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High suspended solids as a factor in reproductive failure of a freshwater mussel

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Abstract. Elevated suspended solids are a widespread stressor of aquatic ecosystems, but their effects on growth and reproduction in freshwater mussels are largely unknown. We fertilized experimental ponds to create a gradient in total suspended solids (TSS) and examined the effects of TSS on growth, nutritional status, reproduction, and clearance rate in *Ligumia subrostrata*. The number of females that became gravid declined sharply with increasing TSS, and no gravid females were found in the highest TSS treatments. The proportion of gravid females was not related to the TSS organic:inorganic ratio. Fertilization was an all-or-nothing phenomenon. In all females that did become gravid, 98 to 99% of eggs were fertilized regardless of TSS, and total fecundity was unrelated to TSS. Clearance rates declined sharply as TSS increased but showed a threshold relationship in which clearance was uniformly low at TSS $> \sim 8$ mg/L. Reproductive failure probably was not caused by poor body condition or nutritional status because growth (length and mass) and energetic status (measured as caloric density) were not related to TSS. We propose 2 mechanisms that implicate interference of TSS with fertilization as the cause of reproductive failure. Reduced clearance rate could decrease the chance of females encountering suspended sperm during filter feeding, or an increase in pseudofeces production could bind sperm in mucus and lead to its egestion before fertilization. Interruption of fertilization coincident with high TSS is a potential mechanism to explain the lack of mussel recruitment in many locations. Monitoring and reduction of TSS, especially during the spawning season, may help create conditions necessary for maintenance and recovery of mussel populations. More research is needed to explore the generality of this pattern across a broad range of mussel species including those adapted to lotic environments or that use different brooding strategies.

Key words: *Ligumia subrostrata*, eutrophication, total suspended solids, fertilization, reproduction, freshwater mussel, Unionidae.

Elevated levels of total suspended solids (TSS) are a ubiquitous water-quality problem in the US (Wood and Armitage 1997, Carpenter et al. 1998, Biggs 2000) and are a threat to many aquatic organisms (Richter et al. 1997). Suspended solids can be composed of organic or inorganic particles. Increased organic solids (phytoplankton, bacteria, fungi, etc.) and eutrophication often result from excess nutrient inputs and can negatively affect aquatic organisms via decomposition and resultant hypoxic/anoxic conditions (Paerl 1988, Vitousek et al. 1997, Camargo and Alonso 2006). Elevated organic solids also can increase food resources, resulting in increased individual growth rates and biomass production (Deegan and Peterson 1992). High concentrations of inorganic solids (sand, silt, clay, etc.) originate from erosion related to agriculture, forestry, and urbanization, and can alter feeding patterns, substrate composition, and foodweb dynamics (Waters 1995). Understanding the net effects of TSS on the health of aquatic organisms is essential for establishing and implementing effective water-quality guidelines and regulations.

Freshwater mussels (Order Unionoida) have declined dramatically in much of North America, but in many cases, the causes of these declines are unknown. Anthropogenic increases in deposited and suspended solids have been widely invoked as causes of mussel declines (e.g., Brim Box and Mossa 1999), but direct

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evidence for these effects is scant (Haag 2012). Furthermore, the effects of TSS on freshwater mussels may vary widely according to context. Translocation of *Hyridella depressa* (family Hyriidae) from an oligotrophic lake to a nutrient-enriched stream below a sewage treatment plant resulted in increased growth rates, potentially because of higher food availability (Walker et al. 2001). Mean annual shell growth of *Diplodon chilensis* (Hyriidae) in Chilean lakes was strongly positively correlated with TSS concentration measured indirectly as Secchi depth (SD; Valdovinos and Pedreros 2007). However, mussels were largely extirpated from lakes with the shallowest SDs (hypereutrophic lakes), possibly indicating a threshold above which increased nutrients and resultant organic solids have a negative effect. TSS loads dominated by inorganic particles can decrease growth rates of zebra mussels (Order Veneroida) (Osterling et al. 2007). Intermittent exposure to extremely high levels of suspended sediment led to decreased clearance rates (the volume of water cleared of particles per unit time) for 3 unionid species and was proposed as a cause of decreased growth or starvation (Aldridge et al. 1987). However, 2-mo exposure to high levels of inorganic suspended sediments (>80 nepholometric turbidity units [NTU]) did not decrease energy density of *Amblema plicata* (Howard 1999).

One of the hallmarks of many mussel declines is absence of recent recruitment, a pattern suggesting that some factor limits reproduction. Sedimentation might negatively affect mussel recruitment (Brim Box and Mossa 1999), but these effects are poorly understood and appear to vary widely. Male mussels (Unionidae) fed excessive food in the laboratory had significantly higher sperm production than individuals in the wild (Galbraith and Vaughn 2009). Fecundity of *Hyridella depressa* below a sewage effluent was ~2× that of mussels upstream of the effluent (Walker et al. 2001), and food availability was positively related to offspring production in fingernail clams (*Sphaerium striatinum*) (Beekey and Karlson 2003). In contrast, recruitment strength of *Margaritifera margaritifera* was negatively related to turbidity (suspended sediment) and deposited sediment, but the mechanism for this relationship was unclear (Osterling et al. 2010). The only direct evidence of negative effects of suspended sediment on mussel reproduction is the observation that very high concentrations of suspended clay (1250–5000 mg/L) caused reduced attachment and metamorphosis success of parasitic mussel larvae (glochidia) on host fishes in the laboratory (Beussink 2007).

