Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*)

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Abstract: To determine the roles of suspended sediment angularity and concentration as contributors to stress and mortality in salmonids, we exposed juvenile coho salmon (*Oncorhynchus kisutch*) to anthropogenically derived "extremely angular" and "round" silicate sediments over a range of concentrations in 96-h experiments. Stress responses (e.g., decreased leukocrit) were elicited by exposure to both sediment shapes when concentrations were >40 g·L^{-1}, corresponding to the minimum concentration at which physical gill damage was observed. Extremely angular sediments also caused stress responses (e.g., elevated hematocrit, decreased leukocrit) at concentrations <41 g·L^{-1}. However, we found no difference between sediment shapes in causing mortality at any sediment concentration. Further, mortalities were not observed until concentrations were about 100 g·L^{-1}, a value that is about an order of magnitude greater than high natural concentrations in salmonid rivers. Natural fluvial suspended sediments cause fish stress and mortality at much lower concentrations than we found with our anthropogenically derived suspended sediments.

Résumé : Pour déterminer les effets de l'angularité et de la concentration des sédiments en suspension en tant qu'agents de stress et facteurs de mortalité chez les salmonidés, nous avons exposé des saumons cohos (*Oncorhynchus kisutch*) juvéniles à des sédiments de silicate d'origine anthropique « extrêmement anguleux » et « arrondis » à des concentrations diverses dans des essais de 96 h. Un stress (p. ex., réduction du leucocrite) a été induit par l'exposition aux deux types de sédiments quand leurs concentrations étaient supérieures à 40 g·L^{-1}, concentration minimale à laquelle des dommages physiques aux branchies ont été observés. Les sédiments extrêmement anguleux ont aussi causé un stress (p. ex., hématocrite élevé, leucocrite réduit) aux concentrations inférieures à 41 g·L^{-1}. Cependant, les sédiments des deux types, à quelque concentration que ce soit, ne causaient pas une mortalité plus élevée l'un que l'autre. De plus, pour qu'il y ait mortalité, il fallait que les concentrations atteignent environ 100 g·L^{-1}, soit une valeur environ dix fois supérieure aux concentrations naturelles élevées observées dans les rivières à saumon. Les sédiments naturels en suspension dans les cours d'eau causent un stress et peuvent tuer des poissons à des concentrations beaucoup plus faibles que celles des sédiments en suspension d'origine anthropique utilisés dans nos essais.

[Traduit par la Rédaction]

Introduction

Laboratory and mesocosm studies have demonstrated that high concentrations of suspended sediment can cause physiological stress and mortality in juvenile fish (McLeay et al. 1987; Servizi and Martens 1987, 1991; reviewed in Newcombe and MacDonald 1991; reviewed in Waters 1995). Yet, juveniles of many fish species occur and thrive in rivers and estuaries that have naturally high concentrations of suspended sediments (e.g., Murphy et al. 1989; Northcote and Larkin 1989). This apparent paradox can be partly explained by the fact that high turbidity, caused by suspended sediments, can reduce the risk of capture by visual predators and thereby enhance survival of juvenile fish prey (Gregory 1993, 1994; Gregory and Northcote 1993; Gregory and Levings 1998). Sediment particle size is also an important factor: high concentrations of suspended sediments composed of relatively small particles can cause fewer deleterious effects to salmonids than those composed of larger particles (Servizi and Martens 1987). Duration of exposure to high concentrations of suspended sediments must also be considered (Newcombe and MacDonald 1991).

It has been frequently speculated that the shape of suspended sediment particles may affect physiological stress and mortality in fish (Noggle 1978; Langer 1980; Berg and Northcote 1985; Servizi and Martens 1987). It is conceivable that high concentrations of highly angular particles could cause significant gill damage and lead to mortality; however, no research has been conducted to examine the effects of suspended sediment particle shape on fish. The purpose of this study was to determine if suspended sediment angularity contributes to mortality and sublethal stress in juvenile coho salmon (*Oncorhynchus kisutch*). We accomplished this by exposing fish to extremely angular and nonangular (i.e., round) suspended sediments over a range of suspended sediment concentration levels in 96-h experi-

