sediments in rainbow trout (Brinkmann et al., 2010; Brinkmann et al., 2013). The applied *in vitro* method is one possibility to screen for differences in particle cytotoxicity between river systems. Results would need to be validated *in vivo* and ideally under more environmentally relevant conditions (e.g. Shaw and Richardson, 2001; Cofalla et al., 2012). The number of influential factors is large, e.g. particle size, geochemistry, microbial contamination, biofilm, and particle bound anthropogenic pollutants (Gerbersdorf et al., 2011). However, multivariate statistical methods can be used to resolve the relative importance of the many possible predictors, and in case of field studies to account for variation between sampling years and locations (e.g. Smolders et al., 2004; Craven et al., 2010; Webb et al., 2010).

Part 2 – Effects on salmonid embryo survival

The second part of my thesis investigated factors affecting salmonid embryo survival in a heavily modified river. This stream was straightened, channelized and the river-bed was stabilized. These modifications are present in many salmonid streams of the northern hemisphere (Brookes, 1988; Gilvear et al., 2002; Wohl, 2006). Despite this, their effect on salmonid embryo survival has been hardly investigated.

Our results confirm that fine sediment transport and deposition is closely linked to the flow dynamics of the river, with suspended transport mostly during high-flow events (**Chapter 4**). Over the entire incubation season, scouring and re-suspension was more important for fine sediment accumulation in redds than the magnitude of suspended fine sediment transport *per*

se (**Chapter 5**). Accordingly, fine sediment accumulation was highest in the upstream site A, where lower water levels limited bed shear stress and particle re-suspension (**Chapter 4**). In sites B and C higher water levels increased bed shear stress and particle re-suspension, and hence less fine sediment accumulated (**Chapter 4**). Thus, river morphology and flow dynamics, rather than fine sediment transport, were important for maintaining gravel permeability over most of the incubation season. Likewise, hydraulic gradients related to the typical "pit-tail" redd morphology (**Chapter 5, Figure 1, page 83**) contributed to water exchange just after redd building (**Chapter 5**). After one month, when the redd morphology was gone and fine sediment had accumulated, water exchange was mostly controlled by flow dynamics, accumulated fine sediment, and the artificial steps (**Chapter 5, Figure 1B and C, page 83**). Oxygen concentration in redds was related to the amount accumulated fine sediment (**Chapter 5**), and hence also to flow dynamics (see above). In summary, over the entire incubation season, processes driven at the intermediate (i.e. individual terraces) and regional scale (i.e. river channel), rather than at the redd scale, affected water exchange and oxygen concentrations in the redds. In support of this notion, gravel permeability and distance to the next upstream step were identified as most important predictors for brown trout embryo survival in our study river (**Chapter 6**). These two parameters are not necessarily linked: Accumulated fine sediment decreased water exchange and oxygen concentrations in the redds, while the artificial steps exerted their effect most likely by creating a more stable incubation environment (**Chapter 6**). The latter could be for example a more stable thermal regime (Hansen, 1975; Bjorn and Reiser, 1991). Therefore, further experiments are needed that incorporate oxygen concentrations and temperature to better understand how the steps affected embryo survival.

I would have expected a measure of oxygen concentration to be an important predictor. Yet for methodological reasons, the suitability of the manual oxygen measurements was limited (**Chapter 5**). Our results indicate that both the duration and concentration of low oxygen, also relative to the developmental stage, were important for embryo survival (**Chapter 6**). Unfortunately, the number of redds with embryo survival, continuous oxygen, and temperature data were too small to be included in our multivariate analysis (**Chapter 6**). Having these data would have allowed approximating critical thresholds for oxygen concentrations that incorporate both the oxygen concentration and the duration of phases of low oxygen.

The methods that we developed and applied (**Chapter 4**) were able to capture the spatial and temporal dynamics of fine sediment transport, deposition and re-suspension in the artificial salmonid redds. One of the biggest practical problems was the loss of sampling devices during flood events. This was especially problematic for devices that could not be replaced, such as the accumulation baskets introduced to quantify fine sediment accumulation over the entire incubation season. However, comparison of our results with other studies and cross-validation between methods demonstrated their general suitability.