The effects of TSS on fertilization of mussel eggs have not been examined directly. Male mussels release sperm into the water, and sperm are captured by the female gills during filter feeding and moved by gill cilia to the suprabranchial chamber where eggs are fertilized (McMahon and Bogan 2001). Consequently, sperm capture and egg fertilization probably are sensitive to changes in TSS that influence feeding dynamics and gill function (Tankersley 1996). High TSS may decrease clearance rate, decreasing the likelihood of capturing sperm, because energetic demands can be met from a smaller volume of water (Winter 1978). Increased TSS may lead to an increase in pseudofeces production and decrease in particle selectivity (Schneider et al. 1998), increasing the chance that captured sperm will be rejected in mucus-bound pseudofeces. Thus, high concentrations of suspended organic particles may present a trade-off between increased growth, caloric density, and fecundity and decreased fertilization success.

We assessed the effects of TSS on clearance rate, growth, caloric density, and reproductive success of mussels in pond experiments. We added liquid fertilizer in experimental ponds to create a gradient of TSS concentrations and explored the balance of potential positive and negative effects. On the basis of available information, we hypothesized that increasing TSS dominated by organic suspended solids would lead to initial increases in growth, caloric density, and reproductive success because of higher food availability, but ultimately would lead to a decline in growth, caloric density, and egg fertilization as TSS exceeded a limiting threshold.

Methods

Experimental design

Our study species was *Ligumia subrostrata*, a sexually dimorphic species that is a common constituent of lentic mussel assemblages. This species typically releases all glochidia by late summer, and the subsequent egg clutch is deposited in the gills and fertilized by October (Gascho Landis et al. 2012). We collected mussels used in the experiment in November 2009 from ponds at the South Auburn Fisheries Research Station, Department of Fisheries and Allied Aquaculture, Auburn University. These individuals recruited to the ponds in early spring 2009 as part of a separate experiment, and despite their young age (<9 mo), 90% of females were reproductively mature and gravid by November (JAS and WRH, unpublished data). Mussels were overwintered in the laboratory in tanks with flow-through pond water until initiation of the experiment in April 2010, at which time all individuals were ~1 y old. We expected all mussels to be reproductively active and

to exhibit high growth rates because of their young age. We tagged all individuals with uniquely numbered Hallprint® tags (Hallprint Pty. Ltd., Hindmarsh Valley, South Australia) and recorded shell length (longest anterior–posterior dimension) and total wet mass (shell plus soft tissue) of each mussel at the start of the experiment.

We conducted experiments in six 0.1-ha ponds (20 × 60 m, 2 m maximum depth) at South Auburn Fisheries Research Station. We increased the organic content of TSS by adding aqueous pond fertilizer (N:P:K = 10:31:0) to ponds to stimulate primary production. We assigned 2 ponds to each of 3 treatments (low, intermediate, and high TSS). We did not fertilize low-TSS ponds. We added fertilizer as needed (1 L fertilizer/pond) to maintain SD measurements between 40 and 75 cm in intermediate-TSS ponds and <40 cm in high-TSS ponds. We used air lifts and baffles to provide oxygenation and even mixing of water and food in each pond. We stocked 10 grass carp, *Ctenopharyngodon idella*, in each pond to control submerged aquatic vegetation and to reduce competition for nutrients between phytoplankton and rooted aquatic plants. We monitored inorganic and organic solids because bioturbation by the fish potentially could have added inorganic particles to the water column.

We calculated the trophic state index (TSI) based on SD (Carlson 1977) weekly for each pond as an index of productivity. The index is scaled from 0 (highly oligotrophic) to 100 (highly hypereutrophic). The threshold between eutrophic and hypereutrophic is 70. Trophic state was calculated using the equation $TSI = 10(6 - \log_2 SD)$.

We placed 38 mussels in each pond on 24 April 2010 and removed them on 11 November 2010. We suspended mussels 45 cm from the surface in pocket nets (20 × 30 cm) made from 1.25-cm^2 plastic mesh (2 pocket nets/pond with 19 mussels/net; Fig. 1A) because mussels can obtain resources from the sediments by pedal feeding (Raikow and Hamilton 2001, Nichols et al. 2005). Suspension prevented contact with the sediment and allowed us to link feeding and growth rates to TSS loads more directly. Sex ratios in each pocket net were 1 male: 2.4 female. The number of individuals in each pocket net decreased from 13 female and 6 males at the start to an average of 12 females and 5 males at the end of the experiment because some mussels escaped. The sex ratio of 1:2.4 (male:female) was relatively constant throughout the experiment. We placed males and females side by side in pocket nets to minimize sperm limitation. We did not handle mussels after we placed them in ponds, but we cleaned pocket nets periodically to maximize water circulation.

