EFFECTS OF EXCESSIVE SEDIMENTATION ON THE GROWTH AND STRESS RESPONSE OF WHITETAIL SHINER (*Cyprinella galactura*) JUVENILES

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Abstract. This study investigates the effects of increased suspended sediment concentration (SSC = 0, 25, 50, 100, and 500 mg/L) on the growth and physiological stress of *Cyprinella galactura*. Correlation analyses show a significant negative relationship between mean daily growth rate of *C. galactura* and suspended sediment concentration, and a significant positive relationship between SSC and stress, measured as whole-body cortisol concentration. Mean daily growth rates and mean cortisol levels of juvenile *C. galactura* differ significantly among SSC treatments. Growth rates were significantly higher and cortisol levels were significantly lower at the 500 mg/L treatment level.

INTRODUCTION

Excessive sedimentation of rivers and streams has been linked with the imperilment of southeastern freshwater fishes. Highland species requiring clean substrate are most at risk. While broad-scale studies suggest that increased sedimentation negatively impacts community composition, diversity and population density (Walsh et al. 1995, Waters 1995, Newcombe and Jenson 1996, Burkhead et al. 1997), few studies have determined the mechanisms behind these changes.

There is evidence that suspended sediment concentration is negatively correlated with spawning success in *Cyprinella* (Burkhead and Jelks 2001). Researchers have also found that increased levels of suspended sediment may reduce feeding and growth rates of larval salmonids (Sigler et al. 1984). However, there is limited work on the direct physiological effects of increased sedimentation on fishes.

One study on salmonids documented a positive correlation between suspended sediment level and physiological stress, measured as an increase in plasma cortisol levels (Redding et al. 1987). However, this study exposed yearling fish to relatively high levels of sediment (high treatment = 2 – 3 g/L; low treatment = 0.4 – 0.6 g/L). Very little research has been done 1) on the larval life stage, 2) on non-game species or 3) with low sediment concentrations (< 100 mg/L).

Stress in fish has been related to growth reduction, abnormal behavior and immunosuppression (Wedemeyer 1984, Schreck et al. 1997). The latter is associated with increased susceptibility to disease and increased mortality. The objective of this study was to investigate how increased suspended sediment concentration affects growth rate and stress in juvenile whitetail shiners (*C. galactura*), a surrogate for the federally threatened spotfin chub (*Erimonax monachus*).

METHODS

Experimental Tank Design

The apparatus used to create and maintain suspended sediment treatment levels consists of 20 experimental units, each composed of a 40 liter glass aquarium and a motor-driven paddle which moves back and forth along the bottom of the tank (Figure 1). The 20 paddles are connected to a central drive shaft that is powered by a 0.5 horsepower variable-speed gear motor. Each time the paddle travels along the bottom, any sediment that has settled is resuspended. To aid in resuspension, air lines have been attached to each paddle allowing it to act

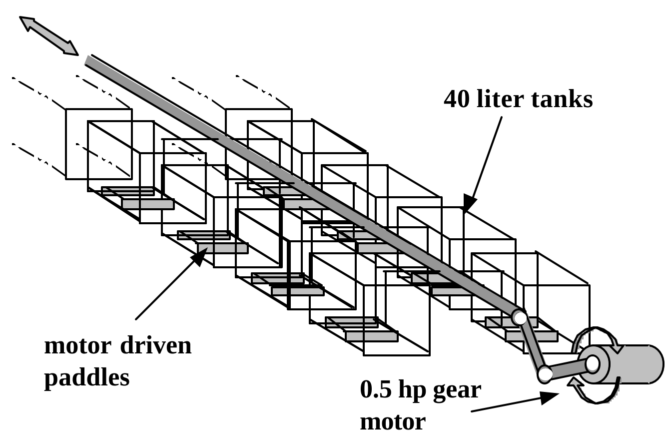

Figure 1. Apparatus used in growth and stress trials, consisting of a half-horsepower variable-speed gear motor powering a reciprocating drive shaft.

as a slow moving air diffuser. This combination of air bubbles and paddle movement is sufficient to maintain the highest treatment level (i.e. 500 mg/L).

Growth Trial

A single 30-day growth trial was conducted using juvenile *C. galactura*. Suspended sediment treatment levels were 0, 25, 50, 100 and 500 mg/L. Sediment used in growth and stress trials was collected from the Little Tennessee River watershed. After collection, sediment was wet-sieved to obtain the < 45 μm size fraction. Preliminary testing of experimental tanks determined that 45 μm particles are the largest that can be kept continually in suspension in the experimental apparatus. This particle size fraction is similar to the size of suspended sediment transported in the Little Tennessee River during baseflow. During water year 2001, 90% of baseflow suspended sediment (sampled at Lake Emory gauge) was finer than 62 μm (USGS 2001). Suspended sediment treatments are within the range of conditions observed in the Little Tennesee River (turbidity range: 10 – 1500 mg/L, W. O. McLarney unpublished data).