Bed scouring was a threat for embryo survival in the Enziwigger (**Chapter 5**). In Switzerland and Central Europe numerous salmonid streams are channelized, which alters their flow dynamics and causes increased water levels as well as flow velocities. This can trigger increased bed scouring during high flow events. In support of this notion, half of our redds were destroyed by high flow events in the downstream sites B and C, while only 8% were lost in the most upstream site A. In the Enziwigger this was mostly related to increasing water levels from upstream to downstream, since the artificial steps equalized slopes among study sites. In some rivers, climate change might further increase the frequency and intensity of winter floods in the future (Scheurer et al., 2009; Goode et al., 2013). From their investigation on the scour risk of salmonid redds in Rocky Mountain streams Goode et al. (2013) concluded that: “Scour risk for all species is reduced when changes in channel morphology (width, depth, and grain size) keep pace with climate-driven changes in streamflow”. Therefore, I would expect that in channelized rivers like the Enziwigger, where channel morphology is strongly confined by the steps and lateral stabilization, future increases in winter-flood events might be a particular risk for salmonid recruitment.

By integrating the fields of hydrology (Huber et al., in press) with geomorphology and fish ecology (**Chapters 3–5**) we were able to develop a holistic understanding of the factors affecting brown trout embryo survival in our study river (**Chapter 6**). Altogether, our results underline the importance of appropriate flow dynamics and suitable river structure to sustain salmonid incubation success (Newson et al., 2012). In some channelized salmonid streams where channel form cannot adjust to altered flow dynamics, river bed stabilization might be necessary to mitigate the risk of redd scouring during winter flood events. In this regard our results indicate that artificial bed-stabilization structures could even support salmonid embryo survival. Likewise, our results corroborate that the processes driving water exchange and oxygen supply in salmonid redds are intimately linked to the river system (Greig et al., 2007;

Malcolm et al., 2008). Therefore, interdisciplinary collaboration to develop a process based understanding of the river system is, in my opinion, essential to develop appropriate management strategies in salmonid streams (see also Montgomery, 2004).

Outlook

My results clearly illustrate that it is essential to apply a holistic perspective to understand how fine sediment can affect salmonid fish. I could show that for effects on juvenile salmonids both physiological adaptations of the organism as well as specific particle properties need to be considered. Similarly, for effects on salmonid embryos, the particular river system, with its hydrological and geomorphological setting, as well as the developmental stage of the embryo has to be considered. Therefore, similar to Stuart and Losos (in press, quoted above), also for fine sediment research it is essential to conduct more complete, rather than simply more, case studies. I believe we ultimately need to integrate fine sediment as one aspect of environmental change, and from there to develop strategies to sustain salmonid populations in the 21st century.

References

Berg L, Northcote TG. Changes in territorial, gill flaring, and feeding behavior in juvenile coho salmon (*Oncorhynchus kisutch*) following short term pulses of suspended sediment. Canadian Journal of Fisheries and Aquatic Sciences 1985; 42: 1410-1417.

Bjorn T, Reiser D. Habitat requirements of salmonids in streams. In: Meehan W, editor. Influences of forest and rangeland management on salmonid fishes and their habitats, Special Publication. 19. American Fisheries Society, Bethesda, 1991.

Brinkmann M, Hudjetz S, Cofalla C, Roger S, Kammann U, Giesy JP, et al. A combined hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events. Part I-multiple biomarkers in rainbow trout. Journal of Soils and Sediments 2010; 10: 1347-1361.

Brinkmann M, Hudjetz S, Kammann U, Hennig M, Kuckelkorn J, Chinoraks M, et al. How flood events affect rainbow trout: Evidence of a biomarker cascade in rainbow trout after exposure to PAH contaminated sediment suspensions. Aquatic Toxicology 2013; 128: 13-24.

Brookes A. Channelized Rivers: Perspectives for Environmental Management. Chichester: Wiley, 1988.

Cofalla C, Hudjetz S, Roger S, Brinkmann M, Frings R, Wolz J, et al. A combined hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events-part II: an interdisciplinary experimental methodology. Journal of Soils and Sediments 2012; 12: 429-442.

Craven SW, Peterson JT, Freeman MC, Kwak TJ, Irwin E. Modeling the relations between flow regime components, species traits, and spawning success of fishes in warmwater streams. Environmental Management 2010; 46: 181-194.

