

A simple reciprocating apparatus for maintaining long-term turbidity in biological experiments

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Abstract

Elevated turbidity adversely affects the behavior, physiology, and distribution of marine and freshwater organisms. Although laboratory turbidity studies vary in topic, scope, and design, they all require an experimental apparatus with the ability to maintain constant sediment concentrations (or turbidities) for extended periods of time. Here, I describe a low-cost alternative to more complex systems, a reciprocating apparatus that uses motor-driven paddles and compressed air to keep fine sediment in suspension for extended periods of time in at least 20 tanks simultaneously. With this apparatus, suspended sediment levels, ranging from 25 to 500 mg/L, were maintained within 90% to 95% of initial values for 7 days. In addition, because sediment was kept in suspension using a very slow curtain of air bubbles, this apparatus did not induce a stress response in test organisms, as measured by stress hormone concentration.

Introduction

Turbidity is a well-documented correlate of impairment in aquatic ecosystems (see Waters 1995, Newcombe and Jenson 1996). A growing body of research illustrates that increased turbidity can have adverse effects on both marine and freshwater organisms (Lemly 1982, Bruton 1985, Cyrus and Blaber 1987, Newcombe and MacDonald 1991, Lenat and Crawford 1994). Excessive sedimentation in aquatic systems negatively affects resident biotic communities directly and indirectly at multiple spatial scales (Roth et al. 1996, Allan et al. 1997, Jones et al. 1999). Many studies on the effects of sediment have focused on large-scale linkages between excessive sedimentation and the abundance, diversity, and structure of fish and macroinvertebrate assemblages (Berkman and Rabeni 1987, Richards et al. 1996, Wang et al. 1997, Lammert and Allan 1999, Sutherland et al. 2002). Many of these field and laboratory studies suggest that population- and assemblage-level effects of elevated sediment loading are in part caused by increased suspended sedi-

ment concentration (SSC). To determine the mechanisms behind these effects, some researchers have investigated the effects of turbidity on growth, survival, stress response, foraging behavior, and reproduction (Swenson and Matson 1976, Gradall and Swenson 1982, Sigler et al. 1984, Berg and Northcote 1985, Redding et al. 1987, Barrett et al. 1992, Gregory 1994, Burkhead and Jelks 2001). The majority of this research has been conducted using salmonids and other game fishes (see reviews in Waters 1995, and Newcombe and Jenson 1996).

Understanding how increased turbidity affects aquatic organisms (e.g., through physical abrasion, visual impairment, disruption of fish spawning cues, physiological stress, reduced growth) is necessary for development of science-based turbidity standards and wise land-use planning.

Previous turbidity studies have varied in scope and design, yet each has required an apparatus that is able to maintain constant sediment concentrations (or turbidities). Difficulty maintaining a constant turbidity for long duration is partially a function of sediment particle size. Consequently, many studies are conducted with very fine clay, even though the particle size distribution of near-bed suspended load in many rivers and streams is dominated by larger clay, silt, and even sand (Gordon et al. 1995). Studies that use natural, larger, locally available sediment are often of short duration (e.g., a few hours to days; Redding et al. 1987, Barrett et al. 1992). Maintaining sediment in suspension is also influenced by sediment mineral composition. Some naturally occurring clays (e.g., kaolinite) are very cohesive and readily form larger particles, which makes maintaining constant turbidity difficult. Because of their structural properties