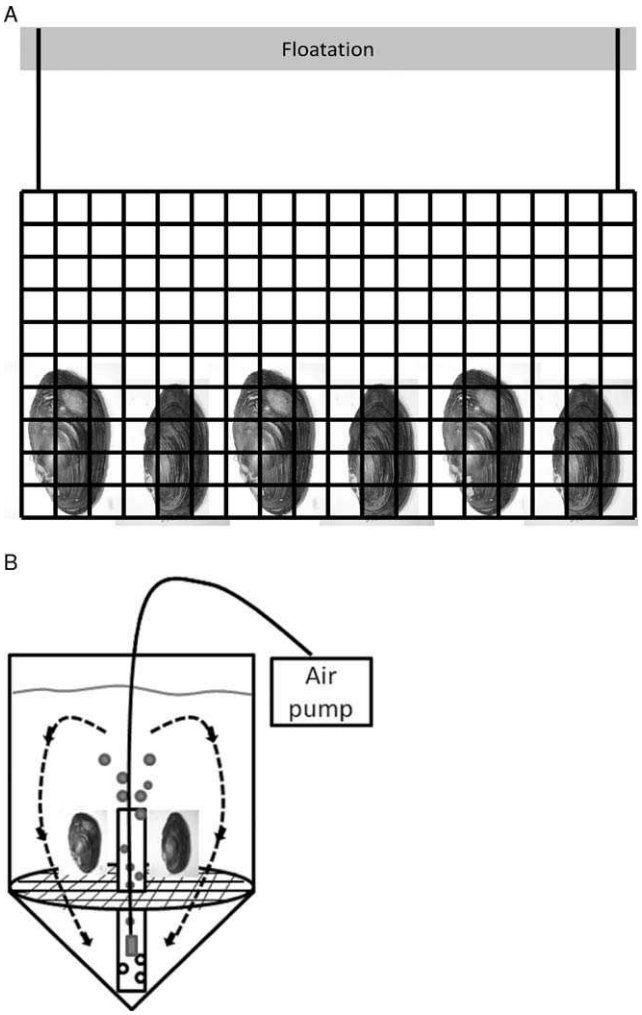

FIG. 1. A.—Floating pocket nets (20 × 30 cm), constructed of 1.25-cm^2 plastic mesh, used to house mussels during the reproduction experiments. Mussels are not drawn to scale. B.—Aerator-driven, upwelling, cone-bottom clearance rate chambers (57 L) with a 1.25-cm^2 mesh platform for holding the mussel while allowing the water to circulate throughout the tank. Arrows represent the direction of the flow.

We did not track the timing of spawning during the experiment to avoid disrupting fertilization of experimental animals. However, we tracked spawning of *L. subrostrata* suspended in pocket nets (pond TSS = 7–11 mg/L) during autumn 2011 to confirm reproductive timing. We held *L. subrostrata* in ponds between the 2010 and 2011 trials. In 2011, we used a 22-gauge needle to extract fluid from the gills of 5 females weekly for 6 wk from 22 August to 26 September (n = 30 females) and examined the extract for eggs or glochidia. We first observed fertilized eggs in the gills on 26 September, indicating spawning probably was initiated during the preceding 6 d (20–25 September). On 31 October, we examined the gills of all females,

and 62% were brooding glochidia. We set the spawning period in 2010 from 7 September to 21 October (from 2 wk before and 4 wk after the earliest spawning in 2011).

Laboratory analysis

We collected water samples once weekly from each pond throughout the 7-mo experiment. We measured TSS directly by vacuum filtration of 50 to 200 mL of sample water (depending on particle concentration) through precombusted, preweighed 47-mm Pall A/E glass-fiber filters (1 μm) (APHA 1995). We dried filters at 105°C for a minimum of 6 h, cooled them in a desiccator for 15 min, weighed then combusted them at 550°C for 1 h, cooled them in a desiccator for 15 min, and reweighed them to determine total dry mass and organic solids content, respectively. We recorded SD weekly coincident with water-sample collection and measured dissolved O_2 (DO) on 5 occasions throughout the experiment.

At the completion of the experiment, we recorded shell length and total wet mass of each mussel to assess growth, which was expressed as % change in length or mass. We used bomb calorimetry (Parr 1417 microcalorimeter; Parr Instrument Company, Moline, Illinois) to measure whole-body (minus the gills) caloric density of 3 fertilized and 3 unfertilized females from each pond when available. We removed gills of unfertilized females before analysis because gills of fertilized females used for calorimetry were removed for quantification of fertilization (see below). We dried tissues at 60°C until a stable dry mass was reached, then pulverized and homogenized them with a mortar and pestle. We analyzed 2 pellets of homogenized tissue (0.02–0.03 g each) from each mussel. If results for the 2 samples differed by >5%, we analyzed a 3rd pellet.

At the end of the experiment, we quantified reproductive effects as: 1) the proportion of females that became gravid in each pond, 2) the proportion of fertilized:unfertilized eggs/female, and 3) individual fecundity (total number of glochidia or fertilized eggs/female). We assessed gravidity of females by gently prying apart the shell valves and examining the gills (Tankersley and Dimock 1993a). Gravid females had swollen, distended gills, and we considered individuals with flaccid gills not gravid. We examined the flaccid gills of a subset of females (n = 3, 70× magnification) and found neither unfertilized eggs nor glochidia in any flaccid gills. To estimate the proportion of fertilized eggs in gravid females, we sacrificed females and examined the gills of up to 6 gravid individuals from each pond. In some ponds, we examined <6 mussels because we found few or no gravid individuals. We flushed gills with water, diluted the contents with 1000 to 2000 mL of water, and counted glochidia and unfertilized eggs in three 1-mL subsamples. We estimated total fecundity by multiplying the mean number of glochidia or fertilized eggs in the 3 subsamples by the dilution volume. We chose 6 males from each pond for gamete extraction (Saha and Layzer 2008) and quantified mature sperm as % of total cells.

Clearance rates

We conducted 8 in-pond clearance-rate trials across a TSS gradient representative of conditions in our experiment (3–48 mg TSS/L; see Results). We had a complete TSS gradient across 5 ponds, and TSS conditions in the 6th pond did not expand the range of TSS. Therefore, we used 5 of the 6 experimental ponds for clearance trials and ran multiple trials in 2 of the ponds. We conducted trials periodically from June to August 2010 on an opportunistic basis that coincided with attainment of desired TSS values across the gradient. Pond temperatures were similar across this period with daily maxima ranging from 30 to 35°C. These temperatures were slightly higher than those during the estimated spawning period (7 September–21 October) when mean daily temperatures were >28°C until 26 September 2010, but fell to 22°C by the end of the spawning period.

We used different mussels in clearance trials than in the fertilization component of the experiment to avoid negative effects of handling on their growth and reproduction. However, all mussels came from the same source stock and were housed in the same experimental ponds. We moved mussels between ponds with similar TSS levels for clearance trials because the number of mussels was limited. Before each trial, we acclimated individuals for ≥48 h in the ponds in which clearance trials were to take place. We ran each trial with 3 replicates conducted in individual static filtration chambers. Chambers were 57-L cone-bottom tanks set up as down-welling systems using an airlift (Fig. 1B). Each chamber contained 25 L of water and 5 mussels (males and nongravid females), and the total mussel biomass was similar across chambers (720–886 g). We set mussels on a platform of 1.25-cm^2 mesh to allow continual flow of water past the mussels and to allow feces and pseudofeces to settle to the bottom of the tank and limit their resuspension in the water column (Fig. 1B). We filled and ran 3 control chambers (no mussels) simultaneously to correct for settling of solids during the trial. We floated the chambers in the pond so

water temperatures would remain similar to pond conditions. We filled chambers in 5-L increments with a sump pump placed near the pond airlift to ensure that the water was well mixed and all chambers had similar starting conditions (Vanderploeg et al. 1995). We collected 500 mL of water from each chamber at time 0 before mussels were added to the chambers. We then added mussels, ran trials for 2 h, and collected 500-mL water samples at the end of the trial. We processed water samples for TSS using methods described above.

