Lesson 5 – Using experimental data from the MSU/MiDNR Black River Stream Side Research Facility to estimate post hatch dispersal distance of lake sturgeon embryos in different substrate types.

Introduction

Behaviors expressed during early life history, including the larval stage, are poorly understood for many fish species. It is widely acknowledged that high mortality is a common feature of the larval stage in most fish species, particularly for species that are broadcast spawners and do not provide post-spawning parental care (like sturgeons). Understanding ecological requirements and behaviors expressed in response to environmental conditions experienced during critical early ontogenetic stages is important for effective fish management. Improved understanding of the ecological requirements of post-hatch larvae and the abilities of individuals at these critical early life stages to behaviorally respond to variability in aquatic environments is necessary to inform fishery managers. Managers require information concerning effects of physical stream features (e.g., stream bottom type and flow) and biotic factors (e.g., density, predation) that affect larvae survival, that will predict where suitable habitat exists, what restoration and protection actions are needed, and where restoration actions should be targeted.

Lake sturgeon (*Acipenser fulvescens*) is a species of management concern because the species is threatened throughout most of its native range. Larval habitat requirements and degree of behavioral plasticity in response to different habitat types, degree of habitat fragmentation, and habitat complexity during the period from egg hatch to dispersal from spawning sites is poorly understood.

Lake sturgeon are broadcast spawners. Eggs and sperm are released by adults into the stream current without construction of a nest. Adults spawn in rivers over coarse substrates that may include large gravel, cobble, boulders, or fractured bedrock. Lake sturgeon eggs are negatively buoyant (heavier than water and thus sink to the bottom) and after fertilization become adhesive and adhere to the substrate. At hatch lake sturgeon have a large yolk sac and are poorly developed, lack eyes and a functional feeding/digestive system, and are poor swimmers. It is widely assumed that upon hatching lake sturgeon larvae do not disperse but continue their development in the substrate interstices in the vicinity of the egg incubation site. However, it is possible that larval lake sturgeon are able to seek and select preferred habitat immediately after hatching despite their poor swimming ability. Further, research is needed to determine whether other intrinsic (genetic) or environmental variables that are known to affect larval phenotype also affect dispersal.

We used experimental flowing streams in a controlled replicated study to quantify larval lake sturgeon dispersal behavior immediately following hatch. Our objectives were to quantify larval lake sturgeon dispersal distance related to stream substrate and to evaluate whether larval lake sturgeon substrate selection depended on availability or distribution (environmental 'grain') for substrate of different type and size. We also were interested in quantifying the effects of family (genotype) associated with differences in dispersal distances, and whether incubation temperature emulating differences between stream conditions experienced during early and late periods in the spawning season influenced larval dispersal distance or substrate selection.

We hypothesized that larval lake sturgeon would not be found in sand substrate but would disperse from the site of egg hatching to gravel substrates with interstitial spaces that would provide cover. We also hypothesized that larvae, because they have poorly developed sensory systems and swimming ability, would be uniformly distributed throughout the upstream-

downstream length of the gravel substrates indicating larval dispersal is a random process and larvae are not able to actively seek and recognize suitable substrates. Improved understanding of larval lake sturgeon behavior and habitat selection relative to the location(s) and types of spawning habitat selected by adults can guide effective lake sturgeon management by identifying critical areas suitable for use by multiple lake sturgeon life stages.

Methods and Materials

Study Site

All work was conducted in our Black River streamside rearing facility (SRF) on the upper Black River, Cheboygan County, MI (see web site under 'About Us' for details). The Black River SRF is located on the upper Black River near the primary spawning habitat for lake sturgeon from Black Lake and uses Black River water pumped from the river in all stages of the rearing process (egg incubation through fish release). Water was either heated or chilled to a constant 18^{o}C or 10^{o}C, respectively for the entire period of incubation. These two temperatures represent the range of natural conditions experienced by eggs deposited by females spawning during 'late' (late May and early June) or 'early' (late April through mid-May).

Fertilization and incubation

Gametes were collected from two adult males and two female lake sturgeon spawning in the upper Black River. One male-female pair was sampled during the early spawning period (May 4) and a second male-female pair was sampled during the late (May 12) 2011 spawning period. Female body size (total length) for the early and late-spawning female was 150 cm (female 1) and 187 cm (female 2), respectively. Gametes were collected by applying pressure to the abdomen and extruding eggs into a plastic bag. Sperm was collected into a 10 ml syringe. Gametes were retained at ambient temperatures in river water in plastic bags in coolers. Gametes were transported to the Black River stream-side rearing facility for fertilization.