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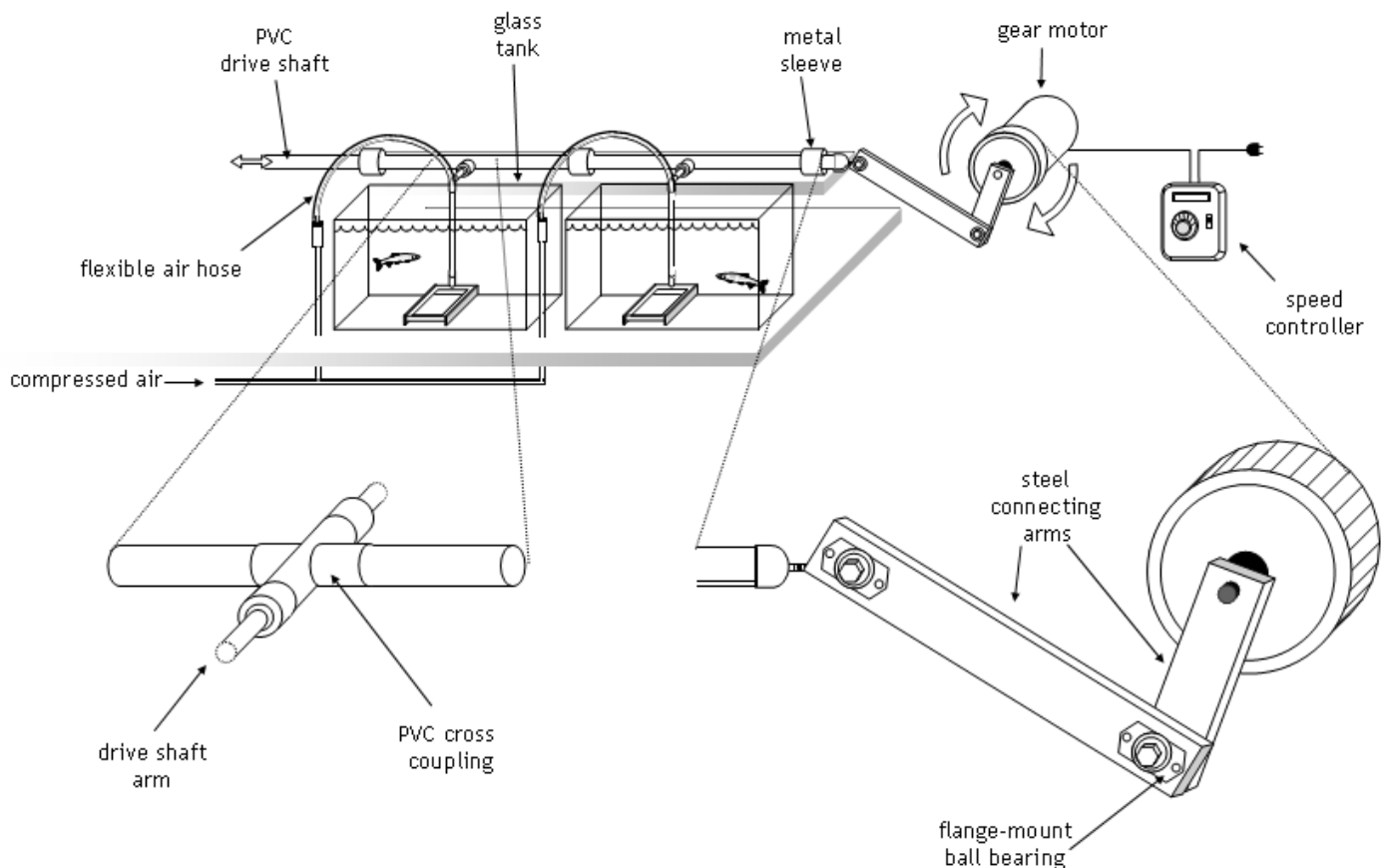


Figure 1. Diagram of sediment suspension apparatus including enlargement of drive mechanism, drive shaft cross coupling, and arm. See text for detailed description.

and availability, some researchers use commercial-grade volcanic clays (e.g., montmorillonite-based bentonite), although in many areas it may not be representative sediment. Using larger clay and silt-sized particles is necessary to more closely replicate the conditions of near-bed suspended load or to test the effects of aspects of suspended sediment other than turbidity (e.g., particle scour of mica-based silt on gill tissue).

Efficient techniques have been developed that are able to maintain near-constant turbidity levels indefinitely. One apparatus uses a computer-controlled beam transmissometer to continually measure turbidity and add turbid water from a source tank as needed (Greycay 1989), but elegant, complex methods such as this may be too costly if numerous tanks and high replication are needed. Other researchers have conducted turbidity tests in large artificial stream environments or in situ, with channels constructed in or next to streams (Sigler et al. 1984, Berg and Northcote 1985, Barrett et al. 1992). Some of these researchers have had success maintaining near constant SSC (e.g., ≤ 3 g/L for 2–3 weeks; Sigler et al. 1984). However, because of logistical and cost concerns, these studies are usually limited to one or two channels, allowing only a small number of treatment replications per trial.

Here I describe an apparatus designed to keep fine sediment ($< 45 \mu\text{m}$) in suspension for extended periods of time in numerous experimental tanks simultaneously. This sediment suspension system is a low-cost alternative to large artificial stream systems. This apparatus allows for high replication and extended periods of sediment exposure at near-constant turbidities.

Materials and procedures

General description—The design of this apparatus is not complex. It consists of a motor-powered drive mechanism that moves a paddle within each of a variable number of experimental tanks (Figures 1 and 2). While two baffles on each paddle slowly sweep the floor of a given tank, the paddle also delivers a column of compressed air that resuspends settled particles. The number of experimental tanks that are possible with this design is a function of length of drive shaft, size of tanks, and power of the motor.

The inspiration for the design of this apparatus came from a system commonly used to hatch and rear game fishes (personal communication, E. Henderson; E & K Fisheries, Dearing, GA, USA). The basic premise behind the design is the use of a single motor to power a reciprocating drive shaft, to which



Figure 2. Photograph of sediment suspension apparatus in use.

multiple paddles are connected. In the fish-hatching prototype, these paddles are used to fan clutches of eggs of nest-guarding game fishes (e.g., channel catfish, *Ictalurus punctatus*). This fanning motion mimics parental behavior and provides the same vital purpose (i.e., oxygenation and removal of metabolic waste and sediment).

