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NOTE

Short-term exposure to elevated suspended sediment increases oxygen uptake of gilled larval Eastern Hellbender (*Cryptobranchus alleganiensis*) salamanders

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Abstract: Freshwater ecosystems are increasingly impacted by anthropogenic elevated levels of suspended sediment that may negatively affect aquatic organisms, including salamanders. Although increasing fine sediment in streams has been suggested as a reason for population declines, to date no study has empirically assessed the effect of suspended sediment on gilled larval Eastern Hellbenders (*Cryptobranchus alleganiensis* (Sonnini de Manoncourt and Latreille, 1801)), a critical life-history stage and a species of conservation concern. We used custom respirometers to elucidate effects of suspended sediments on larval Eastern Hellbender oxygen uptake in trials conducted in situ in Georgia (USA) streams. Mean oxygen uptake increased and was significantly higher in trials when larval salamanders were exposed to suspended sediment (mean = 5.06 mg O_2/L for 800 mg/L of sediment treatment vs. 2.25 mg O_2/L for 0.00 mg/L of sediment control). This may indicate elevated physiological stress in response to short-term exposure to suspended sediments. Qualitatively, individuals in both groups exhibited rocking behavior in response to low oxygen (hypoxia), albeit at different frequencies (sediment exposure = 7.6 rocks/min and control = 2.1 rocks/min). Larval salamanders may be able to temporarily compensate for low oxygen through increased rocking behavior when high suspended sediment loads are present, with future respirometry research needed.

Key words: respirometry, sedimentation, salamander conservation, physiology, amphibian, hypoxia, Eastern Hellbender, *Cryptobranchus alleganiensis*.

Résumé : Les écosystèmes d'eau douce subissent les impacts croissants de teneurs élevées en sédiments en suspension d'origine humaine qui peuvent avoir des effets négatifs sur les organismes aquatiques, y compris les salamandres. S'il a été proposé que l'augmentation des sédiments fins dans les cours d'eau pourrait être une des raisons des baisses de populations, aucune étude à ce jour n'a évalué empiriquement l'effet des sédiments en suspension sur les larves à branchies de ménopome (*Cryptobranchus alleganiensis* (Sonnini de Manoncourt et Latreille, 1801)), un stade clé du cycle biologique de cette espèce dont la conservation est préoccupante. Nous avons utilisé des respiromètres adaptés pour élucider les effets des sédiments en suspension sur la consommation d'oxygène dans des essais menés in situ dans des cours d'eau de la Géorgie (États-Unis). L'apport d'oxygène moyen augmente et est significativement plus grand dans les essais dans lesquels les larves de salamandres sont exposées à des sédiments en suspension (moyenne = 5,06 mg O_2/L pour le traitement de 800 mg/L de sédiments contre 2,25 mg O_2/L pour le traitement témoin de 0,00 mg/L de sédiments). Cela pourrait indiquer un fort stress physiologique en réaction à une exposition de courte durée à des sédiments en suspension. Du point de vue qualitatif, des individus des deux groupes présentaient un comportement de balancement en réponse à la faible oxygénation (hypoxie), bien que de différentes fréquences (exposition aux sédiments = 7,6 balancements/min et témoin = 2,1 balancements/min). Les larves de salamandre pourraient être capables de compenser provisoirement une faible oxygénation par un balancement accru en présence de fortes charges de sédiments en suspension, mais plus de travaux de respirométrie sont nécessaires. [Traduit par la Rédaction]

Mots-clés : respirométrie, sédimentation, conservation des salamandres, physiologie, amphibien, hypoxie, ménopome, *Cryptobranchus alleganiensis*.

Introduction

Conservation management should incorporate projected environmental changes and ensuing physiological effects on species of conservation concern. Freshwater ecosystems are increasingly threatened by a myriad of anthropogenic-induced environmental stressors, including increased temperature extremes, pollution, and habitat degradation (Dudgeon et al. 2006), and in particular, exposure to elevated suspended solids in streams (i.e., sedimentation) has been identified as a major threat to aquatic biota (Newcombe and MacDonald 1991; Waters 1995). It is estimated that ∼50% of streams in the United States are impaired by excessive

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sedimentation and turbidity (Henley et al. 2000), with hypoxia increasingly occurring in aquatic habitats as a result of nutrient enrichment and increased temperatures lowering dissolved oxygen availability (Rogers et al. 2016). It is therefore increasingly critical that the physiological effects of increased stream sedimentation be evaluated for vulnerable freshwater taxa.

