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ARTICLE

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

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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

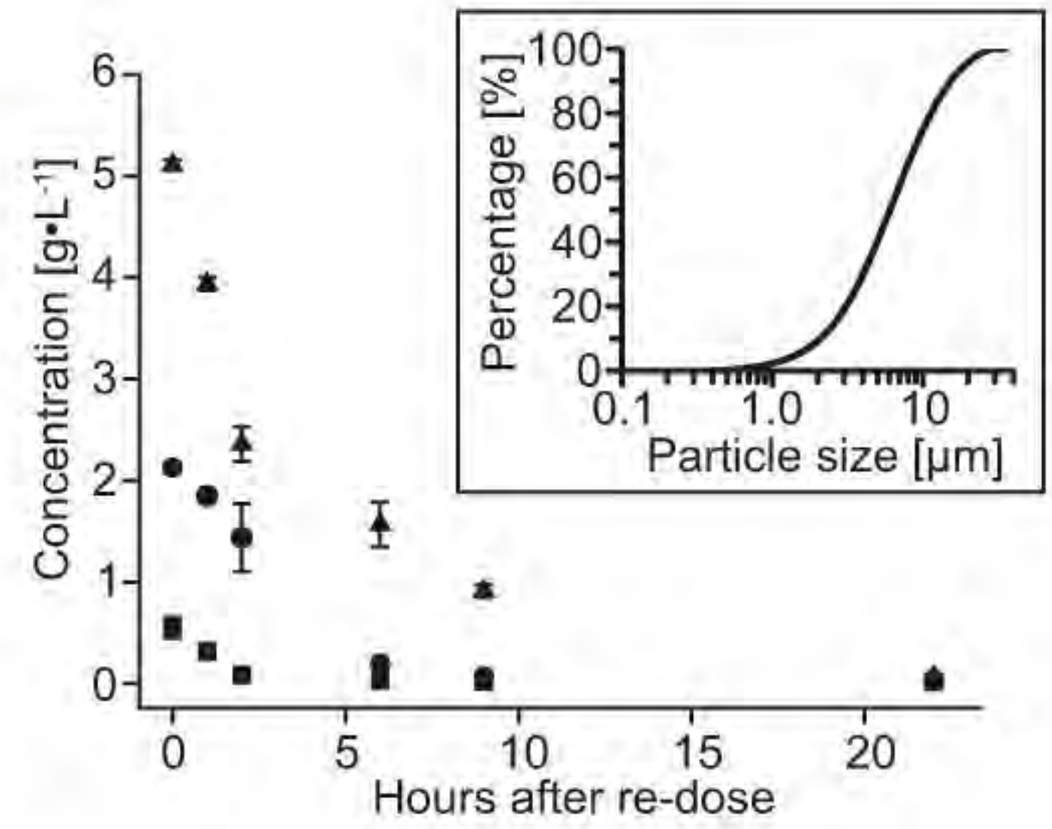
Experimental procedures

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-

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 (5000g, 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 (5000g, 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).