The apparatus described below is based on the same principle: the transfer of power from one source to many experimental units. In the fisheries prototype, single-baffle paddles move back and forth several inches above clutches of eggs in the bottom of a raceway. In the apparatus described here, paddles have been re-designed with two baffles that move along the bottom of individual experimental tanks (Figures 1 and 2). Similar to the fisheries hatching machine, paddles are connected to a central drive shaft that is powered by a variable-speed gear motor. Each time the paddle travels slowly along the bottom, sediment suspension is aided by the movement of the baffles over the tank bottom. However, the primary means of sediment suspension is compressed air. Air is introduced into each paddle and emanates from the bottom of each paddle through a series of small holes, thus creating a slow-moving screen of air bubbles. This moving wall of diffuse air bubbles

creates upward water movement, suspending sediment particles while increasing the level of dissolved oxygen. This combination of air bubbles and baffles is sufficient to maintain a suspended sediment concentration of approximately 500 mg/L for extended periods.

The design of this apparatus is intrinsically flexible and lends itself to specific adaptation. The dimensions and materials for each component can be changed to suit the needs and resources of the investigator. Design details and construction specifications given below are for an apparatus used by the author in several turbidity experiments.

Detailed description and construction

Motor—The drive mechanism power source, used in experimental trials of this apparatus, is a Dayton 1/2 HP, 5.8 amp, 90 volt DC variable-speed gear motor (Model 6Z413A; Dayton Electric, Niles, IL, USA; Figure 1). This type of gear motor is fan cooled, which is preferable because high workloads for long durations can cause increased heating of the motor. In experimental trials, this motor easily powered 20 paddles in 38-L tanks. The motor was capable of powering all 20 paddles at very high speeds, indicating that it could have powered many more paddles at the low speed required for experimental trials. A variable-speed gear motor of this type is recommended, as it allows the investigator the ability to determine optimal paddle speed for maintaining a given turbidity, without causing stress to test organisms. A speed controller, which can easily be connected to most gear motors, allows precise and replicable velocity. A Dart speed controller was used in experimental trials of this apparatus (Model 253G-200E; Dart Controls, Zionsville, IN, USA).

Because of high rotational force, the motor must be securely mounted with bolts to an immovable bench or table. If the motor is allowed to move, even slightly, the whole drive mechanism can become misaligned and unstable. This can cause sudden and destructive movement of drive shaft, and breakage of drive mechanism, paddles, or tanks.

Connecting arms—Two connecting arms transfer the circular motion of the gear motor to the reciprocating motion of the drive shaft and paddles (Figure 3). The distance traveled by the drive shaft and paddles is equal to twice the effective rotation radius (R) of the shorter connecting arm (arm A). The effective radius of arm A is slightly smaller than its length and equals the distance from the center of the motor shaft to the center of the carriage bolt attaching connecting arm A and B. The effective radius of arm A can be determined by measuring the distance a paddle needs to travel to cover the bottom of a given tank, and then dividing this distance by two. This system therefore can be adapted to any length (or width) tank. This apparatus can also be adapted to a series of different-sized tanks by simply determining the drive shaft travel distance based on the smallest tank (i.e., effective radius of arm A equals 1/2 length of smallest tank). To cover the distance of the longest tanks (and intermediate-length tanks) within this

