The effects of Suspended Sediment on Japanese Medaka (*Oryzias latipes*) and Mosquitofish (*Gambusia affinis*) Metabolism

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Sedimentation is recognized as a significant environmental stressor in aquatic ecosystems and high amounts of suspended sediments (SS) in streams are known to negatively affect aquatic organisms. In particular, it has been hypothesized that many fish species may exhibit increased respiration rates when exposed to elevated SS. To evaluate this hypothesis, we evaluated the acute response (3 hour exposure) of two small, freshwater fish species exposed to high suspended sediment loads using experimental respirometry chambers which measure oxygen consumption as a proxy of metabolism. Our results indicate that Japanese Medaka (*Oryzias latipes*) did not exhibit a significantly greater oxygen consumption, as compared to control fish, when exposed to chamber sediment of 0.17 g/L (p-value of 0.41), though oxygen consumption was higher for sediment treatments. However, mosquitofish (*Gambusia affinis*) exposed to 0.17 g/L did exhibit noticeably greater oxygen consumption compared to fish in no-sediment control test, but not at statistically significant levels (p-value of 0.07). Further study is needed with increased sample sizes and across SS levels to determine the threshold of increased metabolic rate among freshwater species exposed to SS.

Introduction

Sedimentation is the largest source of contamination in North American streams and rivers (Waters 1995). Anthropogenic activities such as logging, development, erosion, removal of riparian vegetation, and agricultural practices have increased the amount of suspended sediments (SS) in many stream ecosystems (Richards and Host 1994). Elevated levels of SS can be detrimental to aquatic species either through stream habitat alteration (decreased critical refuge sites), behavior (reduced spawning activity) or decreased physiological function (impaired gill function, increased metabolic stress, and thus energy demands associated with respiratory systems) (Sutherland 2007, Sutherland & Meyer 2007, Moyle & Leidy 1992). Chronic exposure to elevated concentrations of SS may reduce gill surface area available for respiration, alter the amount of dissolved oxygen available to ectoderms, impairing metabolism (Newcombe & Jensen 1996, Rosewarne et al 2014).

Closed respirometry systems are often used by physiologists to measure standard metabolic rates of fish species due to their high repeatability and effective design (Steffensen 1989, Norin & Malte 2011). Closed respirometry experiments involve measuring the dissolved oxygen content of water over a set time interval within a closed chamber of a known volume. Individual fish are placed within chambers and oxygen depletion within the chamber is measured throughout the timed experiment to estimate oxygen demand of fish when exposed to sediment. Small freshwater fish, such as Medaka (native to Asia) and mosquitofish (widespread throughout the U.S.), are ideal for use in respirometry trials designed to quantify the physiological effects of SS as they are model species readily available and easily maintained in the laboratory (Newman & Doubet 1989, Wittbrodt et al. 2002).

While there is accumulating research demonstrating the negative effects of SS on fish at levels as low as 25 mgL, the specific physiological effects of SS across a range of sediment levels are largely unknown (Newcombe & Jensen 1996, Smith et al. 2003). There also are limited data addressing the effects of SS on nongame freshwater fish species, as the majority of studies of SS on fish have focused on salmonids. Therefore, given the growing evidence that SS negatively affect fish species, it is critical to evaluate the potential sources of stress that increased levels of suspended sediments may have on a variety of fish species. Our primary goal in this study was to investigate how high levels of suspended sediment would affect short-term metabolic parameters in Japanese Medaka (*Oryzias latipes*) and mosquitofish (*Gambusia affinis*). We predicted that both of these small, freshwater fish species would increase oxygen consumption due to stress associated with high sediment loads and interference of normal gill oxygen uptake.

