Imitating the Physical Properties of Drifting Semibuoyant Fish (Cyprinidae) Eggs with Artificial Eggs

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ABSTRACT

Six Rio Grande basin cyprinid taxa compose a reproductive guild that spawns during increases in stream flow and broadcasts semibuoyant (drifting) eggs in the current. Spawning by guild members is naturally initiated by elevated flows during spring snow-melt or summer rainstorms, but reservoir releases now also result in spawning. Location or development of a material that mimics the physical properties of semibuoyant eggs of guild members would enable quantification of the displacement of drifting eggs and assessment of the additive impacts of stream flow, instream barriers, and habitat modifications on populations of these fishes. To initiate this search, the size and terminal velocity of guild members' eggs was determined and their specific gravity (SG=1.00589 $\pm$ 0.00011) calculated. Products with specific gravities similar to semibuoyant eggs were obtained, and tests were conducted on their rates of travel and settling times when exposed to current. Materials with specific gravities slightly greater than semibuoyant cyprinid eggs (SG>1.01) sank too rapidly, while materials less dense than water (SG<1.00) traveled downstream more rapidly than guild members' eggs. Particle size, not shape, noticeably affected rates of travel and settling times. Laboratory and field tests indicated that a modified nylon 12 particle (SG=1.00457 $\pm$ 0.00017) most closely mimicked the properties of semibuoyant cyprinid eggs.

INTRODUCTION

Stira et al. (1976) were among the first to report a technique for producing artificial eggs that could be used to monitor downstream movement (drift) of fish eggs. They developed a "gelegg" that was used to mimic eggs of American shad (*Alosa sapidissima*) in the Connecticut River and described techniques to manufacture artificial fish eggs of different sizes or specific gravities. Ottaway (1981) reported the use of a material (nylon 6) that approximated the size and specific gravity of eggs of brown trout (*Salmo trutta*), and this material was subsequently employed in studies on downstream displacement of eggs (Crisp 1989a, 1989b).

Our primary concern in obtaining a material that would act as a surrogate fish egg was to ensure that its buoyancy closely approximated that of eggs of our study species. A precise means of determining an object's buoyancy is to calculate its specific gravity. Early efforts by Milroy (1898) describing eggs of several marine fishes included calculations of the specific gravity of those eggs (range=1.02 to 1.09), and more recent work on salmonids (Berg and Grimaldi 1965, Bonham 1976) provided specific gravity estimates between 1.06 to 1.20. Subsequent attempts to produce artificial fish eggs that mimicked the properties of real fish eggs recognized the importance of matching specific gravity of surrogate eggs to that of real eggs (Stira et al. 1976, Ottaway 1981).

Studies of Rio Grande basin fishes resulted in the identification of a reproductive guild of mainstream cyprinids comprised of Rio Grande silvery minnow (*Hybognathus amarus*), plains minnow (*H. placitus*), speckled chub (*Macrhybopsis aestivalis*), Arkansas River shiner (*Notropis girardi*), Rio Grande shiner (*N. jemezanus*), and Pecos bluntnose shiner (*N. simus pecosensis*). These fishes are broadcast spawners that produce nonadhesive, semibuoyant eggs (Platania and Altenbach 1998). Soon after spawning, eggs swell to about 3 mm in diameter and become semibuoyant as water fills the large perivitelline space surrounding the 1 mm diameter embryos (Platania and Altenbach 1998).

Spawning occurs during natural increases in flow (spring runoff and summer convectional storms) and during extended periods of constant high flow (reservoir releases). Eggs are displaced downstream from the point where spawning occurs. Hatching time of the eggs is temperature dependent but generally occurs about 24-48 h after fertilization (Moore 1944, Bottrell et al. 1964, Platania and Altenbach 1998). Recently-hatched larval fish are displaced downstream and individual protolarvae do not develop sufficient mobility to move out of main-channel flow until at least three to four days post-hatching. Two potential factors limiting recolonization by guild members and attainment of historic (pre-impoundment) population size appear to be the downstream transport of eggs and larvae beyond barriers that block upstream movement of fishes and displacement into highly degraded downstream riverine habitats and reservoirs.