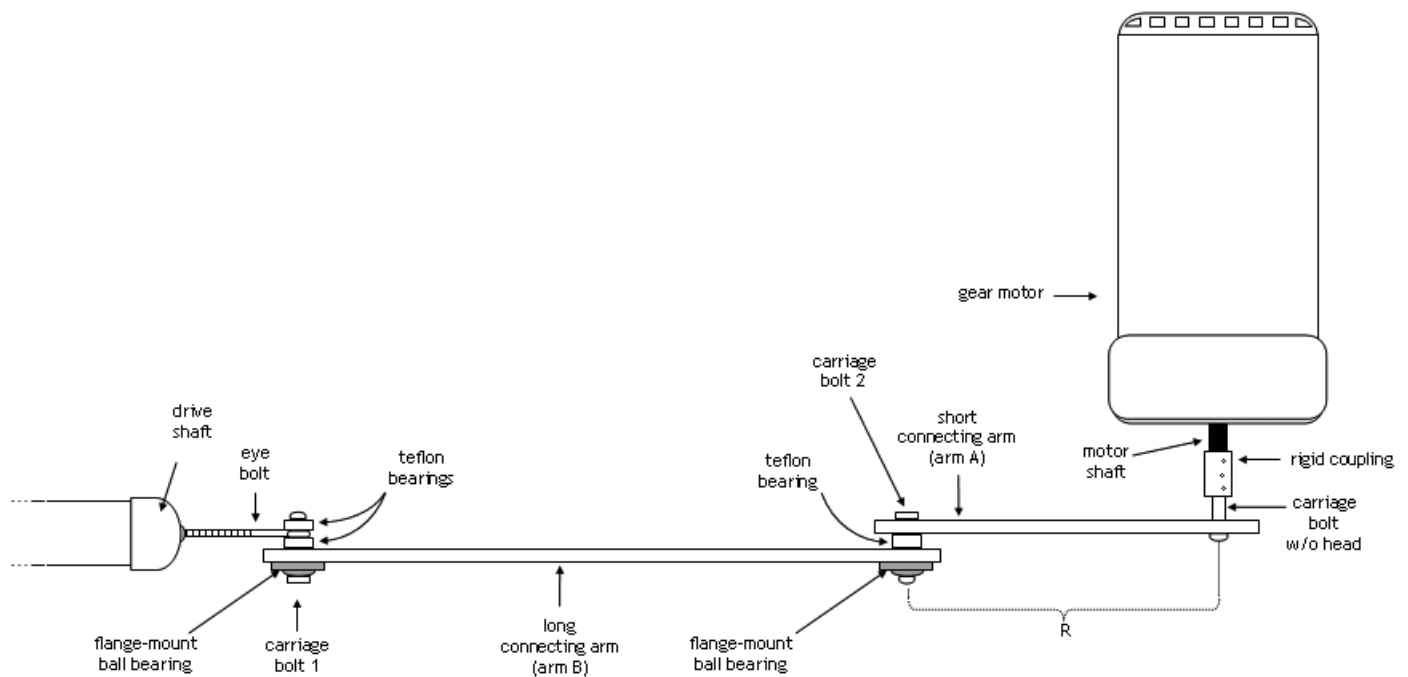


Figure 3. Diagram of drive mechanism as seen from above, showing how motor, connecting arms and drive shaft are attached to each other. See text for detailed description.

series of variable-length tanks, one must increase the width of each paddle frame (see Figure 4 and description below) to fit each tank. The length of arm B is not as precise, although it must be longer than arm A, so that the drive shaft does not hit the motor shaft upon rotation of arm A. If arm B is too long, there may be excessive flexing, which creates drive shaft instability. A length for arm B that has proven successful is approximately 1.5 to 2 times length of arm A.

Connecting arms are constructed of quarter-inch (0.64-cm) steel to insure a minimal amount of flexing during operation. Flexing of the connecting arms can result in a sideways motion of the drive shaft, which increases friction and potential for apparatus breakage. Connecting arms are attached to motor, drive shaft, and each other using steel carriage bolts (0.5 × 4 inch; 1.27 × 10.16 cm). Arm A is attached securely to and rotates with the motor shaft (Figure 3). To attach arm A to the motor, a rigid steel coupling must first be attached to the motor shaft. To the other end of the rigid coupling is attached the smooth end of a carriage bolt, with the head removed. Arm A is then threaded onto the other end of the bolt and secured with a Teflon-coated nut. Carriage bolts 1 and 2 (see Figure 3) are threaded only on the last 2.5 cm of their length, allowing them to smoothly rotate within the two flange-mount ball bearings attached to connecting arm B. Teflon-coated nuts are used to secure bolts 1 and 2. Teflon bearings are used where ever spacers are needed to separate or secure parts.

Drive shaft—The purpose of the drive shaft is to simultaneously transport a variable number of paddles within the exper-

imental tanks. The drive shaft is made of 1-inch (2.54-cm) schedule-80 PVC pipe. Small sections of pipe are connected together with 1-inch (2.54-cm) cross couplings to which paddles are attached (Figure 1). Using appropriate bushings, a half-inch (1.27 cm) piece of pipe is attached to the 1-inch (2.54 cm) cross coupling, creating a drive shaft arm. Paddles are then attached to this arm by using a metal screw (see Figure 4). Drive shaft arms are plugged with silicone sealant to prevent compressed air from escaping. The end of the drive shaft is capped with a standard rounded PVC cap. The drive shaft is connected to the connecting arms by using a quarter-inch (0.64-cm) eyebolt (Figure 3). After drilling a hole in the cap, the threaded end of the eyebolt is secured within the cap with two Teflon-coated nuts, one inside and one outside the cap. The eye of the bolt is then held onto carriage bolt 1 by using Teflon bearings as spacers. This allows for smooth movement of the carriage bolt within the eye of the eyebolt, which in turn allows connecting arm B to move freely up and down.

The most important factor in the efficient performance of the drive shaft is precise alignment, which reduces friction and prevents apparatus breakage. Alignment of the drive shaft is achieved using several metal sleeves (Figure 1). Accurate alignment of each sleeve in all three planes is essential. Even a slight misalignment could result in undue friction, misalignment of paddles, or the breakage of one or more parts.