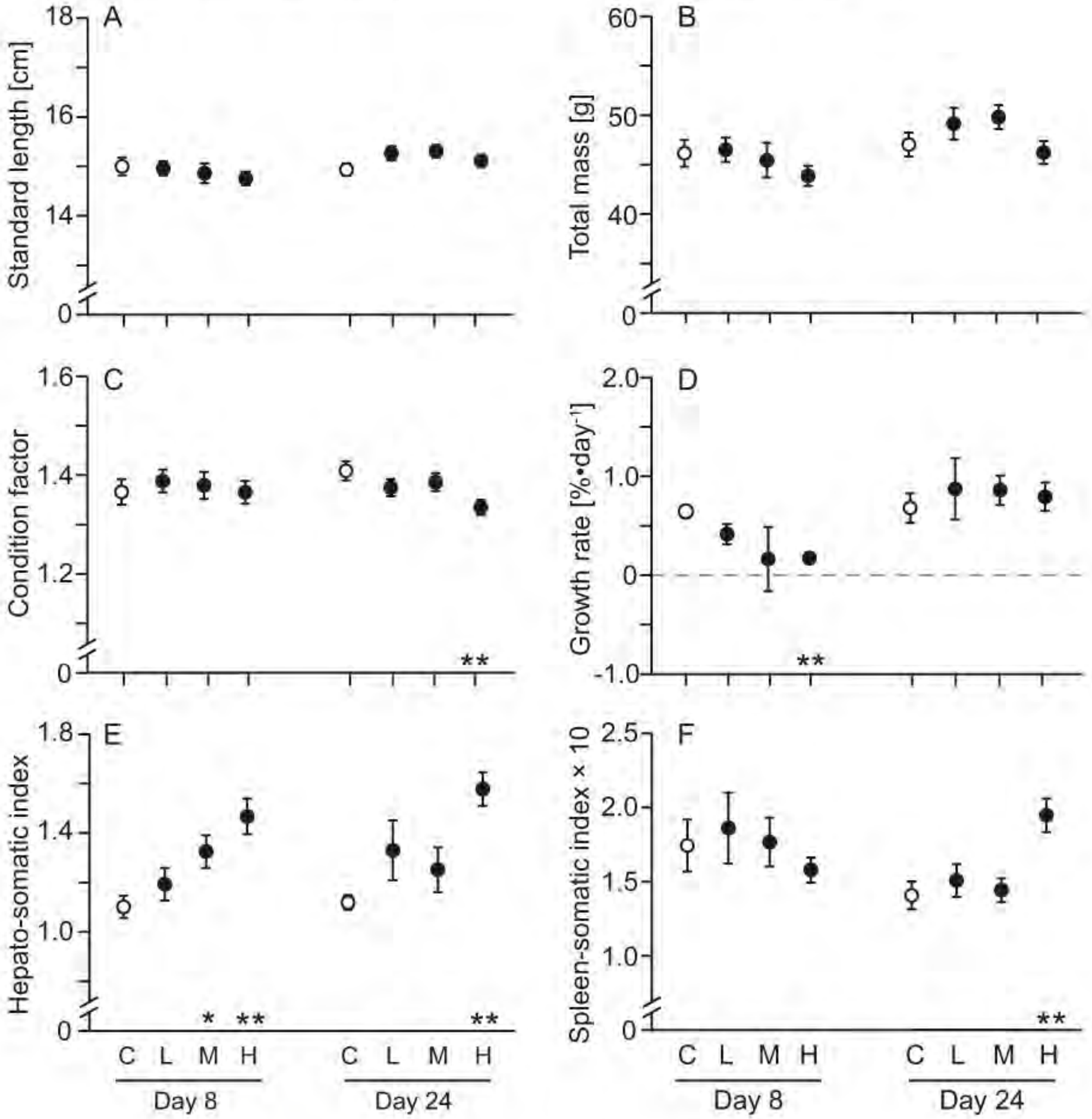
In spleen and kidney, the number and area fraction of melano-macrophage aggregates (MAs) was quantified (Schwindt et al. 2006), with four digital images analyzed for each of two sections ($n = 8$ images per fish). In the red pulp of the spleen, accumulations of individual macrophages containing distinct 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).