Crosa G, Castelli E, Gentili G, Espa P. Effects of suspended sediments from reservoir flushing on fish and macroinvertebrates in an alpine stream. Aquatic Sciences 2010; 72: 85-95.

Dayeh VR, Schirmer K, Bols NC. Applying whole-water samples directly to fish cell cultures in order to evaluate the toxicity of industrial effluent. Water Research 2002; 36: 3727-3738.

Gerbersdorf SU, Hollert H, Brinkmann M, Wieprecht S, Schuttrumpf H, Manz W. Anthropogenic pollutants affect ecosystem services of freshwater sediments: the need for a "triad plus x" approach. Journal of Soils and Sediments 2011; 11: 1099-1114.

Gilvear DJ, Heal KV, Stephen A. Hydrology and the ecological quality of Scottish river ecosystems. Science of the Total Environment 2002; 294: 131-159.

Goode JR, Buffington JM, Tonina D, Isaak DJ, Thurow RF, Wenger S, et al. Potential effects of climate change on streambed scour and risks to salmonid survival in snow-dominated mountain basins. Hydrological Processes 2013; 27: 750-765.

Greig SM, Sear DA, Carling PA. A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. Hydrological Processes 2007; 21: 323-334.

Häfeli N, Schwartz P, Burkhardt-Holm P. Embryotoxic and genotoxic potential of sewage system biofilm and river sediment in the catchment area of a sewage treatment plant in Switzerland. Ecotoxicology and Environmental Safety 2011; 74: 1271-1279.

Hansen EA. Some effects of groundwater on brown trout redds. Transactions of the American Fisheries Society 1975; 104: 100-110.

Harvey BC, Lisle TE. Effects of suction dredging on streams: A review and an evaluation strategy. Fisheries 1998; 23: 8-17.

Huber E, Huggenberger P, Epting J, Schindler Wildhaber Y. Zeitliche und räumliche Skalen der Fluss-Grundwasser-Interaktion: Ein multidimensionaler hydrogeologischer Untersuchungsansatz. Grundwasser in press: DOI 10.1007/s00767-013-0220-x.

Lake RG, Hinch SG. Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 1999; 56: 862-867.

Malcolm IA, Greig SM, Youngson AF, Soulsby C. Hyporheic influences on salmon embryo survival and performance. In: Sear D, DeVries P, editors. Salmonid Spawning Habitat in Rivers: Physical Controls, Biological Responses, and Approaches to Remediation. 65. American Fisheries Society, Bethesda, USA, 2008, pp. 225-248.

Montgomery DR. Geology, geomorphology, and the restoration ecology of salmon. GSA Today 2004; 14.

Newcombe CP, Jensen JOT. Channel suspended sediment and fisheries: A synthesis for quantitative assessment of risk and impact. North American Journal of Fisheries Management 1996; 16: 693-727.

Newson M, Sear D, Soulsby C. Incorporating hydromorphology in strategic approaches to managing flows for salmonids. Fisheries Management and Ecology 2012; 19: 490-499.

Scheurer K, Alewell C, Banninger D, Burkhardt-Holm P. Climate and land-use changes affecting river sediment and brown trout in alpine countries - a review. Environmental Science and Pollution Research 2009; 16: 232-242.

Shaw EA, Richardson JS. Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. Canadian Journal of Fisheries and Aquatic Sciences 2001; 58: 2213-2221.

Smolders R, De Coen W, Blust R. An ecologically relevant exposure assessment for a polluted river using an integrated multivariate PLS approach. Environmental Pollution 2004; 132: 245-263.

Stuart YE, Losos JB. Ecological character displacement: glass half full or half empty? Trends in Ecology & Evolution in press: http://dx.doi.org/10.1016/j.tree.2013.02.014.

Webb JA, Stewardson MJ, Koster WM. Detecting ecological responses to flow variation using bayesian hierarchical models. Freshwater Biology 2010; 55: 108-126.

Wendelaar Bonga SE. The stress response in fish. Physiological Reviews 1997; 77: 591-625.

Wingfield JC, Kelley JP, Angelier F. What are extreme environmental conditions and how do organisms cope with them? Current Zoology 2011; 57: 363-374.

Wohl E. Human impacts to mountain streams. Geomorphology 2006; 79: 217-248.