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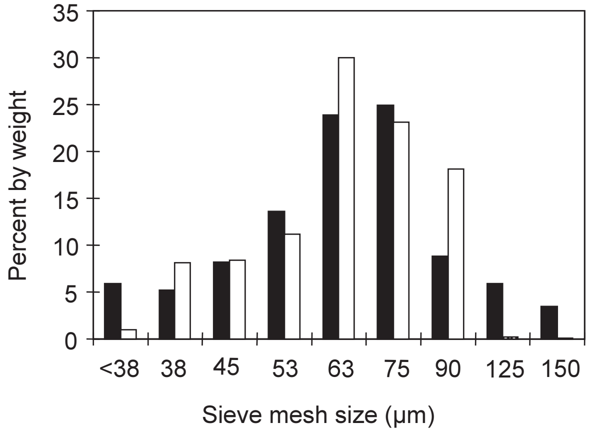
Fig. 1. Histograms showing the percent by weight of extremely angular (solid bars) and round (open bars) suspended sediments that were retained on U.S. Standard stacked sieves. The values represent the mesh size of each sieve. The value to the far left represents sediments not retained on any sieve.



ments and evaluated mortality rates, morphological body changes, and hematological stress responses.

Methods

Angular sediments (crushed silica) were obtained from OCL Industrial Materials Ltd., Surrey, B.C. Crushed silica is used in sand-blasting and can be purchased in graded sizes. The smallest size was used because its size distribution is similar to locally occurring suspended sediments from the Fraser River (see the particle size distribution given in Servizi and Martens (1987)). Round sediments (silica glass beads) were obtained from Canasphere Industries Ltd., Burnaby, B.C. Silica beads are manufactured for use in sand-blasting and for hardening metal surfaces (termed "peening"). We obtained a graded size distribution similar to that of the crushed silica. We determined the exact size distribution of both sediment types by screening particles through U.S. Standard screens (Fig. 1). A sediment extract was obtained from both angular and round silica by mixing 200 g silica·L water^{-1} and allowing the sediment to settle from suspension. Silica particles have residual amounts of hydroxyl ions on their surface as a by-product of their manufacturing; therefore, they were soaked in glacial acetic acid until the sediment extract approximated pH 7. These "treated" sediments were used in all experimental trials. X-ray diffraction revealed that both the round and angular sediments had the same mineral makeup, which was primarily quartz with some feldspar and traces of calcite, mica, and chlorite (L. Lavkulich, Institute for Resources and Environment, University of British Columbia, Vancouver, B.C., personal communication).

The aspect of particle shape that we were most interested in was "roundness", which refers to the sharpness of the corners and edges of the grain. A common method and the one that we used to estimate roundness is visual comparison of grains with standard images of grains of known roundness (Blatt et al. 1972). Natural sediments fall into one of six roundness categories ranging from very angular to well rounded. Our angular sediments had much sharper edges than those classified as very angular by Blatt et al. (1972); thus, we subjectively termed ours extremely angular. Our round sediments were similar in shape to the well-rounded sediments (Blatt et al. 1972). For comparative purposes, we refer readers to Servizi and Martens (1987) who provided photomicrographs of the sediments used in their bioassays. Based on the Blatt et al. (1972) scale, about 90% of Servizi and Marten's (1987) sediments would be classified as subangular to very angular whereas <5% of their sediments would be considered extremely angular. None of their sediments would be classified as well rounded.

Coho salmon eggs, obtained from Inch Creek, Mission, B.C., were fertilized and embryos were reared to the parr stage at the Department of Fisheries and Oceans' Cultus Lake Laboratory, Cultus Lake, B.C. Both facilities are situated near one another in the southern portion of the Fraser River watershed near Vancouver, B.C. Fish were reared under a natural photoperiod in troughs with Cultus Lake water at 6–8°C. Fish were fed a maintenance ration throughout the period of our experiments but were not fed 2 days prior to or during trials. Fish used in all trials ranged from 80 to 111 mm in length.

Our study was conducted using the same laboratory facilities, apparatus, protocol, and water supply as those of Servizi and Martens (1987, 1991). Their papers should be consulted for additional methodological details not presented herein. Eight 35-L inverted cone-shaped vessels, each containing 30 L of water, were used to expose juvenile coho to suspended sediments for a 96-h period that we termed a "trial". A circular cage, 30 cm diameter × 18 cm high, held five fish and was placed at a depth of 22 cm in each vessel. All vessels were partially submerged in a large circular tank to maintain constant temperatures. A 1-hp recirculation pump was externally mounted on each vessel to keep sediments in suspension. Sediments were pumped out a threaded hole in the narrow inverted tip of the vessel and back into the open top of the vessel.