Amphibians are among the most imperiled stream taxa and are generally considered important indicator species due to their highly permeable skin and reliance on cutaneous respiration (Welsh and Ollivier 1998; Stuart et al. 2004). In the Appalachian region, stream salamanders form assemblages that are integral to ecosystem processes (Davic and Welsh 2004; Keitzer and Goforth 2012), and thus, fully aquatic larval salamanders are likely to be ideal model species for studying anthropogenic pollution and environmental disturbance of streams in this region (Lowe and Bolger 2002). Surprisingly, despite the widespread persistence of sedimentation in lotic ecosystems of the United States, few studies have focused on sedimentation as a source of stress for stream-dwelling amphibians (Keitzer and Goforth 2012). The deposition of fine sediments in lotic ecosystems can increase substrate embeddedness, reduce larval food quality (Gillespie 2002), limit the availability of larval shelter sites through inundation of interstitial spaces in stream bed substrata (Welsh and Ollivier 1998; Nickerson et al. 2003; Brannon and Purvis 2008), and alter foraging and cover habitat (Waters 1995). These effects can interact synergistically to reduce aquatic salamander abundance (Bank et al. 2006). Suspended sediment loads in freshwater habitats can also negatively impact aquatic juvenile gilled vertebrates by altering feeding rates and aerobic scope, abrasion or clogging gills, or lowering growth rate (Martens and Servizi 1993; Lake and Hinch 1999; Rosewarne et al. 2014; Cumming and Herbert 2016). Stream salamander larvae have shown greater sensitivity to sedimentation than adults through both direct acute effects (lethal concentrations that can clog gill rakers and gill filaments; Price et al. 2011) and sublethal effects (including reduced physiological function such as reduced growth or reproduction; Henley et al. 2000).

The natural history of the Eastern Hellbender (*Cryptobranchus alleganiensis* (Sonnini de Manoncourt and Latreille, 1801)) salamander makes it an ideal model species to study the impacts of increased fine sediment loads (i.e., larval reliance on gills and use of cutaneous respiration; Nickerson and Mays 1973). This salamander species uses a fully aquatic mode of respiration whereby ~90% to 97% of gas exchange occurs cutaneously in adults (Guimond and Hutchison 1973). Previous research has documented adult Eastern Hellbenders exhibiting rocking behavior in hypoxic conditions if oxygen saturation drops below 75%, performing this behavior to increase the amount of cutaneous gas exchange and possibly to maintain blood oxygen tension (Harlan and Wilkinson 1981). Gilled larvae may be exposed to elevated suspended sediment levels during critical growth periods if they inhabit streams with high stream bank soil erosion input from anthropogenic terrestrial sources, or following rain events that may temporarily increase suspended sediment concentration and deplete oxygen (Kemp et al. 2011). Increased suspended sediment exposure in aquatic ecosystems has the potential to be detrimental to growth (Cumming and Herbert 2016) and preferentially impact smaller fish species with smaller gills (Chu et al. 2020). Unfortunately, little is known regarding juvenile or gilled larval salamander tolerances to increased sediment loads because early life-history stages of this species are rarely encountered across its geographic range (Foster et al. 2009). At present, Eastern Hellbender populations throughout much of the United States suffer from extremely low recruitment and declining populations are heavily skewed towards adult age classes (Wheeler et al. 2003; Burgmeier et al. 2011). Purported reasons for these declines include siltation, water pollution, collection for the pet trade, and angling mortality (Wheeler et al. 2003; Nickerson and Briggler 2007). Eastern Hellbender larvae have external gills that they use until they are ~1.5 years old, or ~125 mm total length (Nickerson and Mays 1973), making them ideal candidates to examine how suspended sediment affects physiology. However, we are aware of no study in which sediment has been empirically tested as a source of potential physiological stress, as well as potentially a driver of declines, in this species.

Respirometry is a non-invasive tool for estimating oxygen consumption of aquatic organisms, often used as a proxy of metabolism (Stiller et al. 2013). Closed system respirometry studies place individuals in sealed chambers and measure the decrease in oxygen concentration over time (Svendsen et al. 2016). While previous researchers have used respirometers to investigate the effect of sediment load on aquatic organisms, such as juvenile fishes (Neumann et al. 1982; Hess et al. 2017) and a select few amphibian species (Bucher et al. 1982; Strickland et al. 2016), no studies have used respirometry to investigate the effects of sediment on oxygen consumption of gilled larval Eastern Hellbender salamanders, a critical life-history stage of hellbenders for population stability and recruitment (Unger et al. 2013). This is due in part because it is difficult to use respirometer techniques in situ due to lack of control over actual suspended sediment levels and the interaction of temperature and sediment as stressors in a natural environment (Alexander et al. 1994; Rodgers et al. 2016). Thus, preliminary data are largely lacking to demonstrate how hypoxia and increased suspended sediment interact to alter oxygen consumption in fully aquatic salamanders.