Approximately 7-10 mls of eggs from each female were fertilized with 1.0 ml of sperm from one male in 200 mls of UV-filtered river water. Eggs were fertilized in individual trays onto 1200 micron mesh screens without use of de-adhesive compounds so eggs would adhere to the screen mesh. Eggs were incubated in heath trays using a recirculation water system (20 L/min). Water in each tank was maintained at either a constant 10^{o}C or 18^{o}C temperature representing the ambient temperatures during the early and late spawning periods, respectively in the Black River (Forsythe et al. 2012). A TrimLine Titanium TLD 3 chiller and Easy Plug 230v Heater (Aquatic Ecosystems, Inc) was used to control temperatures. We utilized different incubation temperatures because incubation temperature significant affects incubation time and larval size at hatch (Adkinson 1994) which may affect larval dispersal distance and substrate selection. Eggs reared at colder temperatures have longer incubation times and larval size at hatch is generally larger compared to larvae from eggs reared in warmer water (Crossman 2008).

Embryo developmental stage was monitored following fertilization and developing embryos and screens onto which the eggs were adhered were removed from the heath trays and placed on a brick surface at the levels of the upstream most substrate in experimental flowing streams (Fig. 1). Eggs from each female and incubation temperature were placed into each of 4 flowing streams.

Experimental Treatments

Larval dispersal trials were conducted in four flowing streams (7.32 m x 0.61 m x 0.61 m) that differed in order of substrate (Fig. 1). Substrate was sorted into three categories; sand, large stones (> 4.00 cm) and small stones (< 2.50 cm). A sub-sample of 100 large and 100 small stones, were digitally photographed to measure greatest linear distance (mm) and total area (mm^2), using Image-J software (v1.43u). Flowing streams representing four substrate treatments were filled with substrate to an average depth of 7.62 cm in different substrate orientations (Fig. 1). Substrates in 3 treatments (flowing streams 1-3) were 1.52 m in length while the length of the fourth treatment (flowing stream 4) representing a 'finer' grain environment was 0.76 m in length. The upstream to downstream order of substrates differed, beginning with either sand, large gravels or small gravels. The outflow from each treatment tank was fitted with 1200 um mesh screen to create an entrainment area to ensure that larvae did not disperse from the down-stream end of the flowing streams unobserved. Entrainment areas were cleaned and checked multiple times daily for larvae. No larvae were found in any entrainment area during the course of the experiment.

A distance scale was drawn at 1 cm intervals on each of the four flowing streams so the distance traveled by hatched larvae could be measured. All raceway treatments were supplied with unfiltered river water pumped directly from the Black River. Velocity was measured daily in each flowing stream at three locations in the middle of each substrate type using a Marsh McBirney Flow-mate model 2000. Water velocity was maintained at approximately 0.2m/sec in all flowing streams and during all trials. Three days after all eggs hatched, the water flow was stopped and substrate was sampled from the most downstream location to the most upstream location within each treatment tank. Substrate was gently sorted in two to three centimeter increments to search for larvae in the substrate. Once a larval lake sturgeon was found it was removed to eliminate the possibility of counting larvae multiple times. The distance traveled (cm) and substrate type was recorded for each larvae.

We photographed 50 eggs from each female with a digital camera and measured each egg using Image J software (v1.43u). We also photographed 30 larvae per female and incubation temperature. Larvae were anethsetized using MS222 (25 mg/ml) and were photographed using a digital camera. A mm-scale was included in each egg and larval photo to insure accurate measurements. Yolk sac area (mm^2), body area (mm^2), and total length (mm) were measured using Image J software (v1.43u).

Statistical Analyses

Use data summary tools, descriptive statistics, and graphing capabilities to quantify dispersal distances in each tank (substrate treatment order). Estimate mean (and variation) in dispersal distances. Determine evidence for differences among tank treatments, between families, and between eggs subjected to 'warm' and 'cold' incubation water temperatures.

Student Assignment (see full lesson document)

The student's assignment is to take data provided in the accompanying Excel spreadsheet to determine whether there is evidence for differences in embryo dispersal as a function of stream substrate, family, and incubation temperature.