For each trial, four vessels were randomly assigned to contain round suspended sediments and the other four to contain angular suspended sediments. Trials were run from late November 1994 to early January 1995. In the first trial, no suspended sediment was added to the vessels. In each subsequent trial, concentration in the vessels were increased by 50 g·L^{-1} until the concentration was 200 g·L^{-1}, a concentration that preliminary work suggested would cause 100% mortality using either sediment shape. Suspended sediment concentrations were then decreased by 50 g·L^{-1} in each subsequent trial until the last trial, which contained no suspended sediments. In total, 10 trials were carried out. Due to mechanical problems with pumps, some vessels were excluded from some trials.

Due to among-pump variability in performance, the concentration of suspended sediment that fish encountered deviated from that intended. Twice daily over the 96-h trial, we sampled water from the top of each cage using an open scintillation vial that was capped before withdrawal. Each sample was vacuum filtered through preweighed Whatman No. 1 filter disks, dried at 105°C for 1 h, and reweighed to the nearest 0.01 mg. An average of these eight samples was used as the vessel-specific measure of sediment concentration encountered by fish.

Dead fish were removed daily from each vessel throughout the trials. At the end of each 96-h trial, live fish from vessels in which a minimum 60% survived (three out of five fish) were sacrificed by striking the back of the head and used for assessments of sublethal, physiological stress. We examined fish for hematocrit (percentage of red cells in the blood), leukocrit (percentage of white cells in the blood), and percent body moisture. Altered levels of these characteristics, whether they are an increase or decrease relative to baseline levels, suggest that physiological condition has been altered and are general indicators that salmonids are experiencing physiological stress (McLeay et al. 1987; Fagerlund et al. 1995).

Caudal peduncles were severed and blood collected with heparinized microhematocrit tubes. Blood was centrifuged at 11 500 rpm for 3 min. Calipers and a dissecting microscope were used to measure hematocrit and leukocrit as a percentage of the total blood sample volume. Samples were cooled on ice while the measures were made. Body moisture was estimated by removing a cross-sectional piece of the caudal peduncle, patting dry on both

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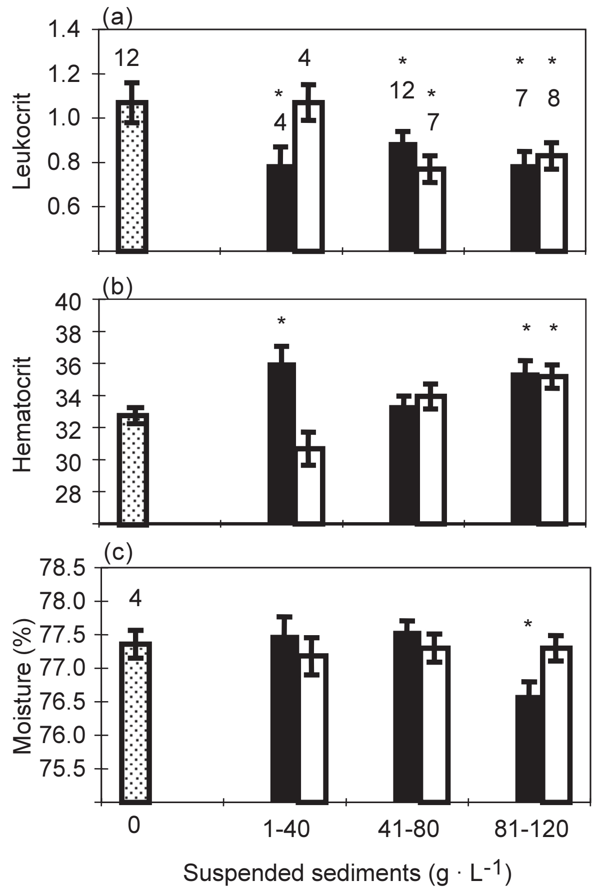