We calculated clearance rates with the following equation to standardize for water volume and mussel biomass in the trial:

$$F = (V/nt)\ln(C_0/C_t)$$

where F is clearance rate (L g^{-1} mussel wet mass h^{-1}), V is the volume of water in the filtration chamber, t is the duration of the trial, n is the biomass of mussels in the chamber (modified from Riisgard 2001 in which n is number of mussels), and C_0 and C_t are the initial and final TSS concentrations, respectively (Riisgard 2001). Before we calculated F, we corrected C_t by subtracting the mean mass of solids that settled in control chambers to yield solids removed by the mussels.

Data analysis

We characterized TSS in each pond by calculating the proportion of days with TSS $>$ 20 mg/L and organic:inorganic ratios (O:I) $>$ 1 over the entire experiment and for the putative spawning season only. We used a TSS threshold of 20 mg/L because previous investigators have shown that filter feeding is disrupted above this level (Hornbach et al. 1984, Way et al. 1990). O:I $>$ 1 indicates dominance by organic particles.

We used a regression approach to examine the effect of TSS (and the related TSI and O:I) on mussel growth, caloric density, and reproductive output. Independent variables were normally distributed (Shapiro–Wilk test: TSS, $p = 0.09$; TSI, $p = 0.44$; O:I, $p = 0.55$). We arcsin$\sqrt{(x)}$-transformed proportional data (growth, proportion of females gravid, and proportion of fertilized eggs) to achieve normal distributions. We used mean TSS in each pond during the entire experiment for analysis of mussel growth (mass and length) and caloric density because growth and energy storage would have occurred throughout this period. We used regression to analyze change in size (growth), caloric density, proportion of eggs fertilized, and fecundity for each individual. We used mean TSS in each pond during the putative spawning season (7 September–21 October) for analyses of fecundity and proportion of fertilized eggs because TSS during this period was most likely to affect reproduction.

We used analysis of variance (ANOVA) to test for differences among clearance rates across 8 TSS treatments (1.9, 7.6, 8.9, 15.3, 20.3, 28.5, 30.1, 48.0 mg/L). We used Tukey's Honestly Significant Difference to identify treatment means that differed (SYSTAT 12; Systat Software, Chicago, Illinois).

Results

We succeeded in creating a gradient of TSS by fertilizing ponds (cf. Fig. 2A, C, E; Table 1), but ponds with high TSS levels were highly variable. Trophic state of all ponds was eutrophic or hypereutrophic, but only fertilized ponds became hypereutrophic. O:I was usually >1 except in 1 pond that was dominated by inorganic suspended solids (O:I $<$ 1) for 60% of the spawning season (Fig. 2B, D, F, Table 1). Overall, O:I was variable within and among treatment levels, and mean TSS and mean O:I were not related during the experiment ($R^2 = 0.39$, $p = 0.184$). DO levels were >4.5 mg/L in the water surrounding the pocket nets throughout the experiment.

All mussels except 1 individual survived the 7-mo experiment, and all mussels grew and showed positive change in length and wet mass during the experiment (Table 2). However, neither measure of growth was related to TSS (Fig. 3A, B). Nutritional status, as indicated by caloric density, also was unrelated to TSS (Fig. 3C).

The proportion of females that became gravid during the experiment was strongly related to TSS, and this relationship was best characterized by an exponential decline (Fig. 4A). At the lowest mean TSS, 88% of females were gravid, but this percentage declined rapidly with increasing mean TSS, and no gravid females were found at TSS >20 mg/L. Ponds with no gravid females also had a higher proportion of days with TSS >20 mg/L for the duration of the experiment and during the spawning season (Table 1). The proportion of gravid females was negatively related to TSI, and no gravid females were found in hypereutrophic conditions (Fig. 4B). The proportion of gravid females was not related to mean O:I ($R^2 = 0.063$, $p = 0.631$).

Neither the proportion of fertilized eggs nor total fecundity of gravid females was related to TSS (Fig. 4C, D). Mean proportion of fertilized eggs ranged from 0.98 to 0.99 even in ponds with relatively high TSS that exhibited a low percentage of gravid