Also important to precise alignment is ensuring that the drive shaft is not flexible. Because the drive shaft is made of PVC, it tends to bend vertically and horizontally under stress.

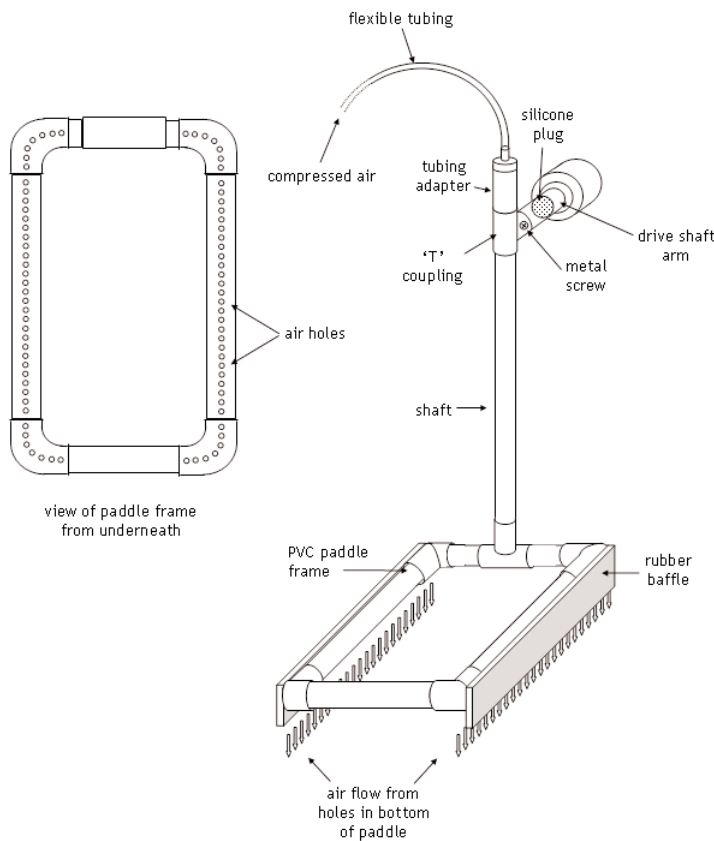


Figure 4. Diagram of paddle assembly, showing paddle frame, baffles, air line and attachment to drive shaft arm. See text for detailed description.

To minimize flexibility, a steel rod is placed inside the entire length of the drive shaft. The diameter of the steel rod is slightly smaller than the inner diameter of the PVC shaft, creating a tight fit. The steel rod is kept in place with several metal screws that are screwed through the PVC shaft, serving as set-screws. A final way to minimize flexing is to increase the number of metal sleeves through which the drive shaft passes. As well as providing reliable performance of paddles, precise drive shaft alignment can reduce the workload of the motor. When friction is reduced, a given motor can operate more paddles, or a given number of paddles can be powered by a smaller motor.

As well as alignment, and reducing flexibility, a further way to reduce friction is by lubricating the path of the drive shaft. An effective lubricant is standard high-temperature, lithium machine grease. Grease can be applied liberally to any surface encountered by the drive shaft. To prevent contaminating tank water, care must be taken that no grease comes into contact with paddles or experimental tanks.

Paddles—The purpose of the motor, connecting arms, and drive shaft is the efficient and reliable transport of the paddles. The paddles are the most crucial component of the apparatus and are responsible for maintaining turbidity levels in the

experimental tanks. Whereas the motion of the baffles can suspend a large percentage of the finest particles, the energy used to suspend larger particles comes from the compressed air introduced into each paddle. The motor is essentially being used to power the movement of this air source. Compressed air is introduced through top of the shaft of each paddle (Figure 4). The air travels down the PVC shaft and into the square paddle frame and emanates through 50 small holes (diameter = 1.5 mm, area = 1.78 mm²) drilled into the bottom of the frame, spaced approximately 5 mm apart. The cross-sectional area of the source pipe (area = 176 mm²) is greater than (approximately two times) the total cross-sectional area of the 50 holes (area = 89 mm²), ensuring that the air is evenly distributed over the entire length of the paddle. A vortex is created by these air bubbles, starting near the floor of the tank and traveling upwards. This slow-moving vortex creates an eddy, into which particles are drawn and resuspended. The air source used for apparatus assessment experiments was a regenerative blower. The air flow rate used for experiments was approximately 142 cm³ s⁻¹. The velocity of the water induced by the air bubble curtain was highly variable but averaged 18–20 cm s⁻¹.