Fig. 2. Average (±1 SE) (*a*) leukocrit, (*b*) hematocrit, and (*c*) percent body moisture for juvenile coho salmon exposed for 96 h to extremely angular (solid bars) and round (open bars) suspended sediments of differing concentrations. Stippled bars represent trials without suspended sediments (e.g., controls). Unless otherwise indicated, the number of trials (e.g., sample size) used in the calculation of the average for that particular sediment concentration is indicated on the top of the leukocrit bars. Asterisks indicate treatments that differed from the control ($P < 0.05$).

surfaces, and weighing to the nearest 0.01 mg. Cross sections from all fish were oven dried at 90° for 24 h and reweighed. Percent body moisture was calculated by dividing the difference in wet and dry weight by the wet weight and multiplying by 100. The responses of individual fish (e.g., leukocrit, hematocrit, moisture percentages) were averaged within vessel, and it is these mean values that were used as individual replicates in the statistical analyses of sublethal responses.

We qualitatively assessed physical damage to fish by dissecting the first left gill arch from each fish and examining it by wet mount under a microscope at 60× magnification for possible damage or abnormalities of filaments and lamella. We also examined fish for the presence of infection or parasites.

During the trials, dissolved oxygen concentrations were maintained at 11.7–12.3 $mg \cdot L^{-1}$ (99–103% saturation). Temperature ranged from 6 to 7.5°C. The pH ranged from 7.0 to 8.2. The lake water supply is regularly monitored and is relatively invariant with respect to turbidity (2 NTU), conductivity (168 $\mu S \cdot cm^{-1}$), and alkalinity (60 mg $CaCO_3 \cdot L^{-1}$) (Servizi and Martens 1991).

We used two-way ANOVA's to examine the relative roles of sediment concentration and shape on hematocrit, leukocrit, and percent body moisture. We used one-way ANOVA's to compare differences in these variables between controls (trials with no added suspended sediments) and treatments (trials with differing combinations of suspended sediment concentration and shape). The effects of sediment concentration and shape on fish mortality were examined by regressing percent mortality on suspended sediment concentration separately for each sediment shape. We then used ANCOVA to compare these linear regression relationships.

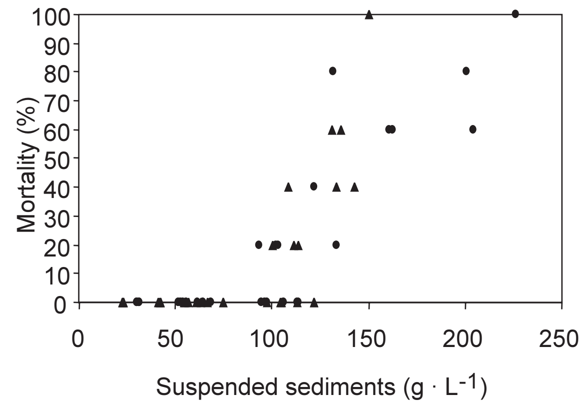

Fig. 3. Percent mortality of juvenile coho salmon after a 96-h exposure to a range of suspended concentrations of extremely angular (triangles) and round (circles) sediments. Twelve observations of 0% mortality are hidden.

Results

Vessel-specific survival rates of ≥60% were required for data to be included into the analyses on sublethal responses. However, few vessels with concentrations >120 $g \cdot L^{-1}$ met this criterion; therefore, we considered this our maximum value for the sublethal analyses and grouped vessels into the following three treatment categories: low (1–40 $g \cdot L^{-1}$), medium (41–80 $g \cdot L^{-1}$), and high (81–120 $g \cdot L^{-1}$). Variation in percent body moisture was weakly associated with variation in sediment concentration ($P = 0.051$); high concentrations produced relatively lower percent body moisture. There was no effect of sediment shape on body moisture ($P = 0.616$). The interaction between concentration and shape was significant ($P = 0.037$). High concentrations of angular sediment caused relatively low percent body moisture, and this category was the only one that significantly differed ($P = 0.032$) from the control category (Fig. 2). High sediment concentrations produced high hematocrit ($P = 0.039$) (Fig. 2) compared with low concentrations. There was no effect of sediment shape on hematocrit ($P = 0.198$). A significant interaction was present ($P = 0.006$), as low-concentration angular sediments produced a relatively high amount of hematocrit (Fig. 2). Hematocrit in the control category was lower than that observed in the high-concentration angular, high-concentration round, and low-concentration angular categories ($P \leq 0.03$ for each) (Fig. 2). Neither sediment concentration nor sediment shape, as main factors, explained significant variation in leukocrit ($P = 0.282$ and $P = 0.743$, respectively). The interaction was significant ($P = 0.027$), however; low concentration of round sediments caused relatively high leukocrit (Fig. 2). Leukocrit in the control category was higher than that observed in any of the other

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categories ($P \leq 0.03$ for each) (Fig. 2) but did not differ from that in the low-concentration round category (P = 0.959).