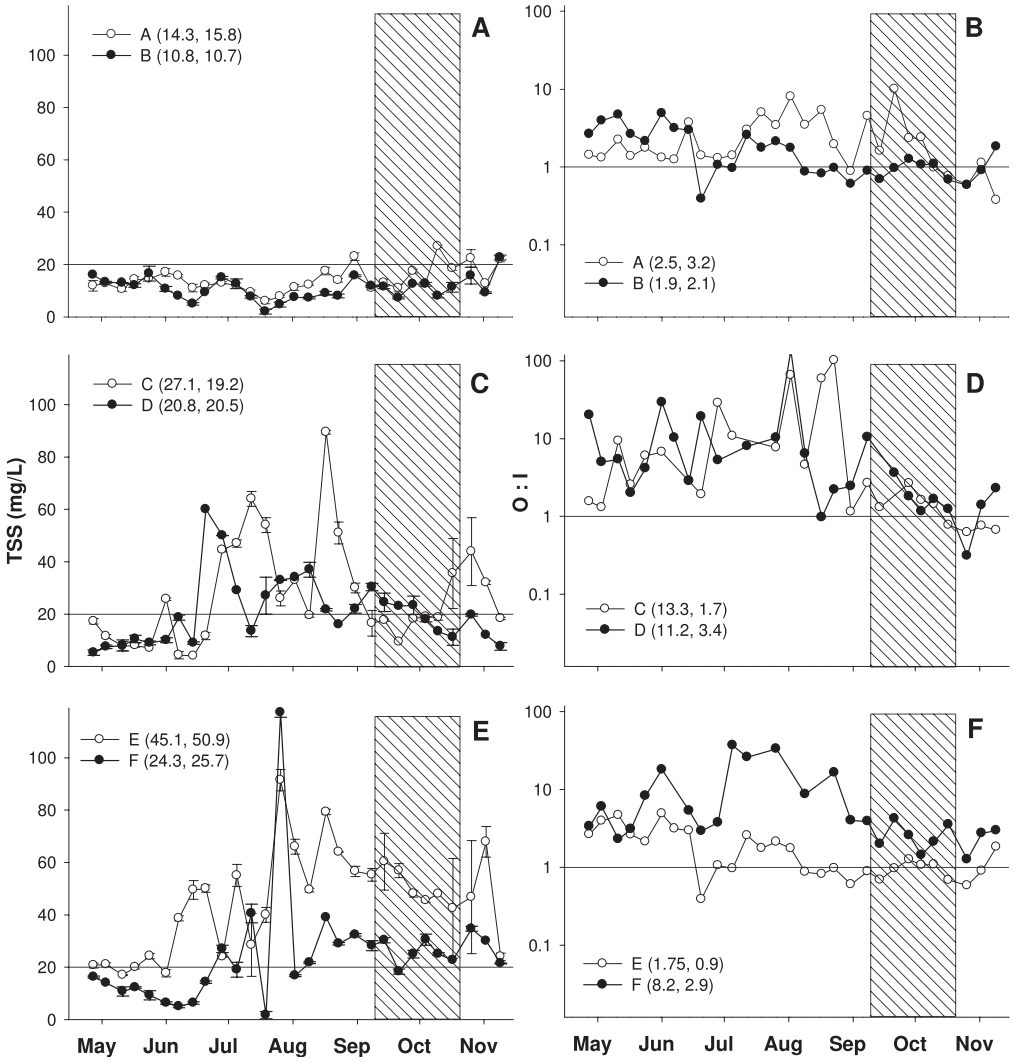

FIG. 2. Weekly total suspended solids (TSS) (A, C, E) and organic:inorganic ratios (O:I) (B, D, F) in experimental ponds in low (A, B), intermediate (C, D), and high (E, F) TSS treatments over a 7-mo growth and fertilization experiment with freshwater mussels. Letters in figure legends correspond with pond letter codes in Tables 1, 2. Shaded areas show the assumed spawning period (7 September–21 October). The horizontal line in A, C, and E indicates the 20-mg TSS/L threshold, above which previous studies have shown disruption of filter feeding (see text). The horizontal lines in B, D, and F indicate O:I = 1, above which TSS are dominated by organic particles. Numbers in parentheses show mean TSS and O:I for the entire experiment and the spawning season, respectively.

females. Sperm production was not related to TSS. At the end of the experiment, mature sperm cells made up >90% of all cells in each gamete extract for 97% of males (n = 36) in all ponds, regardless of TSS, TSI, or O:I.

Clearance rate was negatively related to TSS (Fig. 5). Clearance rate appeared to show a threshold relationship in which clearance dropped abruptly at TSS > ~8 mg/L but remained similar at successively higher levels.

Discussion

Contrary to our expectations, growth of mussels was not related to TSS. We saw neither an increase in growth with moderate increase in TSS nor a decrease in growth or survival at the highest levels of TSS as reported by previous investigators (Walker et al. 2001, Valdovinos and Pedreros 2007). All of our ponds were eutrophic, so food probably was never limiting, even at the lowest TSS. The lack of negative effects of high TSS on growth might have been a result of the prevalence of organic particles, which are potential food items, and lower TSS (maximum ≈ 100 mg/L) than in other studies, which showed decreases in growth or increased catabolism of stored energy reserves at very high concentrations of primarily inorganic suspended sediment (e.g., >600 mg/L; Aldridge et al. 1987, Osterling et al. 2007). In addition, *L. subrostrata* typically occurs in eutrophic environments and probably is well adapted to these conditions.

TABLE 1. Characteristics of total suspended solids (TSS) and trophic state index (TSI) in experimental ponds over a 7-mo experiment (April–November) and during the presumed spawning season (7 September–21 October) exposing *Ligumia subrostrata* to varying TSS levels. TSS = 20 mg/L is the threshold above which previous studies have shown disruption of filter feeding. A ratio of organic:inorganic particles (O:I) >1 indicates dominance of organic particles. TSI was calculated for the spawning season only.

Pond	% days during experiment with TSS > 20 mg/L	% days during spawning with TSS > 20 mg/L	% days during experiment with O:I > 1	% days during spawning with O:I > 1	TSI
A	15	16	84	67	64
B	4	0	83	84	66
C	46	16	86	84	73
D	46	55	93	100	60
E	90	100	56	40	70
F	57	84	100	100	67

In contrast, TSS had profound effects on reproduction. The percentage of brooding females decreased sharply with increasing TSS and TSI, and complete reproductive failure occurred in hypereutrophic ponds with TSS >20 mg/L. However, fertilization of eggs appeared to be an all-or-nothing phenomenon. Fecundity and the percentage of fertilized eggs did not differ between the few females that became gravid at TSS >~15 mg/L and the many females that became gravid in low-TSS ponds. We did not find females that were brooding unfertilized eggs in any treatment. In the high-TSS ponds, females that were not gravid at the end of the experiment might not have produced eggs at all, perhaps in response to high TSS, or they could have produced eggs that were resorbed or released after failing to become fertilized. The percentage of fertilized eggs in gravid females typically is high in most mussel species except those in which unfertilized eggs impart structure to conglutinates (e.g., *Cyprogenia, Dromas*, *Fusconaia, Pleurobema*) (Haag and Staton 2003, Barnhart et al. 2008, Moles and Layzer 2008), and we have consistently observed a high percentage of fertilized eggs in wild populations of *L. subrostrata* (WRH, unpublished data). Brooding glochidia in the gills can reduce respiratory and feeding efficiency (Richard et al. 1991, Tankersley and Dimock 1993b). Thus, the rarity of unfertilized eggs in many species suggests that females do not retain unfertilized or partially fertilized broods to avoid reduced gill function.