A strong positive correlation was found between < 45 μm SSC and turbidity (measured as nephelometric turbidity units – NTU). SSC and NTU were related by the following equation ($p < 0.01$, $r = 0.99$, df = 9):

$$NTU = (0.810 * SSC) + 5.83$$

Three fish were grown out in each of 20 experimental tanks (i.e. 5 treatments * 4 replicates * 3 fish/tank = 60 fish). Growth rates were measured as the mean change in wet weight per day for three fish per treatment replicate (i.e. each treatment replicate = mean daily growth rate of 3 fish). One growth trial has been completed to date. Several more trials are planned for both *C. galactura* and *E. monachus*.

At the start of the growth trial, a sulfa-based antibiotic (Sulfa-4; Fishy Farmacy, Tucson, AZ) was added to each tank to prevent the formation of bacterial blooms. Next, 60 lab-reared whitetail shiner juveniles (mean initial weight = 1.27 g; SD = 0.29) were randomly chosen from holding tanks, quickly patted dry on paper towels, placed in a pre-weighed beaker of water, and weighed to the nearest tenth of a milligram. After weighing, each fish was introduced randomly (using random number table) into one of 20 experimental tanks. The fish were allowed to acclimate to the experimental tanks for 48 hours before sediment was added. Food was provided after 24 hours of acclimation. The fish were fed twice a day, a diet of dry pelleted Purina® AquaMax (D04; 1.5mm) at a rate of 10% initial body weight per day.

Stress Trials

The effect of suspended sediment concentration on the primary stress response of juvenile *Cyprinella galactura* was determined by exposing fish to each of 5 sediment treatments (0, 25, 50, 100 and 500 mg/L) for 48 hours and measuring whole-body cortisol levels as compared to non-stressed controls. The fish were then anesthetized with eugenol (1:5 mixture of eugenol in ethanol) and flash frozen in a dry ice/ethanol bath and stored at – 80°C. Frozen fish were disrupted (i.e. homogenized) by ultrasonication for 2 minutes (Heat Systems-Ultrasonics, Inc.; setting 12), placed in test tubes and the cortisol extracted using diethyl ether (10 ml). The ether layer was evaporated in a 45°C water bath under a stream of nitrogen gas. The resulting hydrophobic residue was dissolved in extraction buffer (250 ul) and cortisol concentration was measured by enzyme-linked immunosorbent assay according to the standard protocol developed by the ELISA kit manufacturer (ELISA kit EA 65; Oxford Biomedical Research, Oxford, MI). Whole-body cortisol concentration (ng/g) was calculated by dividing cortisol concentration by fish mass.

Statistics

Mean daily growth rate and whole-body cortisol concentration were compared among treatments using one-way pairwise ANOVA. Tukey's multiple comparison procedure was used to test for pairwise differences among treatment means. After the completion of two additional growth and stress trials, all three trials will be analyzed together using one-way ANOVA, blocked by trial. Correlation analysis was used to estimate the strength of the relationship between suspended sediment level and both mean daily growth rate and whole-body cortisol concentration. ELISA inter-assay variability was assessed by calculating the coefficient of variation (CV; %) for standard curves assayed on four different assay plates. The precision of the assay was evaluated by calculating the CV for four repeated measures of each sample extract.

RESULTS

Growth Trial

Correlation analysis showed a significant negative relationship between mean daily growth rate (grams wet-weight per day) of *C. galactura* and suspended sediment concentration ($R = 0.98$; $P = 0.003$). Mean daily growth rates differed significantly among suspended sediment treatments (ANOVA, Figure 2, Table 1). Growth rates among the 0, 25, 50 and 100 mg/L treatments were not significantly different, but growth was significantly

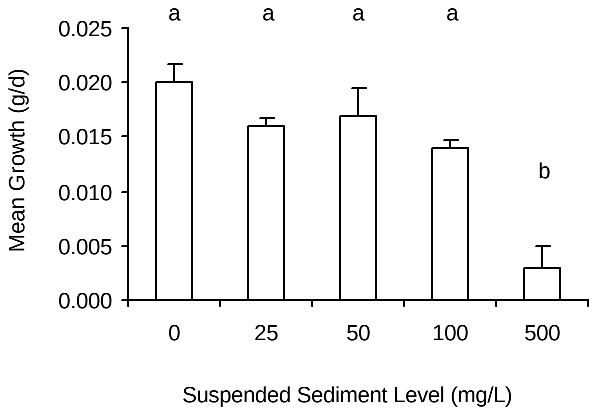

Figure 2. Mean daily growth rate (grams per day) of juvenile *Cyprinella galactura* for 5 suspended sediment treatment levels. Bars with different letters above them are significantly different (*P* < 0.05).