Mortality was not observed, for either sediment shape, until sediment concentration reached about 100 g·L^{-1}. At and above this concentration, mortality steadily increased with increasing suspended sediment concentration (Fig. 3). We found no differences in the slopes (P = 0.470) and intercepts (P = 0.471) of the two linear regression relationships, one for the round and one for the angular sediments, of percent mortality on suspended sediment concentration. The common regression equation, obtained by pooling round and angular data, was as follows: percent mortality = 0.046 × concentration – 25.169 ($P < 0.001$, $r^2 = 0.621$, $n = 59$). The LC_{50} estimated using this equation was 164.5 g·L^{-1}. No mortalities were recorded in the control trials. We were able to run five trials of round sediment at concentrations >150 g·L^{-1} but only one of angular sediment because at these high concentrations of angular sediment, pumps failed due to abrasion of their internal components. However, the slopes of the round and angular treatments did not differ from each other ($P > 0.05$) even when these high concentrations were excluded from the ANCOVA.

Visual examination of gills by wet mount revealed erosion at the distal end of the filament tips in suspended sediment concentrations >41 g·L^{-1} for both angular and round sediments. We could not visually distinguish any obvious differences in the level of erosion caused by round versus angular sediments, nor could we detect higher levels of erosion at the highest sediment concentrations. Because we had very few fish exposed to high concentrations of angular sediments, we were limited in our ability to detect effects at that combination of sediment shape and concentration. We observed no changes to gills for either sediment shape at concentrations ≤41 g·L^{-1}. No gills in live fish were observed to be clogged with suspended sediments, even at the highest concentrations. However, sediments were observed clogging the gills of morbid fish (e.g., fish that had loss equilibrium) and dead fish. Round suspended sediments were observed lined up in a single row between some gill filaments, but this was not observed for angular sediments. No other abnormalities, infections, or parasites were observed.

Discussion

The tolerance of salmonids to suspended sediments has been the subject of several studies (e.g., Noggle 1978; Newcombe and Flagg 1983; McLeay et al. 1987; Redding et al. 1987; Servizi and Martens 1987, 1991; Servizi and Gordon 1990), yet there have been no studies on effects of particle angularity on physiological stress and mortality. We found that stress responses (e.g., decreased leukocrit) were generally elicited by exposure to both sediment shapes when their concentrations were in excess of 40 g·L^{-1}, which corresponded to the minimum concentration at which physical gill damage was noted. Servizi and Martens (1991) suggested that tolerance to suspended sediments is related to oxygen content of the water, oxygen transfer across gill membranes, metabolic demands, and capacity to perform work. Although oxygen in our experiments was near saturation, the gill damage that we observed at high concentrations of suspended sediments could have caused anoxia and stress. In contrast with round sediments, angular sediments elicited stress responses (e.g., elevated hematocrit, decreased leukocrit) at relatively low sediment concentrations (<41 g·L^{-1}). Although we noted no damage to gills at these concentrations, it is possible that gills may have been irritated by angular sediments, resulting in increased mucus production and poorer oxygen transfer. Martens and Servizi (1993) found that juvenile coho salmon that were exposed to natural Fraser River suspended sediment for 96 h at concentrations of 16–41 g·L^{-1} had on average 1500 sediment particles per lamellae lodged intracellularly into gill epithelia. All particles were irregular and angular in shape.