Our results appear most consistent with direct physical interference with fertilization by high TSS. We see at least 2 potential mechanisms for physical interference. First, clearance rates were ~75% lower at intermediate to high TSS than at low TSS, and the likelihood that females encountered sperm during filter feeding may have been similarly reduced. The TSS threshold above which clearance rate was substantially reduced (~8–15 mg/L) was broadly similar to the TSS threshold above which fertilization success decreased sharply (~15–25 mg/L). The apparent discrepancy between these thresholds may be a result of the temporally variable TSS concentrations in the ponds, which could have fluctuated enough to provide brief windows of conditions more favorable for fertilization. Second, high TSS probably increased production of pseudofeces, thereby increasing the probability that sperm were bound in mucus during attempts to clear the gills of heavy accumulations of particulate matter. Asian clams (*Corbicula*

TABLE 2. Growth of female *Ligumia subrostrata* in experimental ponds from April to November. L_0 and M_0 are mean initial length and mass, respectively; L_1 and M_1 are mean final length and mass, respectively. Mean, minimum (min), and maximum (max) change refer to % changes (e.g., $[L_1 - L_0]/L_0 \times 100$) across all individuals in each pond. *n* = number of mussels.

	Length (mm)					Wet mass (g)				
Pond (*n*)	L_0	L_1	Mean % change	Min % change	Max % change	M_0	M_1	Mean % change	Min % change	Max % change
A (24)	48.4	58.9	21.5	6.7	33.4	10.8	18.3	73.8	17.4	131.1
B (23)	48.2	63.8	30.6	7.9	59.0	10.7	24.4	133.3	24.5	331.1
C (27)	48.4	62.4	28.7	16.0	38.4	10.6	22.2	106.3	40.0	193.3
D (18)	48.4	60.1	22.5	5.9	37.3	10.8	20.1	87.2	22.6	155.0
E (17)	49.0	61.8	31.9	13.7	53.7	11.1	21.5	125.2	54.4	225.9
F (26)	47.9	59.3	24.8	1.7	41.2	10.1	18.6	96.3	17.5	180.9

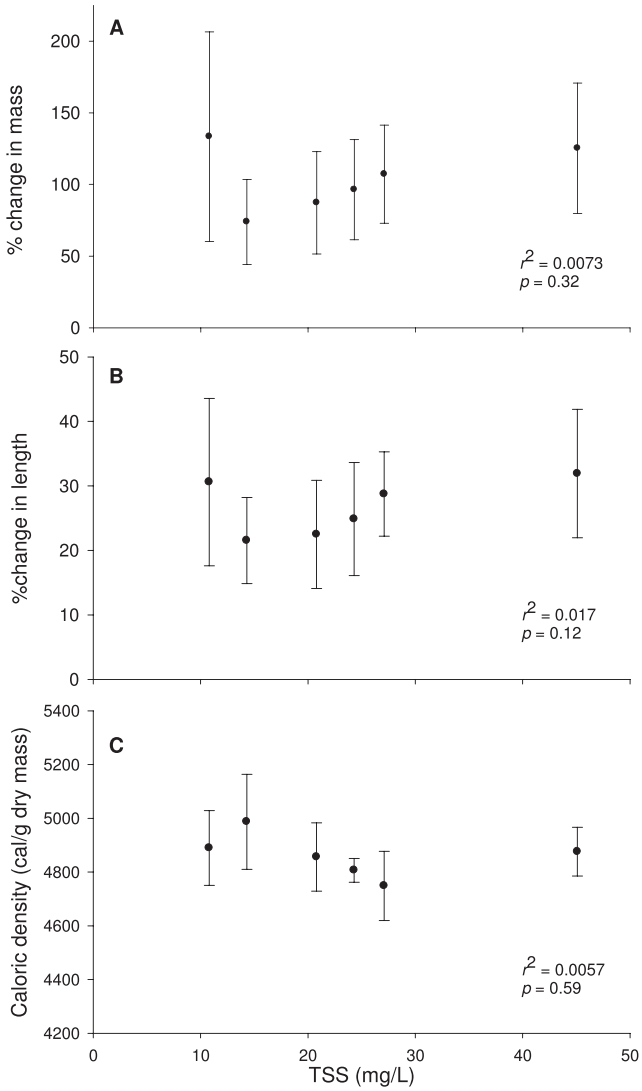

FIG. 3. Mean (±1 SD) % change in mass (A) and length (B), and whole-body caloric density (C) of freshwater mussels in individual ponds along a gradient of total suspended solids (TSS) during a 7-mo experiment.

spp.) and fingernail clams (*Sphaerium*) initiated pseudofeces production at 17 to 20 mg TSS/L (Fuji 1979, Hornbach et al. 1984, Way et al. 1990). Zebra mussels (*Dreissena polymorpha*) initiated production at 27 mg/L, but production continued to increase as TSS increased (Lei et al. 1996, Schneider et al. 1998). These thresholds are remarkably concordant with the TSS threshold above which we saw reproductive failure. However, thresholds for pseudofeces production in unionid mussels are not well known.