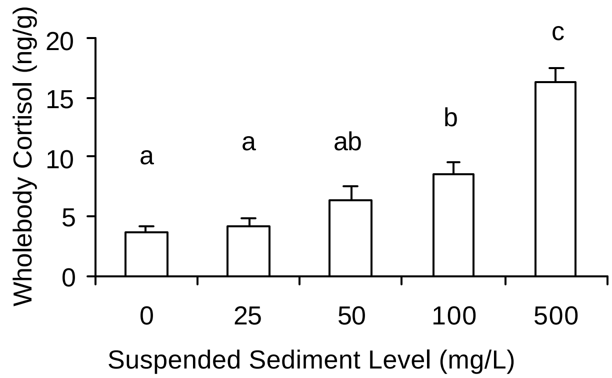

Figure 3. Juvenile *Cyprinella galactura* whole-body cortisol levels (ng cortisol per gram fish mass) for 5 suspended sediment treatment levels. Bars with different letters above them are significantly different (*P* < 0.05).

Table 1. Results of one-way ANOVA for suspended sediment level (mg/L) versus whole-body cortisol levels (ng/g) and mean daily growth rate (g/d) of juvenile *Cyprinella galactura*

Source	d.f.	Sum of Squares	*F*-statistic	*P*-value
Cortisol	4	837.7	24.5	< 0.001
Growth Rate	4	0.001	6.467	0.003

lower in the 500 mg/L treatment. There was no mortality during the growth trial.

Stress Trials

Whole-body cortisol levels (ng/g fish mass) increased with increasing suspended sediment level (Figure 3). Cortisol levels were not significantly different between the two trials and therefore data were pooled for analyses. CV of intra-plate repeated measures (assay precision) was fairly high for the first trial, but decreased below 10% for most samples in the second trial as experimental techniques were perfected. The reproducibility of the assay (inter-assay CV) was within acceptable limits. The average inter-plate CVs of samples containing 0.1, 1.0, and 10.0 ng/ml of cortisol were 5.3, 7.9 and 7.1 %, respectively. Coefficients of variation below 10% are considered acceptable (Dr. Terence Barry, Univ. of Wisconsin-Mad., pers. comm.).

Correlation analysis showed a significant positive relationship between suspended sediment level and whole-body cortisol concentration (r = 0.97; P = 0.004). Mean cortisol levels differed significantly among suspended sediment treatments (ANOVA, Figure 3, Table 1), though pairwise analysis showed that the three lowest treatments were not significantly different. There was also no significant difference between the 50 and 100 mg/L treatments. The highest treatment level was significantly different from all lower treatments.

DISCUSSION AND CONCLUSIONS

Mean daily growth rates of *Cyprinella galactura* juveniles were within the range of values (1 – 2% / day) found for other Cyprinidae species and other families (e.g. Sigler et al. 1984). The significant relationships found in both ANOVA and correlation analyses, between growth rate and sediment level, are primarily a function of the low growth rate in the 500 mg/L treatment. Though the other four treatments did not differ significantly a negative trend is suggested. Future trials with *C. galactura* and *E. monachus* are planned and this increased replication should clarify this relationship.

While there are many studies that report plasma cortisol concentrations (ng/ml) for adult salmonids, there are few data on young-of-year whole-body cortisol levels. Studies on whole-body cortisol levels of cyprinids are non-existent. Although stress response is probably species and stressor specific, it is useful to put these findings in context of previous research results.

Cortisol concentrations for the four lowest treatments were well within the range reported by Barry et al. (1995) for salmonid larvae exposed to acute handling stress (6 – 10 ng/g). However, cortisol concentration for the 500 mg/L treatment (16.2 ng/g) was considerably higher than values reported by Barry et al. (1995). The fact that the 500 mg/L treatment caused a marked stress response is of importance, because stormflow suspended sediment levels in the Little Tennessee River (which harbors one of the few remaining populations of *E. monachus*) are typically many-fold larger than this treatment level. Although these data are preliminary, they suggest a positive relationship between suspended sediment level and physiological stress in *C. galactura*. Understanding the physiological effects of excessive sedimentation of streams is important because they may negatively impact fish populations through increased immunosuppression and decreased growth and survival.

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