It has been suggested that mortality rates of salmonids may increase with exposure to particles of increasing angularity (Noggle 1978; Langer 1980; Berg and Northcote 1985; Servizi and Martens 1987). Indeed, our sublethal measures demonstrated that extremely angular sediments placed fish under stress at lower concentrations than did round sediments. Therefore, we had expected that the concentration for initiation of mortality and the LC_{50} would be lower for extremely angular sediments. Instead, we found that both types of sediment caused similar mortality rates. The causes of mortality are not clear. Sediment did not appear to clog gills except in morbid or dead fish. Even at the highest sediment concentrations, oxygen remained saturated in the vessels, yet anoxia associated with gill impairment, along with other cumulative stressors (e.g., impaired osmoregulation, reduced metabolic capacity to clear sediment from gills), likely contributed to mortality (Servizi and Martens 1991).

Our relatively large LC_{50}, 164.5 g·L^{-1}, was very surprising for several reasons. First, in most natural systems, salmonids are unlikely to encounter the concentrations at which we first observed mortalities (about 100 g·L^{-1}). For instance, highly turbid large Canadian rivers such as the Red Deer and Peace have maximum daily suspended sediment concentrations of only 11–12 g·L^{-1} (Anonymous 1980), and glacial-fed alpine rivers experience occasional peaks of 14–15 g·L^{-1} (Gurnell and Clark 1987). The Fraser River's suspended sediment daily maximum rarely exceeds 1 g·L^{-1} and generally is <0.3 g·L^{-1} (Anonymous 1992). Second, Servizi and Martens (1991) exposed juvenile coho salmon to natural Fraser River suspended sediments using the identical test conditions and found a 96-h LC_{50} of only 22.7 g·L^{-1}. Their sediments had a very similar size distribution to ours but were less angular. Using the identical apparatus and natural sediments, juvenile sockeye salmon (*Oncorhynchus nerka*) had a 96-h LC_{50} of 17.6 g·L^{-1} (Servizi and Martens 1987) and juvenile chinook salmon (*Oncorhynchus tshawytscha*) 31 g·L^{-1} (Servizi and Gordon 1990).

Why natural sediments of intermediate shape would cause mortality at such relatively low suspended concentrations, compared with our values generated with anthropogenically derived sediments, is perplexing. Natural fluvial suspended sediments electrostatically sequester heavy metals and adsorb large organic molecules from solution to their surfaces (Allen 1986; McCallum 1995). Regardless of dissolved aqueous concentrations, these compounds can remain in high concentrations associated with sediments (Giesy and Hoke 1991). Upon contact with gills, electrostatically bound materials could be released from sediments and passively diffuse into epithelial mucus (Coombs 1980; Tessier et al.

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1984). Sediment particles themselves, and their sorbed contaminants, can also be taken up via gill epithelia (Coombs 1980; Tessier et al. 1984; Martens and Servizi 1993). Martens and Servizi (1993) found that only a brief exposure of juvenile salmonids to suspended sediments was needed for small particles to be quickly phagocytosed by epithelial gill cells and then transferred into parenchyma and spleen tissues.

The sediments used by Servizi and Martens (1987, 1991) were taken from deposition zones in the lower Fraser River, an area influenced by non-point-source pollution that contains high levels of sediment-bound contaminants (Hall et al. 1991; K. Hall, Institute for Resources and Environment, University of British Columbia, Vancouver, B.C., personal communication). Although the sediments used by Servizi and Martens (1987, 1991) were repeatedly rinsed in clean water prior to use in their bioassays, compounds sorbed to particle surfaces would not have been removed by this approach. Our anthropogenically derived sediments, on the other hand, were quarried from deposits on land and were not exposed to natural riverine metals and organic compounds after they were crushed and created. Further, the acid bath that we gave to the silica sediments to remove hydroxyl compounds associated with the bead manufacturing process should have removed any residual metal, and possibly organic, compounds that were sorbed on particle surfaces. Thus, the likelihood of contaminant influences on our bioassays was minimal.

The only study that we are aware of that conducted bioassays using suspended sediments of anthropogenic origin and size distribution similar to ours was that of McLeay et al. (1987) who exposed juvenile Arctic grayling (*Thymallus arcticus*) to placer mine sediments. Such sediments are created by crushing gravel. Although not reported in their work, we suspect that these sediments would have been highly angular. They observed 20% mortality at a concentration of 100 $g \cdot L^{-1}$, a finding nearly identical to ours (see Fig. 3). The results of our bioassays with extremely angular sediments, along with this observation from McLeay et al. (1987), suggest that suspended sediment particle angularity may not be a main factor responsible for acute lethality in juvenile fish.

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