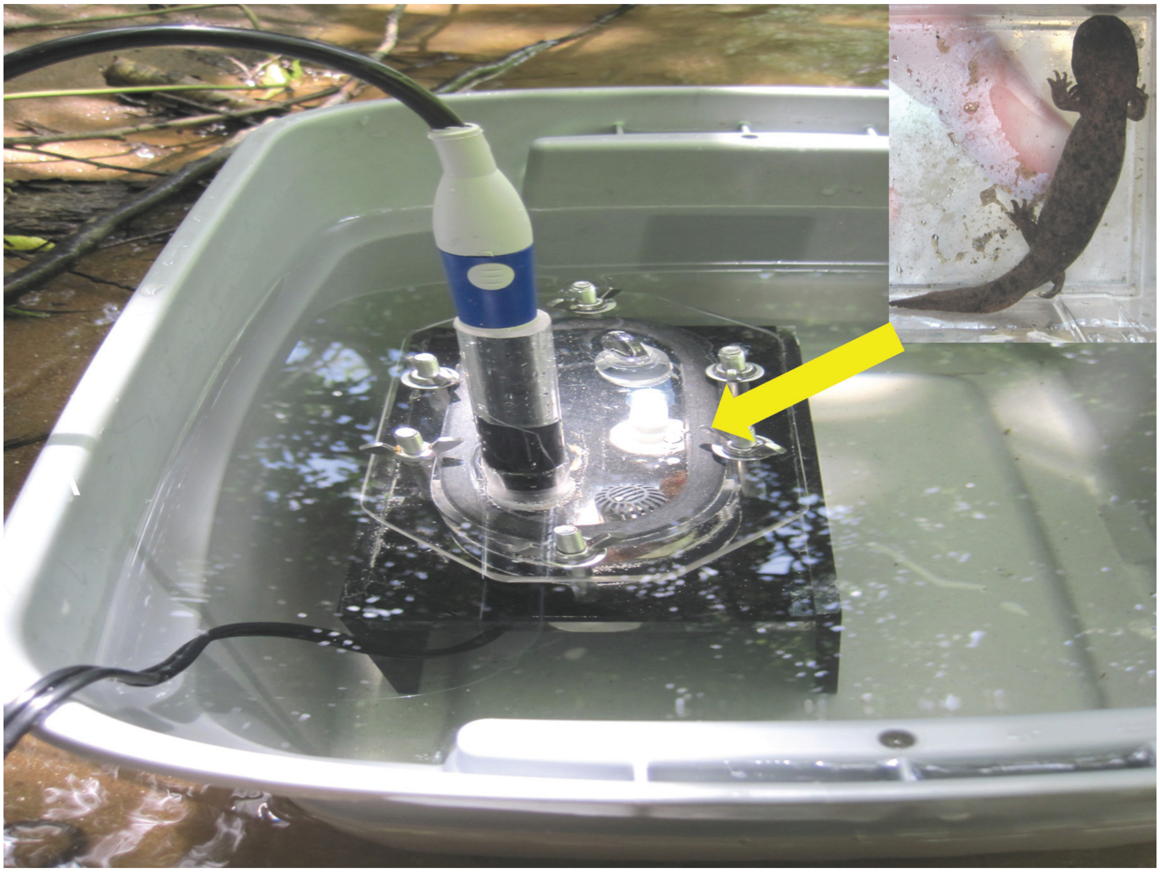
Here, we use in situ respirometry techniques to investigate the effects of elevated suspended sediment on oxygen uptake of salamanders in their natural streams. The aim of the present study was to quantify how reduced oxygen availability or hypoxia would affect the oxygen uptake ability of gilled larval Eastern Hellbenders (measured as uptake of dissolved oxygen in chambers as a proxy for metabolic rate) at high sediment stream loads. We defined hypoxia as the point in time when dissolved oxygen reached 4.0 mg/L (below half of starting dissolved oxygen levels) in respirometry chambers (following Woods et al. 2010; Mahler and Bourgeais 2013; Gilmore et al. 2019). For this purpose, we collected larval Eastern Hellbenders from Georgia (USA) streams and placed them in a portable, in situ closed respirometry system. We tested the hypothesis that (*i*) the presence of suspended sediment would alter dissolved oxygen levels and oxygen uptake rates of larval Eastern Hellbenders and (*ii*) larval Eastern Hellbender salamanders exposed to high sediment loads would have higher oxygen uptake rates. We discuss the potential for sediment in aquatic systems in relation to oxygen uptake to potentially interfere with absorption of dissolved oxygen via skin or gills. We anticipate that this portable respirometer design can be used for future studies on fully aquatic organisms (e.g., fish, mussels, other salamanders) to further investigate oxygen uptake rates in aquatic organisms occupying freshwater streams impacted by varying sediment levels and other factors (temperature, pH, etc.). This study represents the first documentation of sediment impacting oxygen uptake in larval Eastern Hellbenders, a species in decline throughout much of its geographic range.