Paddle dimensions are determined by the shape of experimental tank (Figure 4). The length (i.e., axis perpendicular to the drive shaft) of the frame (i.e., the rectangle from which the air flows) of each paddle is slightly smaller than the tank, so that it can move freely without making contact with the tank. The frame width (i.e., axis parallel to the drive shaft) depends on the distance traveled by the drive shaft per reciprocation. If, for example, laboratory space necessitates that the drive shaft moves only a short distance, the paddle frame width can be increased to travel the entire width of the tank. The height of the paddle shaft depends on the depth of the tank. To minimize friction, the paddle baffles should just barely make contact with the bottom of the tank. It is important that the paddle frame is aligned precisely parallel to the tank bottom to avoid friction at one or more points along the baffle. As with the drive shaft, the paddle must be precisely aligned in all three planes to prevent friction with the tank and undue stress on the drive shaft and motor.

The paddle frame is constructed of half-inch (1.27-cm) schedule-40 PVC pipe and couplings. On top of the shaft is a PVC coupling that accepts a threaded tubing adapter. Air is introduced into each paddle through Nalgene tubing that is connected to a compressed air source. The paddle is connected to the drive shaft arm with a standard T coupling. It should not be cemented to the drive shaft arm; easy removal is necessary if tanks are to be cleaned or if paddles need repair. To attach the paddle to the drive shaft arm, a small hole is drilled through the T coupling and drive shaft while they are aligned, and then they are secured with a metal screw.

Paddle baffles are made from standard weather-stripping rubber. Paddle materials must be tested to determine if they are inert: several fish were reared for 4 months in tanks con-

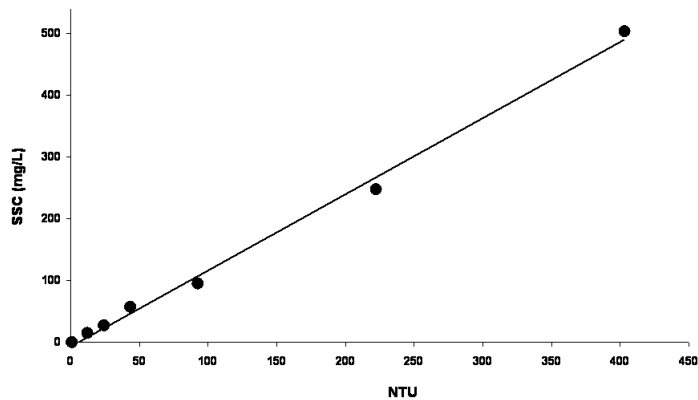


Figure 5. Sediment rating curve describing the relationship between turbidity (NTU) and suspended sediment concentration (SSC, mg/L) for sediment used to test apparatus performance. Turbidity for the rating curve was measured for twenty samples each of 7 SSC treatment levels (0, 10, 25, 50, 100, 250 and 500 mg/L). The relationship between NTU and SSC is described by the following equation: $SSC = 1.2316(NTU) - 6.8426$; $r^2 = 0.99$. Error bars represent standard error ($n = 20$).

taining rubber weather-stripping material. Cortisol levels were measured in these fish, and the weather stripping was found to have no effect on stress response (A.S., unpublished data). Stainless-steel screws are used to attach baffles to paddles. Screw holes are sealed with silicone.

Tanks—Tanks used in experimental trials are standard rectangular 38-L glass tanks. Deeper tanks require longer paddle shafts; longer and wider tanks require different shaped paddle frames. A requirement of all tanks, regardless of their dimensions, is that they remain immovable. To ensure stability of the tanks, metal brackets can be secured to the lab bench and around the base of the tanks. This arrangement also allows precise realignment of tanks in the event that they need to be moved temporarily.

Assessment

Turbidity maintenance was determined during a 21-day growth trial of postlarval spotfin chubs (*Erimonax monachus*), a federally listed species. The apparatus was set up with 20 experimental tanks, with 4 replicates of 5 inorganic sediment concentrations ranging from 0 to 500 mg/L. Sediment composition was, by mass, 60% silt (40–45 μm) and 40% clay (< 40 μm). Turbidity (NTU) was measured daily with a portable Hach model 2100P turbidimeter and converted to SSC using a sediment rating curve determined for test sediment (Figure 5). Because of the inability to use biofiltration during a suspended sediment experiment, metabolic wastes were removed by changing water weekly.

SSCs were maintained within 90–95% of initial values for 7 days (Figure 6). The mean SSC for the highest treatment level (500 mg/L) remained within 94.3% of the initial concentration. The mean SSC for the 100 mg/L treatment ranged from 106.5 to 91.1 mg/L (91.1% of initial). The estimated mean SSC for the 100 mg/L treatment is slightly higher than 100 mg/L

because of the variance associated with the rating curve (i.e., when creating rating curve, the turbidity values for 100-mg/L samples were less than 100 NTU; average = 92.3 NTU). The mean SSC for the 50 and 25 mg/L treatments remained within 95.2% and 89.6% of initial concentrations, respectively. The sediment that settled out of suspension was predominantly clay-sized particles, which bound together forming aggregations in the corners of the experimental tanks. The estimated mean SSC for the 0 mg/L treatment reached a high of 4.7 mg/L, despite the absence of sediment in these tanks. This increase in turbidity may be related to a combination of factors including addition of fish food, growth of bacteria, and presence of fish waste products in the water. Artifacts such as these will vary with experiment design and do not reflect the sediment suspension performance of the apparatus.