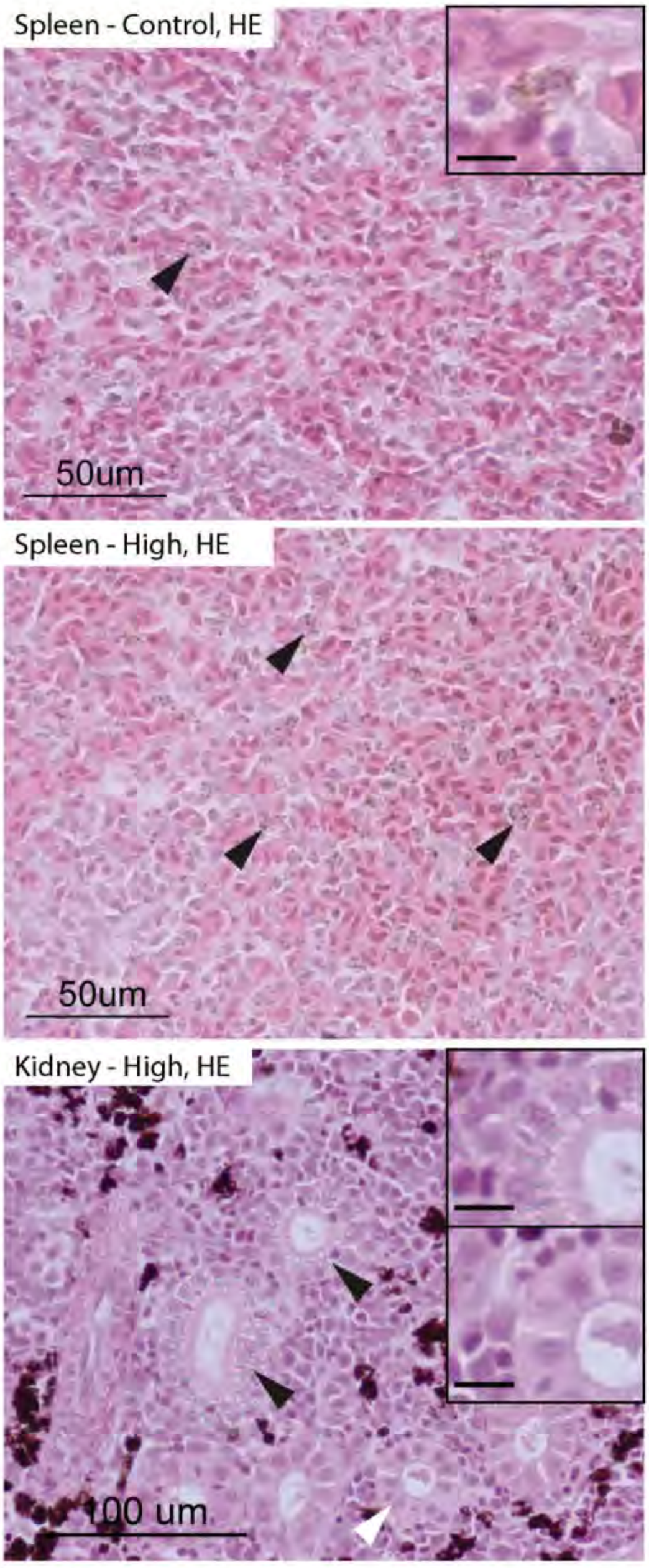


Fig. 3. Cellular responses in spleen and kidney of juvenile rainbow trout exposed to suspended mica particle for 24 days. Spleen: granular macrophage accumulations (arrowheads mark examples for individual macrophages) in a control (upper panel) and a high treatment level fish (middle panel), both stained 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,

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.

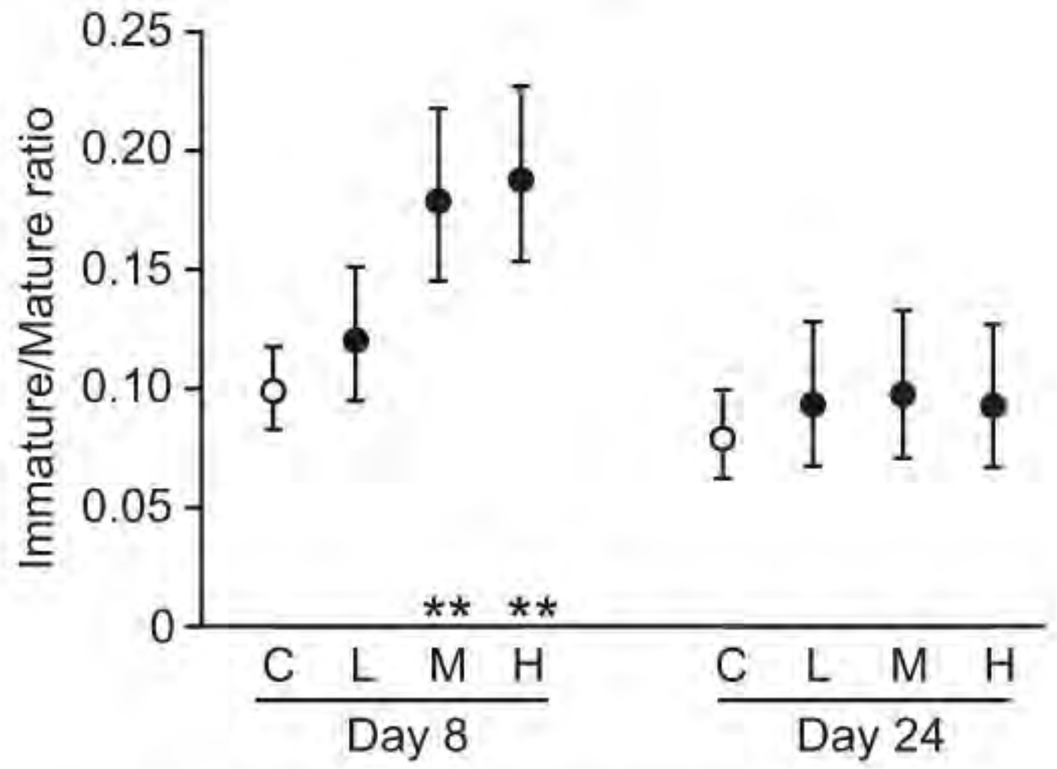
on day 24, plasma ASAT activity was reduced almost twofold in the highest particle concentration ($p = 0.049$; Table 4). The plasma protein content showed a similar pattern of decrease (Table 4). Moreover, plasma protein content and ASAT activity were negatively related to the hepato-somatic index (Fig.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