Materials and methods

Field sampling

Wild-captured larval Eastern Hellbender salamanders were sampled opportunistically during annual stream surveys during July–August of 2013–2015 from three stream reaches (GA1, GA2, and GA3) in the Tennessee River drainages in northern Georgia (location on file with the Georgia Department of Natural Resources and withheld to protect location). Individual larval salamanders were captured by hand during snorkel surveys in streams, were briefly transferred to mesh bags, and were kept in the stream under full shade until the experiment. Salamanders were carefully examined to ensure they possessed full, undamaged gill structures and were of similar size (<100 mm total length; mean mass = 1.6 g (range 1.4–1.7 g), total length = 61.4 mm (range 59.4–

Fig. 1. Custom respirometer setup and field implementation showing the respirometer in a stream with the oxygen probe inserted into the custom tube, the lid held by wing-nuts, a glass stopper, and the internal flow maintained by a modified aquarium air pump (below the housing). Gilled larval Eastern Hellbenders (*Cryptobranchus alleganiensis*) (upper right) were placed in respirometers for both control and sediment trials in northern Georgia, USA. Color version online.



63.3 mm)). Individuals were randomly assigned to either control or treatment groups and then placed singly in respirometers that were transported to field sites (details below). During the last 5 min of respirometer trials, we quantified mean rocking behavior (number of rocking motions per minute) as this represented an organismal response to hypoxic conditions across trials. All individuals were released to their point of capture following post-treatment monitoring for 30 min. Before releasing individuals, we physically examined gill structure using handheld magnifiers to document any potential gill damage or color change. Biological data (i.e., mass (g), total length (mm), and snout to vent length (mm)) and stream water-quality data (i.e., total dissolved solids (TDS), temperature (°C), and pH) were collected using standard calipers, a handheld 250 g balance, and an Ohaus® Instruments portable water-quality meter (Ohaus Corp, Florham Park, New Jersey, USA) before individuals were released.

Respirometry field trials

We used custom-designed and constructed closed acrylic respirometers (0.24 L volume; Bossle et al. 2015; Fig. 1). Sediment exposure and control trials were conducted in situ on the banks of streams within a few metres of larval Eastern Hellbender capture locations and ≤1 h of the time of capture. We performed 16 total respirometry trials (8 for sediment exposure and 8 for control; all different individuals). For control treatments, four trials were conducted in 2013, three trials in 2014, and one trial in 2015, whereas for sediment treatments, four trials were conducted in 2013, one trial in 2014, and three trials in 2015. Individual salamanders were carefully inserted into an opening on the upper surface of the respirometer chambers and an air-tight seal was secured with the placement of a glass stopper. All individuals exhibited normal behavior within 5 min of experiment in all cases (limited exploratory movement). To minimize internal chamber pressure, we slowly inserted an Jenway® 9500 oxygen meter probe into a specially designed tube also positioned on the upper surface of each respirometer chamber. Initial (control) trials were performed using water collected on site at the top of the water column with no addition of sediment, containing <9 mg/L TDS, visually confirmed to be clear at 120 cm with a turbidity tube (Ben Meadows, Janesville, Wisconsin, USA). If streams were visually turbid (e.g., following rain), or if any measurable turbidity was noted with the turbidity tube, then no trials were conducted and streams were revisited the following day. Water chemistry values of the study site streams exhibited little variation, with chamber temperature across field trials ranging from ∼21 to ∼22.7 °C during treatments. As stream water contains some, albeit, low levels of TDS, clear stream water was chosen as a control to more closely mimic natural stream conditions for larval Easter Hellbenders. To conduct our sediment exposure trials, we removed the oxygen meter probe and used the tube to add a premeasured 0.2 g mass of <250 μm particle size sediment that had previously been collected from the same field location, sieved, and oven-dried. We then reinserted the oxygen meter probe into the tube to conduct each trial (following Bossle et al. 2015). Each sediment exposure trial was conducted with a sediment concentration of ~800 mg/L.