Previous studies using artificial eggs examined the extent that demersal eggs had been displaced from the point of spawning over a several hundred meter study area (Crisp 1989b). This study required artificial eggs that could be used to determine the downstream transport of fish reproductive products over large distances (up to 300 km) in several riverine systems in New Mexico. To document the rate, magnitude, and distance of displacement of guild members' eggs, it was necessary to locate or develop a material that mimicked the physical properties of real eggs. In addition, any such material needed to be nontoxic, durable, and affordable in mass quantities. This paper provides the results of laboratory and field research to locate a durable and cost-effective mimic of a semibuoyant cyprinid egg.

METHODS AND MATERIALS

The specific gravity of an object is often determined, in part, by measuring its vertical velocity in liquid. The calculated value of specific gravity is dimensionless since it is dependent on the reference liquid in which the object is tested (i.e., ρ / ρ_{ref}; ρ=object's density (kg/m^3) and ρ_{ref}=density of liquid (kg/m^3)). Specific gravity is calculated from the object's terminal velocity (speed it sinks after initial acceleration) and is largely determined by the object's density. Same-sized objects with only slight differences in their densities will sink at noticeably different rates. In addition, the item with the higher density will settle to the bottom at flows that maintain the particle of lower density in suspension.

Terminal velocities of semibuoyant cyprinid eggs and artificial materials were determined by measuring their sinking rates, after initial acceleration, in a column of water. The test chamber was a vertical 183 cm tall, 8.9 cm diameter, clear acrylic tube. The tube was filled with distilled water (20°C) and terminal velocity of the falling object measured (to the nearest 0.5 sec) between 30 cm from the top of the tube (well beyond the point where the object would reach its terminal velocity) and 100 cm from the top. Terminal velocity (cm/sec) was calculated by dividing distance traveled (70 cm) by time.

Specific gravities of semibuoyant eggs and artificial eggs were calculated using an empirical formula based on the transport properties of Newtonian fluids and the drag coefficient for a sphere. The kinematic viscosity of water at atmospheric pressure was determined from a temperature-based table (from White 1974). The density of water at 20°C and atmospheric pressure (998 kg/m^3) was used in all calculations. The Reynolds number (Re_D) represented the ratio of inertial forces to viscous forces in a liquid and was calculated as a function of the terminal velocity (U), diameter of object (D), and kinematic viscosity (ν) [$Re_D = UD/\nu$]. An empirical drag coefficient (C_D) was calculated from empirical evidence reviewed in White (1974) [$C_D = 24/Re_D + 6/(1+Re_D^{1/2}) + 0.4$]. The final specific gravity (SG) equation was derived from the difference (ΔSG) between the SG of the liquid and SG of the object falling through the liquid. This equation, based on the force balance dynamic of the object at its terminal velocity, includes the gravitational constant (g) and a numeric constant (¾) [$\Delta SG = ¾(U\nu Re_D C_D/D^2 g$ or $\Delta SG = ¾(U^2 C_D/Dg)$].

Fish eggs and artificial mimics were placed in an aquarium with convective flow about a horizontal axis to test their behavior when subjected to current. Flow was driven by an air diffuser that sent a curtain of bubbles up one side of the aquarium. Rate of flow was controlled by the air supply. Observations on the rate and direction of travel were made for the objects as they moved through the tank. The amount of flow needed to maintain objects in suspension was considered a key factor in whether a prospective material was appropriate. If the test product settled to the bottom and did not become re-suspended in the water column at the low flows that kept semibuoyant cyprinid eggs in suspension, it was considered inappropriate.

The prospect of fish ingesting artificial eggs was tested by putting a known number of the particles into separate aquaria containing either adult study cyprinids, green sunfish (*Lepomis cyanellus*), or largemouth bass (*Micropterus salmoides*). The initial response of the fishes to the introduction of the product was noted through direct observation. Fish and artificial eggs also were maintained in aquaria for one week and checked daily to see if particles had been ingested.

Field trials of materials were conducted by releasing small quantities into the Pecos River, NM. Rates of travel were recorded for objects that floated and those that, like semibuoyant cyprinid eggs, remained submerged. Shoreline observations were made to determine dispersal patterns of artificial eggs throughout the river channel as they drifted downstream and the frequency with which they became isolated in areas of low flow or stranded along the river's edge.