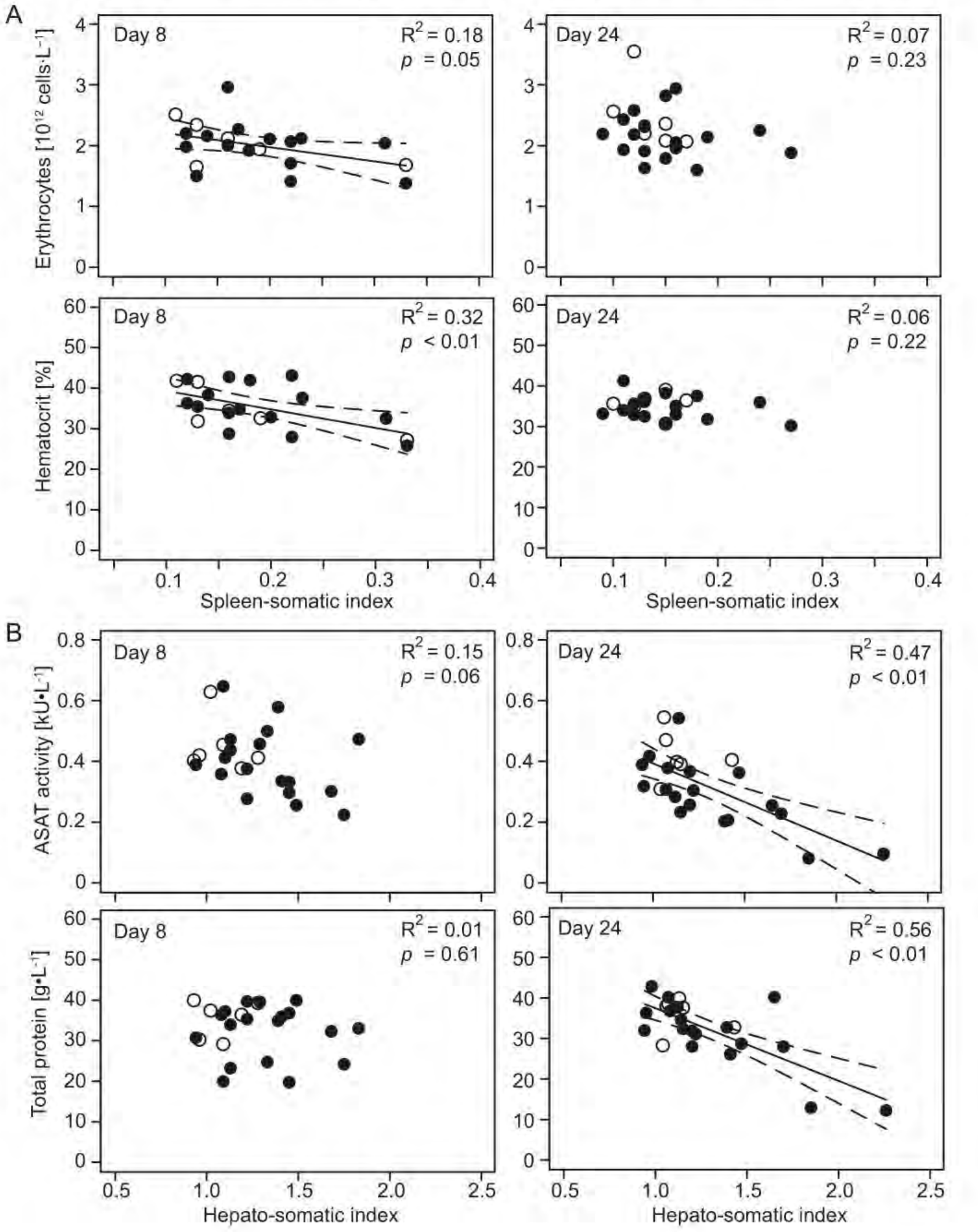


Table 4. Plasma parameters in rainbow trout exposed to suspended mica particles.