Before beginning each trial (control or treatment), we ensured there were no excess air bubbles visible in the chambers. We used a connected aquarium pump to maintain water flow within the respirometer chamber to ensure the sediment remained suspended. To maintain constant stream temperature during each trial, respirometers were placed in 37.8 L plastic containers with flow through openings and positioned directly in streams under full canopy cover. Water temperature was measured by the calibrated oxygen meters, as well as monitored manually during trials using a digital thermometer. Calculated oxygen uptake during trials was used as a proxy for routine metabolic rate or the amount of oxygen required to support metabolic rate of a rested aquatic organism (Chabot et al. 2016). Routine metabolic rate was used over standard metabolic rate, as is likely ecologically relevant, because individuals in the experiments exhibited normal behavior, but were potentially stressed, and were not handled for long periods. Dissolved oxygen concentration in the chamber was measured using the Jenway® 9500 dissolved oxygen reader connected to the previously inserted oxygen probe with values calculated every 2 min to within ±0.01 mg/L, across a total experiment time of 122 min. Dissolved oxygen in the chambers averaged a low of 6.82 mg/L in control treatments and 3.73 mg/L in sediment treatments, respectively, at the end of the experiment. Power was provided to oxygen meters and aquarium pumps using a 600 W power inverter and 12 V portable marine battery that was charged daily following field deployments. After completion of each trial, respirometer chambers were sterilized with 0.05% bleach solution and dried to minimize microbial bacterial oxygen usage and potential spread of infection agents between streams. Respirometer air pump motors were cleaned with cloth and Q-tips® and rinsed with deionized water to remove excess bleach and sediment. Prior to each trial, the oxygen meter was calibrated using a zero-oxygen solution of sodium sulphite according to the manual instructions.

Data analysis

We calculated oxygen uptake during each trial by subtracting the end of trial oxygen water concentration (mg/L) from the initial dissolved oxygen water concentration, similar to Bossle et al. (2015). This provided a concentration value for comparison across sediment treatment versus control treatment for how much dissolved oxygen was in respirometers during the experiment to quantify the potential effect of sediment on larval Eastern Hellbenders. To account for potential bias in size of individuals, we statistically compared mass of individual gilled larvae for control and sediment treatments using a paired *t* test in R using “t.test()” function assuming unequal variance to ensure relative uniformity of mass because previous research has found a correlation between salamander size and oxygen consumption (mL O_2/h or mm^3 O_2/h; Wood and Orr 1967; Homyack et al. 2010). Dissolved oxygen in chambers was compared for sediment exposure and control trials using a paired *t* test similar to the mass comparisons. We calculated the slope of a regression line for dissolved oxygen decline in respirometers using standard linear regression analysis. We tested the difference in slopes between control and treatment regression lines by performing a standard slopes test for significance using sample size for line, standard error for line, and slopes of lines. As an additional metric incorporating body size of individuals, we also calculated oxygen uptake according to Steffensen (1989) and Svendsen et al. (2016) using the formula $y = V_{\mathrm{RE}} \cdot W_{\mathrm{o}} \cdot (\mathrm{d}CO_2/\mathrm{d}t)$, where V_{RE} is respirometer volume, W_{o} is mass of organism, and $\mathrm{d}CO_2/\mathrm{d}t$ is the slope of the linear decrease in oxygen content during trials. We then calculated oxygen uptake as a rate expressed as mg O_2/min. We compared rocking behavior among treatments using descriptive statistics and report standard error (SE).

Ethics approval

Collection and handling of Eastern Hellbenders were approved by the University of Georgia Institutional Animal Care and Use Committee (IACUC) under protocol #A2013 06-023.

Results

A total of 16 trials (eight trials each of control and sediment exposure treatments) were performed between 16 August 2013 and 30 July 2015. Oxygen uptake was significantly faster in the sediment exposed than control treatments, $t_{[15]} = 9.86$, $p < 0.0001$ (Fig. 2). Mean (±SE) oxygen uptake during the 120 min incubation was 5.06 ± 0.81 mg/L for the sediment group, while mean (±SE) oxygen uptake was 2.25 ± 0.32 mg/L for the control exposure treatment, with less dissolved oxygen remaining at the end of sediment treatments. When volume of the respirometry chamber, mass of organism, and slope of declining oxygen in the chamber was taken into consideration, oxygen uptake was 0.0306 mg O_2/g·min for sediment treatments and 0.0144 mg O_2/g·min for control treatments, with higher oxygen uptake in sediment treatments. We observed greater variation in sediment versus control exposure for oxygen uptake when expressed as mg O_2/min (Fig. 3). The sediment treatment reached hypoxia (<4 mg/L) only during the last ~20 min of observation, while control treatments, on average, maintained higher oxygen at the end of the experiment. Mean overall mass and total length of salamanders used in trials was 1.6 g and 61.4 mm, respectively; mass of gilled larval salamanders was not significantly different between individuals selected for the control and sediment exposure treatments ($t_{[15]} = -0.209$, $p = 0.418$). Size range and mean (±SD) for mass and total length were 1.4–1.7 g (1.54 ± 0.119 g) and 59.6–63.3 mm (61.4 ± 1.09 mm), respectively, for control groups, and were 1.4–1.7 g (1.55 ± 0.120 g) and 59.4–63.0 mm (61.4 ± 1.42 mm), respectively, for sediment exposure groups. The regression for control treatment was characterized by a slope of −0.040, whereas the slope of the sediment treatment was −0.085, indicating dissolved oxygen concentration oxygen of the chamber declined at a faster rate in sediment trials (Fig. 2); however, the two slopes were not significantly different ($t_{[120]} = 0.205$, $p = 0.838$). Both linear regressions exhibited high R^2 values (0.968 for sediment treatment and 0.993 for control treatment). The low sample size precluded the establishment of differences between study years.