In addition to testing sediment settling during the spotfin chub growth trial, a separate sediment settling experiment was conducted with no test organisms. As with the growth trial, 4 replicates of the same 5 treatments were used. During the first 7 days SSC was maintained within 6% of initial values, and remained within 10% of initial values for a total of 12 days. The longer duration of high turbidity is likely due to the lack of metabolic wastes and food particles, which may increase particle flocculation and aggregation.

One of the initial concerns with this apparatus was that the moving paddle and curtain of air would cause stress to experimental organisms. A series of stress trials were conducted on juvenile and postlarval whitetail shiners (*Cyprinella galactura*) housed in tanks containing this apparatus (A.S. et al., unpublished data). In each test the speed of the paddle was very slow ($\sim 5 \text{ mm s}^{-1}$) to minimize stress caused by the apparatus. Because of the curtain of air bubbles, this slow speed was sufficient to maintain the highest suspended sediment treatment level ($\sim 500 \text{ mg/L}$). Data suggest that there was not a significant stress response due to the apparatus being in the tanks. Stress hormone levels were not significantly different between fish reared in apparatus tanks (turbidity control tanks) and those not reared in apparatus tanks. Cortisol levels were < 5 ng/g for both groups of fish. Shiners quickly adjusted to the presence of the slow-moving wall of air. Many individuals repeatedly swam through the bubbles, riding the upward current. No adverse effect of the paddle movement was detected.

The apparatus described here is a closed system. Because the control and maintenance of turbidity precludes water column filtration, the buildup of metabolic wastes must be factored into experimental design (i.e., size and number of organisms per tank, volume of water/tank, duration of experiment, water temperature, and feeding rates). In addition, the constant screen of air bubbles, which creates the water movement necessary for sediment suspension, increases the concentration of dissolved oxygen in the water.

Although this apparatus maintains suspended sediment (< 45 μm) concentrations of near 500 mg/L for extended periods, upper limits of performance have not been established.