		Treatment level			
Parameter	Day	Control	Low	Medium	High
Na ($mmol \cdot L^{-1}$)	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 ($mmol \cdot L^{-1}$)	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 ($mmol \cdot L^{-1}$)	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 ($mmol \cdot L^{-1}$)	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 ($U \cdot L^{-1}$)	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 ($kU \cdot L^{-1}$)	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 ($g \cdot L^{-1}$)	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 ($mmol \cdot L^{-1}$)	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.

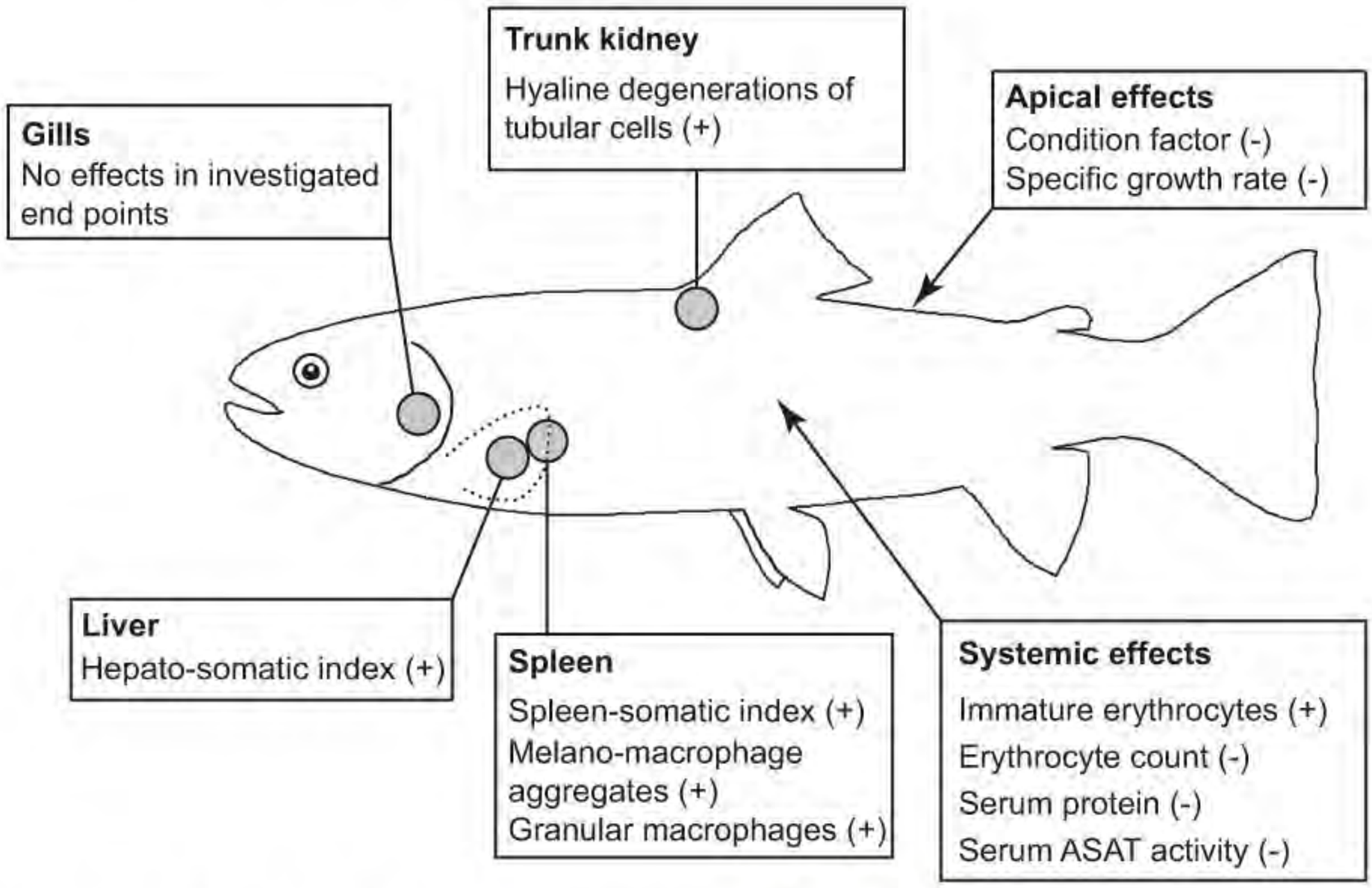
As with the gills, no large-scale histo-pathological effects were observed in liver or spleen. Thus, the increase of liver and spleen indices in particle-exposed fish was not related to inflammatory cell infiltration or other proliferative changes, at least at the time of sampling. Nonetheless, we observed 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

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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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.