Methods

We chose Mosquitofish and Japanese Medaka for our experiments as both are well established as model species for ecological and genetic research and each of these species was readily available at the University of Georgia's Savannah River Site Ecology Laboratory located in Aiken, SC. Japanese Medaka and mosquitofish were utilized for closed respirometry experiments from July 15 to August 1, 2013. All fish used in experimental trials were denied food for 24 hours prior to experimentation and were measured for total length (± 0.5mm) and mass (± 0.01g). Individual responses of fish to SS were measured based on oxygen consumption (i.e., via respirometry) in custom-designed and constructed respirometers (Goforth 2009). These respirometers are closed systems with an integrated water pump for unidirectional flow that maintains fine sediment (i.e., < 250 µm particle size) in suspension during trials (Fig. 1A, 1B). Sediment was collected from Horse Creek, near Aiken South Carolina, heated to 120°C for 24 hours (to remove active bacteria), then filtered through a 250 µm particle size sieve to obtain fine particle size. Exactly 0.192 g of sediment was utilized in each sediment treatment trial to achieve values of 800 mg/L equated to ~0.17 mg/L SS (determined by initial validation as not all sediment added to chambers suspended in water). Each experimental respirometry chamber (total volume 0.24 L) was

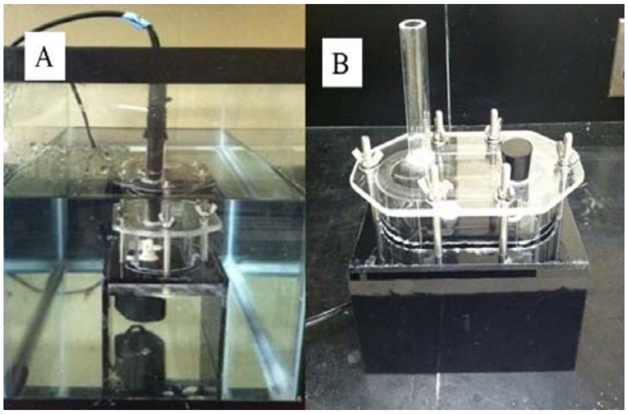

Figure 1. Experimental conditions with respirometer with oxygen probe placed in aquaria (Panel A) and closed respirometer design (Panel B).

fitted with a Jenway 2000 dissolved oxygen probe (± 0.01 mg/L) to monitor oxygen levels during trials. Individual fish were sealed in experimental chambers, each of which was incubated in larger aquaria maintained at 22 ºC for the duration of the trial. Trials lasted 3 hours and 10 minutes, and oxygen consumption was determined based on the starting (10 minutes after the trial begins) and ending (190 minutes after the trial began) oxygen concentrations in the chamber. All respirometry experiments were conducted under similar experimental conditions with either sediment added (sediment treatment) or no sediment added (control). Individual fish were used only once for either sediment treatment or control experiments.

Oxygen consumption was calculated in mg/L following Goforth (2009). To analyze the data, a T-test, assuming equal variances, was used to test for significant differences in mean oxygen consumption from the beginning to the end of trials for each species and treatment (SS or control) combination.

Results

Thirty-one complete respirometry experiments were analyzed for Japanese Medaka (N=10; 5 sediment treatments, 5 controls) and mosquitofish (N=21; 11 sediment treatments, 10 controls). Mean total length and mass for Japanese Medaka and mosquito fish were 33.3 ± 2.7 SD, 0.4 ± 0.18 SD and 41.8 ± 3.5 SD, 0.76 ± 0.18 SD, respectively. Japanese Medaka exposed to a high sediment concentration of 0.17 g/L consumed more oxygen during the trial period than did fish exposed to no sediment, but the difference was not significantly different (p-value = 0.41). Mean oxygen consumed in the Medaka control experiments was 2.57 mg/L, while the mean oxygen consumed by Medaka subjected to the sediment treatment was 2.71 mg/L (Fig. 2). Mosquitofish exhibited marginally greater oxygen consumption at the sediment concentration of 0.17 g/L than did those fish in sediment-free controls (p-value = 0.07). The average oxygen consumed by mosquitofish in control experiments was 2.48 mg/L while the average oxygen consumed by mosquitofish subjected to the sediment treatment was 3.03 mg/L (Fig. 3). Both species expended more energy and consumed more oxygen in response to the high SS concentration experiments.