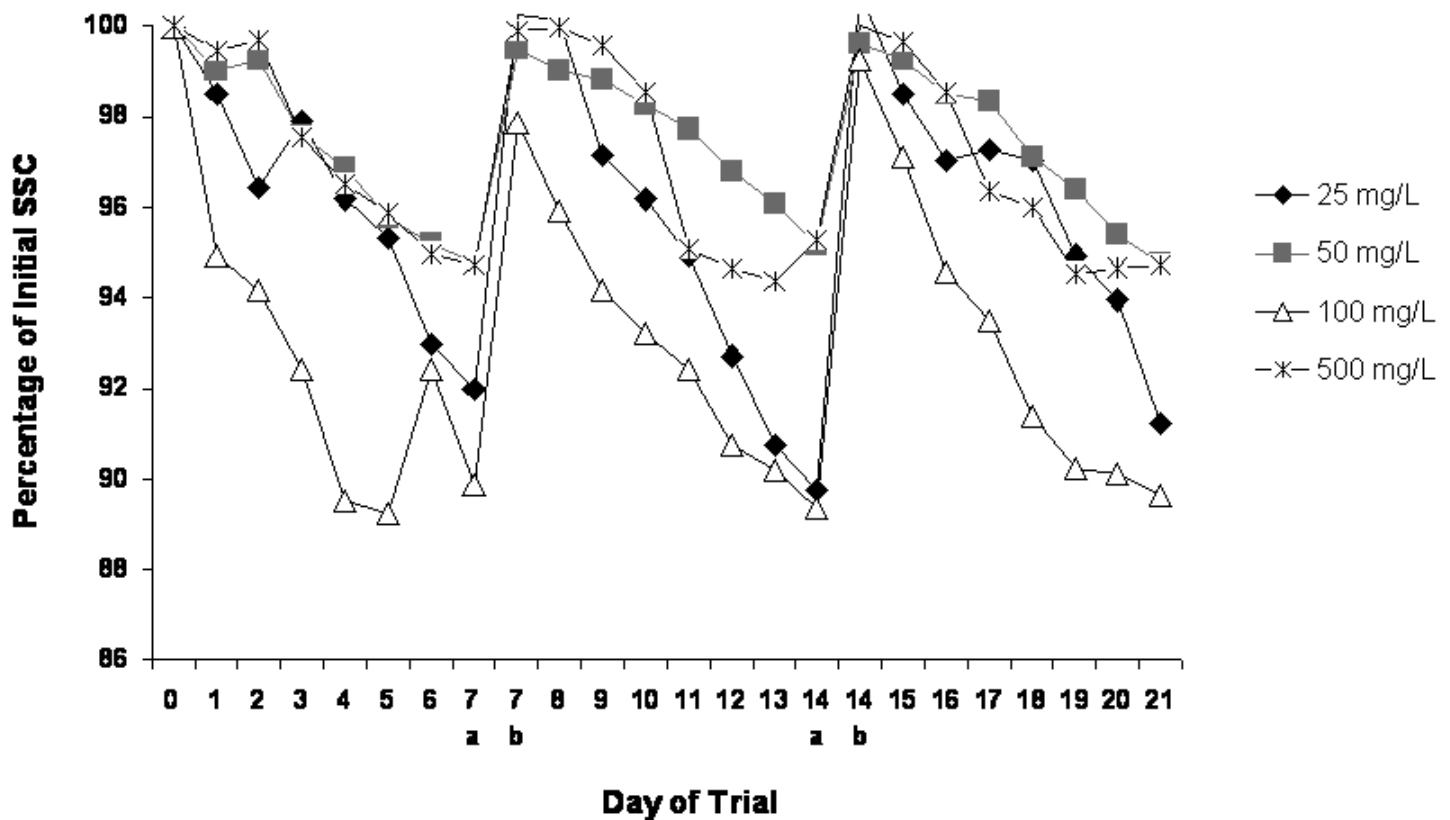


Figure 6. Percentage of initial mean suspended sediment concentrations (SSC) for 25, 50, 100 and 500 mg/L sediment treatments, measured during 21 day spotfin chub growth trial. SSC (mg/L) were calculated from measured turbidity (NTU) using a sediment rating curve (Figure 5).

Paddle movement and air flow rate can both be increased and may result in the ability to keep larger particles in suspension or suspend similar particles for longer periods of time.

This apparatus was tested with, and did not adversely affect, small minnows. Despite the very slow movement of the paddles, other types of organisms may be affected if they are not mobile. To alleviate this problem, a false bottom of mesh or other material could be placed above the paddles, so that they move freely below.

Apparatus performance has also not been established using salt water. Because salt can increase particle flocculation (especially clays) in water entrained with air bubbles, it seems likely that turbidity may be reduced in salt water due to particle aggregation. Increased flocculation and particle aggregation may preclude the use of this apparatus for marine experiments.

Discussion

Research on effects of turbidity on the physiology of aquatic organisms is limited. With the exception of research on commercial and game fishes, there is a dearth of information regarding the mechanisms regulating biological impacts of excess sediment in aquatic environments. Many questions

remain unanswered regarding the effects of elevated turbidity on invertebrates, nongame fishes, and other vertebrates.

Techniques have been developed for maintaining turbidity that are not prohibitively complex or costly and that are easily replicable (Redding et al. 1987, Gregory 1994, Burkhead and Jelks 2001). However, a primary problem with many of these methods is that the turbulence necessary to maintain high constant turbidity for long periods of time can create an inhospitable environment for test organisms, potentially resulting in artificial behavioral responses or increased stress in sensitive organisms. Eliminating all zero-flow areas (i.e., eddies where sediment settling occurs) is difficult; therefore, maintaining a near-constant turbidity for extended periods is also difficult. Some researchers are able to partially compensate for this difficulty by conducting short-duration experiments (Berg and Northcote 1985, Breitburg 1988, Gregory 1994). However, if research goals require a longer-term exposure to turbidity (e.g., studying the effects of turbidity on growth or spawning behavior), then short-term methods are not sufficient.

The device described here is designed to keep fine sediment (< 45 μm) in suspension for extended periods of time in numerous experimental tanks simultaneously. This sediment suspension system allows for high replication and is an alter-

native to complex costly laboratory techniques and large flow-through systems.

Comments and recommendations

The objective of this article was to present a simple, flexible device that will enable efficient turbidity-related biological research. The design presented here is an adaptable model that is functional and cost-effective. The most expensive piece of equipment is the variable-speed gear motor, whose size and cost will vary with the number of paddles being transported. Because additional drive shafts and tanks can be easily connected to the connecting arms, expanding the design to include more experimental tanks does not markedly increase the cost.

This design is offered as a framework on which to make improvements. One modification that may improve its performance is replacement of the metal sleeves with linear ball bearings and replacement of the drive shaft with a high-precision linear bearing shaft. These changes would markedly reduce friction, thereby increasing the number of paddles (and thus replicates) that a given motor could operate. Multiple drive shafts could also be powered from one motor, increasing the number of treatments and replicates, without markedly increasing costs.

Although aquatic scientists agree that excessive sedimentation negatively affects aquatic communities, quantifiable relationships between sediment concentration and effect remain elusive. However, understanding these relationships is vital when developing scientifically based turbidity standards. The apparatus described here offers a cost-effective approach for quantifying the response of aquatic organisms to suspended sediment.

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