Reduced clearance rates and increased pseudofeces production are not mutually exclusive and would be expected to occur synergistically to reduce sperm acquisition and egg fertilization. Furthermore, the mode of sperm transfer in mussels helps to explain the all-or-none basis of egg fertilization. Male freshwater mussels do not release sperm singly, but rather in hollow, spherical aggregates called spermatozeugmata, each of which contains ~3600 to 9000 sperm (Barnhart and Roberts 1997, Waller and Lasee 1997). Spermatozeugmata have not been reported in *L. subrostrata*, but they occur in at least 18 other species, including all 5 North American unionid tribes, a pattern suggesting that they are a general feature of freshwater mussels (Haag 2012 and references therein). A brood of a 61-mm *L. subrostrata* (the approximate mean size of females at the end of the experiment) contains ~75,000 eggs (Haag, in press, and references therein), and could be fertilized completely by only 15 spermatozeugmata (assuming 5000 sperm in each spermatozeugmata). However, reduced clearance rate and increased production of pseudofeces could incrementally reduce acquisition of spermatozeugmata and egg fertilization below some critical level necessary for retention and brooding by female mussels.

Our results are concordant with physical interference by TSS with egg fertilization, but we find this mechanism puzzling as an explanation for reproductive failure in mussel populations. The extent to which high TSS may interfere with reproduction in the wild depends on several factors. Levels of organic suspended solids sufficient to cause reproductive failure in our study are probably frequent in eutrophic lentic habitats, and it is interesting that mussel species like *L. subrostrata* that are characteristic of these habitats should be so sensitive to high TSS. However, we have occasionally observed a very low incidence of gravid females of *L. subrostrata*, *Pyganodon grandis*, and *Utterbackia imbecillis* in lentic habitats in Mississippi (see Haag, in press, and references therein). The typical late summer and autumn fertilization and subsequent overwintering of the brood in the female gills of these and other species of the tribes Anodontini and Lampsilini, which dominate lentic habitats, may have evolved, in part, to coincide with decreases in day length and primary productivity and resultant lower TSS.

Previous explanations for reproductive failure or variation in female reproductive output do not account for the patterns in our study. Several authors proposed food limitation as a factor in determining female mussel reproductive output. In *M. margaritifera*, egg production occurred only when females exceeded a minimum body-mass threshold, and increasing energetic surpluses above this threshold were associated with increased fecundity (Bauer 1998). However, food limitation or other energetic

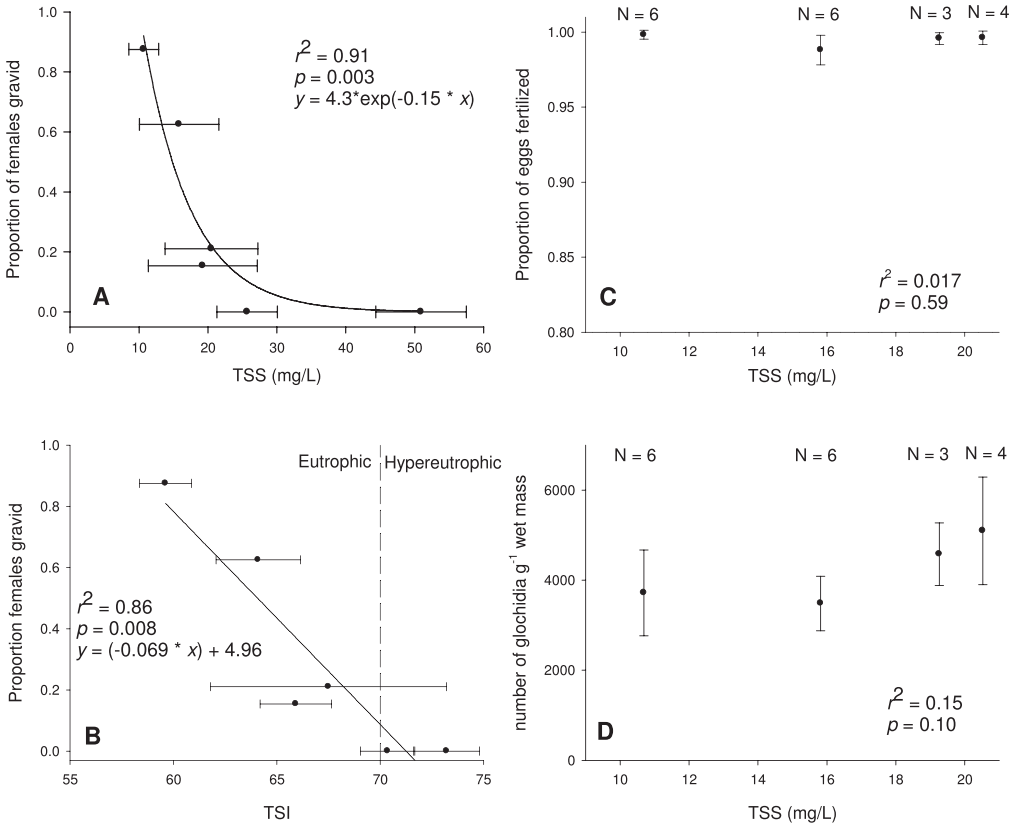

FIG. 4. Relationships between mean proportion of mussel females gravid and mean (±1 SD) total suspended solids (TSS) (A) and trophic state index (TSI) (B), between proportion of eggs fertilized and mean (±1 SD) total suspended solids (TSS) during the presumed spawning period (C), and between mean (±1 SD) number of glochidia/female and mean TSS (D) in each pond during a 7-mo experiment. In all panels, data points indicate mean values for individual ponds. On panel B, the dashed line shows the threshold between eutrophic and hypereutrophic conditions. On panels C and D, sample sizes are the number of female mussels examined. No females were examined from ponds lacking reproduction ($n = 2$).

constraints do not seem likely explanations for complete reproductive failure in our study because neither growth nor caloric content were related to TSS. Sperm limitation also has been proposed as an explanation for reproductive failure (Downing et al. 1993, Galbraith and Vaughn 2009). This explanation is unlikely for our results because males and females were in close proximity in experimental pocket nets. Moreover, no evidence indicated that sperm production was affected by TSS. Mature sperm cells dominated gamete extracts of all males regardless of treatment.