RESULTS

The physical attributes of semibuoyant cyprinid eggs were calculated from 100 trials. Mean diameter of eggs was 2.672 ± 0.022 mm, and they were slightly negatively buoyant ($\Delta SG = 0.00589 \pm 0.00011$) in water. Terminal velocity of eggs was low ($U = 9.291$ mm/s ± 0.120).

Several materials that absorb many times their mass in water and become slightly negatively buoyant were tested. Polyurethane foam had a low terminal velocity, similar to that of semibuoyant cyprinid eggs, and remained in suspension in low currents generated in the aquarium. Field experiments using polyurethane foam demonstrated that particles remained in suspension as they drifted downstream and behaved like semibuoyant cyprinid eggs. Similar results also were noted for a cross-linked homopolymer of acrylamide consisting of amide groups (-$CONH_2$-) linked to CH and CH_2 groups. This material absorbed several hundred times its mass in water and swelled from a small hard crystal (<1 mm) to a large (several mm) irregular gel-like sphere.

In addition to products that absorbed water, non-absorbing solid materials (SG range= 0.97 to 1.01) that approximated the size of semibuoyant cyprinid eggs were tested. The most durable and cost-effective non-absorbent materials located were in the polyamide family. The chemical structure of polyamides is a ring of CH_2 groups linked by a single amide (peptide or protein) bond (-CONH-).

While unmodified nylon 12 polyamide has a specific gravity of 1.01, there are structurally modified resins of nylon 12 with lower specific gravities. Medium and low viscosity grades of nylon 12 are less dense and more flexible than their unaltered parent compound resulting in a lower specific gravity. The modified nylon 12 particles evaluated were medium-soft to soft grades.

Nylon products tested (both modified and unmodified) were non-toxic extruded cylinders about 3 mm in both diameter and length (Table 1). The potential effect of shape on nylon 12 was investigated by molding a small number of the cylinder shaped particles into spheres; there was no appreciable difference between the terminal velocity of nylon 12 spheres versus cylinders and both acted similarly in aquarium tests. However, commercially molding large quantities of nylon 12 into spheres was cost prohibitive.

Unmodified nylon 12 behaved like semibuoyant cyprinid eggs but sank about 1.5 times as fast despite a difference in specific gravity of only 0.00435. Nylon pellets sank and stayed on the bottom of the aquarium at the low flows (<2 cm/s) which kept semibuoyant cyprinid eggs in suspension. In field tests, unmodified nylon 12 particles remained in suspension in moderate to high flows but settled in low-velocity areas or along shorelines.

One form of modified nylon 12 had a near-neutral buoyancy (ΔSG=0.00457 $\pm$ 0.00017; N=100) and most closely approximated that of semibuoyant cyprinid eggs (ΔSG=0.00589 $\pm$ 0.00011). The terminal velocity of these semibuoyant nylon particles (U= 7.597 mm/s $\pm$ 0.175) was quite similar to that of eggs (U= 9.291 mm/s $\pm$ 0.120). This type of modified nylon 12 particle also mimicked the movement of semibuoyant cyprinid eggs in aquarium tests and did not settle out in low velocity areas during field trials.

Plastics whose specific gravity was greater than 1.01 were also evaluated. Polystyrene was the principal material in this class that was tested (SG=1.04). Spheres of polystyrene that were similar in size to semibuoyant cyprinid eggs had a higher terminal velocity than eggs. Conversely, small polystyrene beads had a terminal velocity similar to semibuoyant cyprinid eggs but settled to the bottom in aquaria at the low velocity currents that kept semibuoyant cyprinid eggs in suspension.

The final category of materials tested had specific gravities <1.00. Principal materials examined were expanded polystyrene and modified nylon 12 particles.

Expanded polystyrene beads (2-3 mm) traveled on the water's surface, were affected by surface winds, and were often stranded along the shoreline or among partially exposed instream debris. In field tests of the modified nylon 12, with a specific gravity of 0.97, the particles traveled below the water's surface while in areas of current. The rate of travel of this positively buoyant form of modified 12 nylon was faster than that observed for the semibuoyant form of modified nylon 12 (SG=1.005). In low velocity aquatic habitats, positively buoyant nylon 12 particles (SG=0.97) floated to the surface and were often stranded along the shoreline.