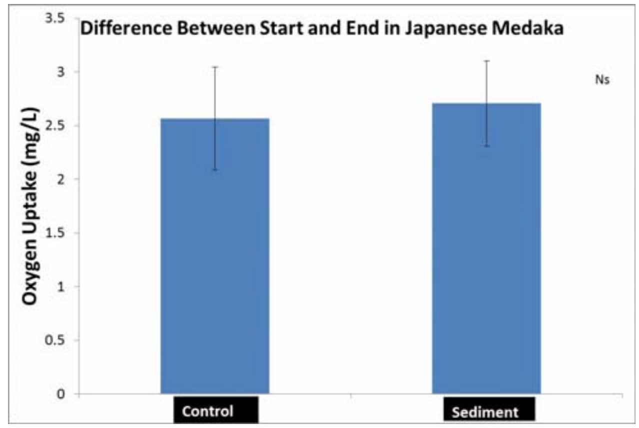

Figure 2. The difference of oxygen uptake measured in mg/L between start and end of experimental trials in Japanese Medaka *(Oryzias latipes). *Ns denotes non-significant value.*

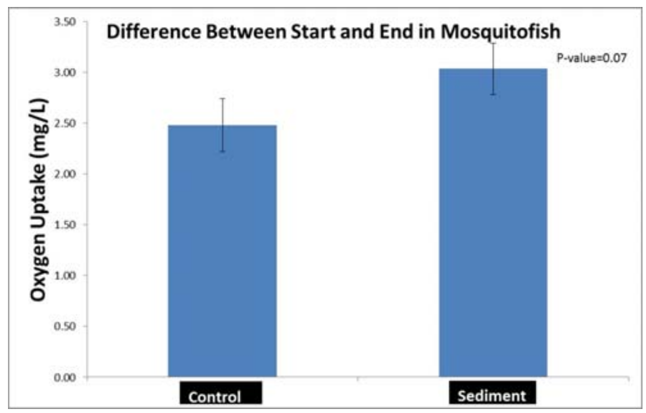

Figure 3. The difference of oxygen uptake measured in mg/L between start and end of experimental trials in mosquitofish (*Gambusia affinis*); p value = 0.07.

Discussion

The primary objective of this study was to determine if elevated levels of SS affect the physiology (oxygen consumption measured as a proxy for metabolism) of model fish. Our results support the hypothesis that fish increase respiration rates when exposed to increased loads of suspended sediment. While we observed increased consumption of oxygen as a consequence of exposure to SS in both fish species, the lack of statistical significance in our results is likely due to the small sample size tested within treatments. Larger numbers of experimental trials may have increased our ability to detect a difference in oxygen consumption as a function of SS loads in these species. Within species, marginal increases in metabolic rates we observed in our experiments likely can be attributed to impairment of respiratory gill function. Gill damage, reduced gill condition across the respiratory surface of the gill, has been documented for other fish species at similar levels of SS (Sutherland & Meyer 2006). In addition, the physical characteristics of sediment (particle composition, type, chemistry, etc.) also may have contributed to variation in rates of oxygen consumption during experiments, inflating the observed variance in outcomes and lowering our power to detect a response. Differences in gill shape and size between Medaka and mosquitofish may have driven the magnitude of the response in oxygen consumption rates observed between species. For example, mosquitofish used in our experiments were larger than Medaka, and presumably have larger gill area, possibly affecting the potential for this species to respond to increased SS in the environment relative to the smaller Medaka. In addition, mosquitofish are tolerant to low oxygen conditions (Porte et al. 1992) in the wild, which suggests that this species has evolved physiological mechanisms to respond to low oxygen environments such as those in our experiments. Clearly, further study is needed using adequate replication if the variance in biological response to elevated suspended sediment loads is to be elucidated for these fish species.

This study was limited by the numbers of experimental replicates and relatively short period over which the individual trials were conducted (~3 hours). Nonetheless, given these constraints, we did document changes in fish physiology tending toward statistical significance. Our study provides a valuable first step towards understanding the impact of sediment on small freshwater fish and suggests that further research using larger numbers of experimental replicates over longer time periods as well as a range of SS loads would be useful for characterizing the biological responses of these fish species to SS.

Notes and References

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