Water chemistry values of study site streams exhibited little variation, with chamber temperature across field trials ranging from ~21 to ~22.7 °C during treatments (Table 1). The amount of TDS in study site streams ranged from 8.2 to 8.9 mg/L and pH values ranged from 6.9 to 7.1. We observed a greater occurrence of rocking behavior in our sediment treatments (7.6 ± 0.93 rocks/min) compared with control treatments (2.1 ± 0.74 rocks/min). Qualitative examination of larval gill structure of individuals immediately following respirometer trials found no observable direct damage to gills as a result of our short-term field experiment with all lamellae appearing normal across all trials.

Discussion

Our preliminary study is the first investigation of oxygen uptake associated with high suspended sediment on gilled larval Eastern Hellbender salamanders. We found that short-term exposures to elevated sediment loads increased oxygen uptake in larval Eastern Hellbenders based on field respirometer trials. We caution interpretation of these preliminary findings because hypoxic conditions were reached more frequently in the sediment treatment. In comparison, other studies of short-term suspended sediment exposure have detected declines of oxygen consumption in aquatic organisms that may be able to alter their activity (respiration) in response to increases in suspended sediment or turbidity (Grant and Thorpe 1991). Interestingly, the response of amphibians to high influxes of suspended sediment varies widely across species.

Fig. 2. Mean (±SE) oxygen uptake (mg/L) across all trials measured in control (light gray boxes; $n = 8$) and sediment (dark gray triangles; $n = 8$) treatments for Eastern Hellbender (*Cryptobranchus alleganiensis*) in field-deployed respirometers in northern Georgia, USA.

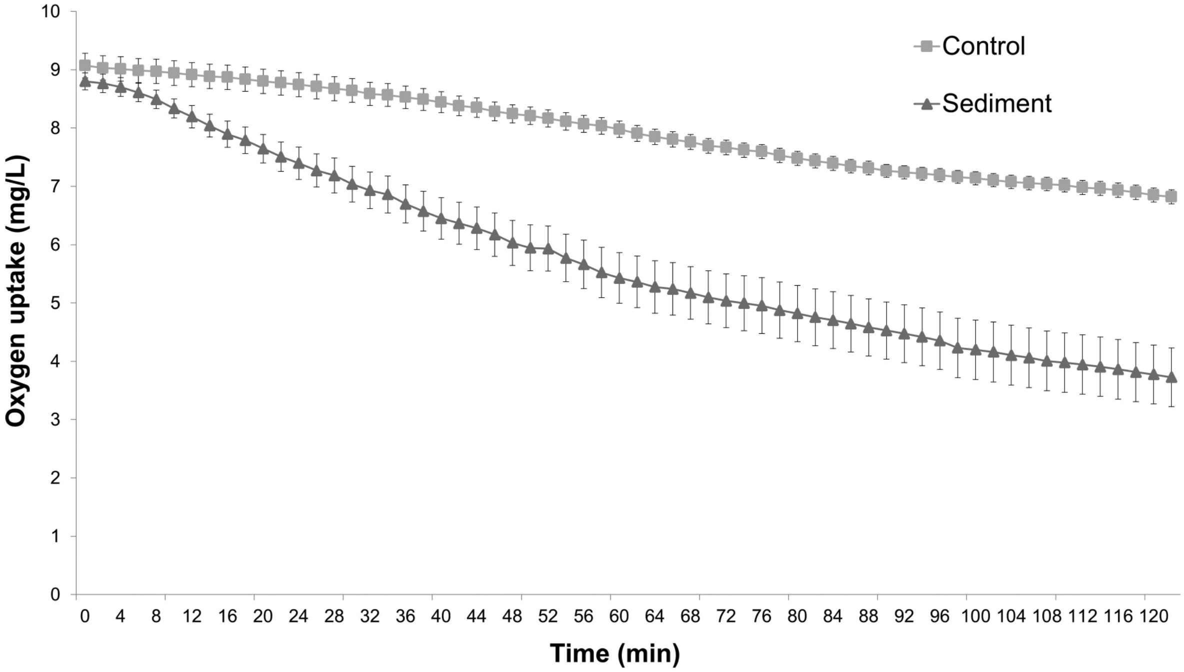
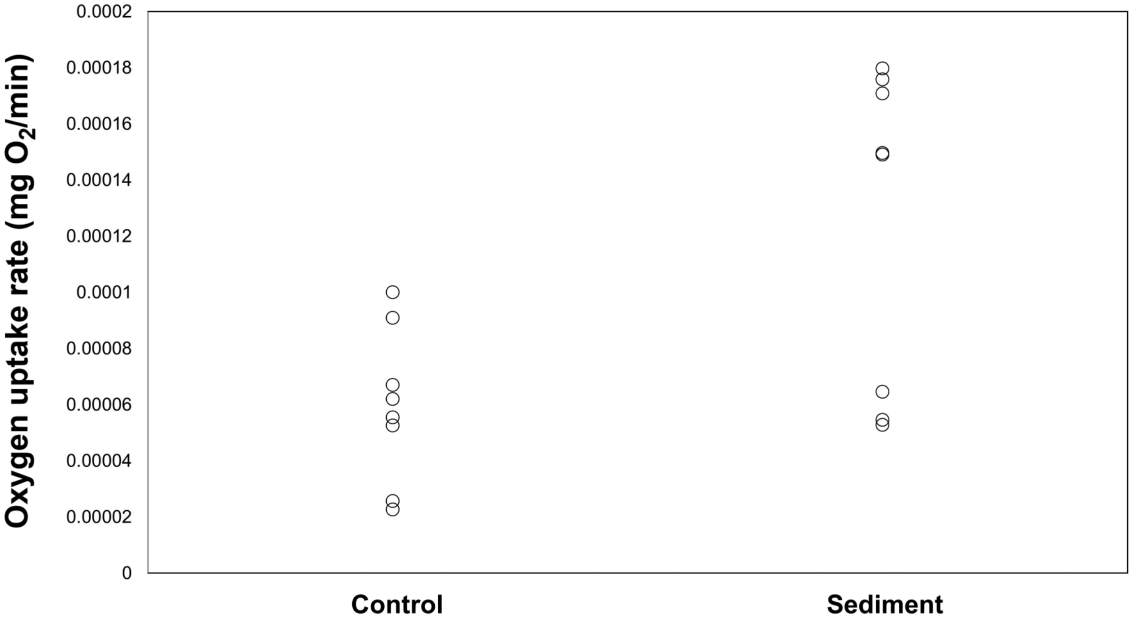