The different nylon 12 products, the polyacrylamide, and polyurethane pieces were introduced and held in aquaria containing fishes for one week but no materials were consumed. Fish responded to all objects and on occasion drew particles into their mouths, but appeared to recognize the pieces as non-food items and rejected them. This "mouthing" behavior was only noted immediately after products were introduced.

DISCUSSION

While the relative buoyancy of an object was the most important factor determining its suitability as an artificial fish egg, knowledge of its behavior in an aquarium or natural stream setting was also crucial. Small unexpanded polystyrene beads had a higher surface area to volume ratio than larger ones and achieved the drag necessary for force equilibrium at a terminal velocity nearly equal to that of semibuoyant cyprinid eggs. However, small beads settled to the bottom of the aquarium under low currents because the force exerted on their reduced surface area was insufficient to transport them back into suspension. Products were considered inappropriate if they did not remain in suspension at the low velocities that kept semibuoyant cyprinid eggs suspended.

Although positively buoyant materials were initially considered potential mimics for eggs, they did not behave like fish eggs. Expanded polystyrene pieces floated and consequently could be blown onto the shore and stranded. These particles also were attracted by electrostatic charges to each other and objects they contacted as they drifted downstream (e.g., instream debris and shoreline areas). Positively buoyant nylon 12 pieces (SG=0.97) traveled downstream faster than semibuoyant nylon 12 particles (SG=1.005). The difference in travel times between the two modified nylon 12 products appeared to be due to the different distribution patterns; the positively buoyant particles remained in the upper portion of the water column as they traveled downstream, while the semibuoyant nylon 12 pieces dispersed throughout all portions of the water column. The actual downstream distance traveled by semibuoyant nylon 12 particles, including their vertical movements within the water column, was greater than that traveled by either floating objects or positively buoyant nylon 12 pieces. Differences in the downstream movements of semibuoyant nylon 12 particles (SG=1.005) versus partially submerged or floating particles (SG<1.00) were sufficient to render the latter group unsuitable as mimics of semibuoyant cyprinid fish eggs.

While several products performed as well as semibuoyant nylon 12 particles in laboratory experiments and behaved much like semibuoyant cyprinid eggs, they were not appropriate mimics for other reasons. Particles of different colors were necessary for tests of downstream movement through adjoining river reaches but

Table 1. Principal materials tested and results of experiments.

MATERIAL	SG	Shape	Diameter (mm)	Cost per million	Color-Coding	Comments
Semibuoyant Cyprinid Eggs	1.006	Sphere	2.7	-	-	-
Semibuoyant Nylon 12	1.005	Cylinder	3	ca. $500	easy	mimicked real eggs
Semibuoyant Nylon 12	1.005	Sphere	3	ca. $8,500	easy	mimicked real eggs
Positively buoyant Nylon 12	0.97	Cylinder	3	ca. $500	easy	fast downstream transport time
Unmodified Nylon 12	1.01	Cylinder	3	ca. $500	easy	settled out of suspension
Expanded Polystyrene	<1.00	Sphere	2-3	<$100	moderate	fast downstream transport time
Unexpanded Polystyrene	1.04	Sphere	1	<$100	moderate	settled out of suspension
Polyacrylamide Gel Particles	ca. 1.00	Roughly Spherical	all sizes	<$100 [a]	difficult	particles were delicate
Polyurethane Foam	ca. 1.00	Ovate Sphere	3	ca. $3000	moderate	sediment infiltrated pores

[a] Cost of the raw material not including labor required to grind and sieve appropriate sized particles.

some materials were difficult to permanently mark. Ottaway (1981) described the advantages a more durable and cost-effective product (i.e., nylon 6) had compared to the gelatin-based eggs proposed by Stira et al. (1976). Similar advantages were noted in this study for the nylon 12 particles compared to naturally-based (e.g., carbohydrate and lipid-cellulose) and manufactured (e.g., polyacrylamide and polyurethane) products. One of the most challenging factors in developing and providing a usable artificial fish egg was the quantity (i.e., 10^7) required to track their movements and dispersion through long river reaches, especially when considering the small percent of the discharge that could be sampled for these products. After all laboratory and field results were considered, semibuoyant nylon 12 (SG=1.005) was selected as the optimum mimic for a semibuoyant cyprinid egg.

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