Hypoxia is often associated with hypereutrophic environments because of high microbial O_2 demand and could negatively affect female metabolic processes or egg survival in the gills. For example, O_2 stress has been proposed as a trigger for females to abort their broods to alleviate reductions in gill efficiency associated with brooding (Aldridge and McIvor 2003). All of our DO measurements were >4.5 mg/L, but these measurements were made during the day, and nighttime DO levels may have dropped sharply during suspension of phytoplankton photosynthesis. Nighttime drops in DO are a characteristic feature of lentic environments and could have caused females to abort broods, particularly in high TSS ponds, but all of our ponds were constantly aerated to minimize O_2 stress. Moreover, reproductive failure was observed in a high TSS pond dominated by inorganic solids during the spawning season (Pond E, Fig. 2F) and in a high TSS pond dominated by organic solids (Pond F, Fig. 2F). Diurnal variation in DO should have been less severe in ponds dominated by inorganic solids because of lower biological O_2 demand.

Enrichment of water bodies by agricultural fertilizers and resultant increases in primary productivity and decomposition rates can lead to increases in NH_3. Mussels, especially in the glochidial and juvenile stages, are inordinately sensitive to NH_3, and NH_3 toxicity is proposed as a cause of mussel declines in many areas (Augspurger et al. 2003, Wang et al. 2008, Strayer and Malcom 2012). Consequently, high NH_3

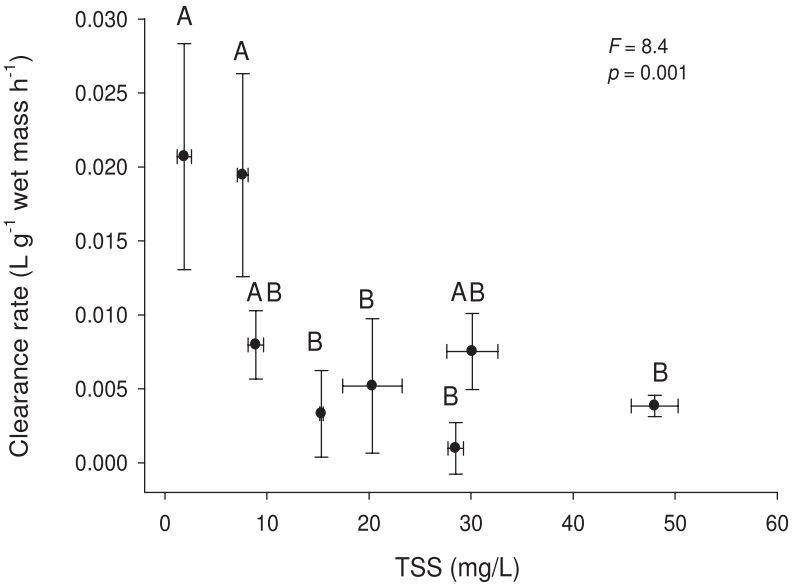

FIG. 5. Mean (±1 SD) clearance rate of mussels and mean (±1 SD) total suspended solids (TSS) during the period of clearance trials in a 7-mo pond experiment. Mean clearance rates with the same letter were not significantly different.

levels in hypereutrophic ponds could have caused death and abortion of the brood but allowed survival of adult females. We did not measure NH_3 levels in ponds, but several observations are inconsistent with this factor as a cause of reproductive failure. First, adult mussels in all ponds experienced negligible mortality. If adult mussels were stressed by NH_3, we would have expected to see at least some mortality or depressed growth in hypereutrophic ponds. Second, NH_3 levels are typically higher in sediments than in the water column. In the sediments, NH_3 can adsorb to sediment particles and lower DO can reduce the ability of bacteria to detoxify NH_3 (Frazier et al. 1996, Strayer 2008). In our study, mussels were suspended in the water column where they presumably would have been exposed to low NH_3 concentrations.

Primary productivity is typically lower in streams than in lentic habitats, and suspended sediment generally is of less direct importance to aquatic organisms than deposited sediments because, even in agricultural watersheds, very high levels of suspended sediments usually occur for only short periods after storm-flow events (Waters 1995, Borah et al. 2003, Schwartz et al. 2011). Our data show that negative effects on mussel reproduction could occur even with the modest increases in TSS routinely associated with an array of human landscape impacts. For example, 2 y after clear cutting, average monthly TSS concentrations in an Appalachian stream at base flow ranged from 22 to 57 mg/L for much of the year (including in October, the main period of egg fertilization for many mussel species) and were ~10× higher than in an undisturbed watershed (Gurtz et al. 1980). In the Kaskaskia River basin in Illinois (USA), mean TSS at base flow was 17 mg/L in areas of intensive agricultural, and 14 mg/L in urbanized areas (Miller et al. 2011). These levels approach or exceed thresholds above which complete reproductive failure occurred in our study. Inputs of suspended sediment in streams may be composed primarily of inorganic particles from landscape erosion, and these effects may further limit reproduction by reducing food acquisition and nutritional status of female mussels below critical levels needed for egg production.

Our study provides some of the first data demonstrating a negative effect of suspended sediment on mussel reproduction, and they suggest that elevated TSS could be an important factor in mussel declines in some situations. Additional research is needed to clarify the mechanisms responsible for reproductive failure at high TSS and to examine other factors affecting early reproductive stages. The generality of our results should be evaluated across a range of species' life histories and habitat conditions. Measurement of TSS, especially during periods of egg fertilization, should be part of efforts to identify causes of mussel declines and of evaluations of sites for mussel reintroduction.

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