Fig. 3. Variation in oxygen uptake rate (mg O_2/min) in control ($n = 8$) and sediment ($n = 8$) respirometers for gilled larval Eastern Hellbenders (*Cryptobranchus alleganiensis*).



For example, Welsh et al. (2019) found increases of sediment led to declines of both Tailed Frogs (*Ascaphus truei* Stejneger, 1899) and Southern Torrent Salamanders (*Rhyacotriton variegatus* Stebbins and Lowe, 1951), with concomitant increases in Coastal Giant Salamanders (*Dicamptodon tenebrosus* (Baird and Girard, 1852)) within the same streams. Moreover, the effects of increased sediment on salamanders may be stage-specific, as interstitial spaces and shelter habitat reduce refugia, potentially increasing exposure of larvae to predators (Lowe et al. 2004). We hypothesize that the increased oxygen uptake that we observed in sediment treatments could be the result of increased rocking behavior (in the presence of higher suspended sediment) or represent a shift in cutaneous respiration. This rocking behavior could have potential physiological and energetic costs if sediment levels are persistent in streams with gilled larval Eastern Hellbenders. To elucidate the mechanism of increase in oxygen uptake, we recommend further study using divided-chamber respirometry along with additional treatments of suspended sediments in both field and laboratory conditions. We also recommend future respirometry studies incorporate normoxic controls to test the effect of interactions between oxygenation level

Table 1. Mean values of water-quality parameters for three unique stream-reach sections (GA1–GA3) sampled for Eastern Hellbenders (*Cryptobranchus alleganiensis*).

	GA1	GA2	GA3
Dissolved oxygen (DO; mg/L)	9.6	9.9	9.1
pH	6.9	7.1	7.1
Total dissolved solids (TDS)	8.9	8.2	8.7

Note: Stream reaches are within Tennessee River drainages in Georgia; locations are withheld to prevent illegal collection of Eastern Hellbenders, but are on file with the Georgia Department of Natural Resources, Atlanta, Georgia, USA.

and sediment occurrence. Finally, oxygen consumption trials should be conducted in controlled laboratory conditions to more accurately measure changes in dissolved oxygen levels over time and develop oxygen consumption curves unique to the species.

Increased oxygen uptake rates can lead to detrimental effects, particularly if they reduce aerobic scope. However, the duration of high sediments likely varies temporally across streams within the geographic range of Eastern Hellbenders and unfortunately little is known about the effects of long-term exposure to elevated sediment loads for this species. However, elevated suspended sediment due to precipitation are likely to be short lived and be limited in direct impacts on salamander larvae because sediment concentrations often return to pre-rainfall levels within 72 h in many Appalachian streams (Miller et al. 2015). Although larval Eastern Hellbenders may be able to withstand short-term elevated sediment loads, we caution interpretation of these results because the stress of elevated sediment loads in combination with other chronic stream pollutants may have detrimental sublethal effects on larval Eastern Hellbender populations across their geographic range. Alternatively, elevated sediment may impact food sources (macroinvertebrates), the availability of interstitial habitat, and potentially affect predator–prey dynamics, as well as have other indirect effects.

It is likely that elevated sediment, if persistent, can lead to indirect effects, potentially affecting growth of aquatic organisms. Suspended sediment concentrations above 450 mg/L may negatively affect aquatic organisms, such as brown trout (*Salmo trutta* (Linnaeus, 1758)), by reducing feeding rate (Greer et al. 2015). Moreover, elevated suspended sediment may be indirectly altering local macroinvertebrate prey densities that may be sensitive to increasing sediment loads and sediment-associated contaminants (Moran et al. 2017). Larval Eastern Hellbenders are known to rely on mayfly nymphs as a major source of food (Hecht et al. 2017), and mayfly nymphs exhibit significant increases in mortality in response to elevated suspended sediment (Everall et al. 2018). Alternatively, gilled larvae may be able to counter elevated sediment levels by relying on cutaneous respiration in combination with increased rocking behavior as we documented for larvae in our sediment treatments. The greater rocking behavior in sediment treatments could explain the higher oxygen uptake rates for sediment trials. Future studies could position cameras on chambers during all trials to allow ease of behavioral observations, such as rocking, for both sediment and control treatments. Moreover, oxygen consumption in salamanders may be correlated with overall respiratory surface as studies have shown with lungless and lunged salamanders (Feder 1977). In addition, scaling of size (either gill or surface area) may have impacted our results as size of individuals varied slightly in our study. As research on oxygen consumption in response to sediment loads in stream-dwelling salamanders is lacking compared with other aquatic organisms (fish, mussels, etc.), further research should be conducted in salamanders under realistic water-quality stream conditions of sediment, pH, and temperature.

Although we noted treatment effects in our experiments, it is important to note that the trials were conducted in streams with existing, small variations in TDS, pH, and temperature, and were not under laboratory conditions with water chemistry and temperature controlled. Moreover, we did not correct oxygen capacitance for temperature. We acknowledge that variation in these factors could have affected oxygen uptake in our study, but we suggest that these effects were likely minimal because both control and treatment trials were conducted within the same streams in close temporal proximity with no significant effect of year. As our study was limited to individuals sampled across three stream reaches and across multiple years, we recommend future work in aquatic respirometry occur within the same stream or within the same season to minimize the effects of acclimation history (e.g., warmer than normal spring) or overall temperature. Future respirometry studies in closed systems should closely monitor levels of individual activity to ensure increases in consumption are not due to initial movement of experimental animals or stress of being placed in chambers. Ultimately, as this species is of conservation concern, we did not want to remove any larvae from their natural habitats for more than the time required to conduct respirometer trials and biological processing. Eastern Hellbenders are thought to be living at the physiological limits of their gas exchange capabilities (Ultsch and Duke 1990), as large vertebrates with cutaneous respiration are ideally adapted to well-oxygenated aquatic habitats (Boutilier and Toews 1981). Moreover, cutaneous respiration in combination with rocking behavior of Eastern Hellbenders may allow them to persist at elevated temperatures during summer to fall when larvae are active. Similar fully aquatic larval Brook Salamanders (genus *Eurycea* Rafinesque, 1822) increase their oxygen uptake at higher temperatures (Norris et al. 1963). Previous research on Eastern Hellbender adults has documented blood pH decreases and CO_2 increases with increases in temperature (Moalli et al. 1981). However, Terrell et al. (2013) found Eastern Hellbender adults exhibited no physiological responses to fluctuations in water temperature.

In conclusion, we noted differences in oxygen uptake for gilled larval Eastern Hellbender salamanders exposed to elevated suspended sediment in natural streams, with oxygen depletion occurring more rapidly when high suspended sediment was present. Accordingly, there appear to be no overtly negative effects in the short term, and gilled larvae may be able to shift to cutaneous respiration and increased rocking behavior when dissolved oxygen declines to hypoxic conditions. Although preliminary, these results suggest high sediment loads if persistent may have physiological effects on larval Eastern Hellbenders via increased energy costs or metabolic demands. Based on our results, we encourage zoos that are conducting captive rearing programs to consider assessing sediment and its potential effect on growth under more controlled laboratory settings and compare those trials to conditions in naturally occurring streams.

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