Lesson 6 – Embryo behavior - Ecological conditions experienced during early larval stages affect larval lake sturgeon phenotypes and behavior

Background

Organisms have the ability to behaviorally, physiologically, and phenotypically respond to new or changing environmental conditions that affect their ability to survive and reproduce. Within-generation accommodation in response to ecological factors (plasticity) results from both genetic and environmental effects and is often differentially expressed during sequential ontogenetic stages. Environmental conditions at all life stages can influence survival and reproduction. In early ontogenetic stages, environmental conditions can affect traits that are associated with survival and life-history characteristics. For example, temperatures experienced through the period of embryonic and early post-incubation development have been shown to affect growth and developmental rate and offspring size. Development can also be influenced by resource availability and foraging risks and environmental conditions experienced during early development can modify developmental trajectories and can induce or constrain plastic responses. The ages and sizes when organisms transition between life stages can also vary in response to environmental conditions. Further, the temperatures experienced by adults can affect life-history traits of offspring, including age at first reproduction, fecundity, and embryonic development time.

Because environmental conditions in early life can have lasting effects into adult life-stages, empirical data are needed that identify how environmental conditions influence phenotypic and behavioral traits over sequential ontogenetic stages.

For many broadcast-spawning fishes, eggs (and by extension larvae), are exposed to spatially heterogenous conditions and potentially a wide suite of environmental variables, even within the same stream and at the same time of deposition. Additionally, eggs are often fertilized by multiple males, which presents the opportunity for substantial variation in genetic by environment interactions. In the absence of nest preparation and without post-ovulatory parental care, eggs and larvae of same or different pedigree are exposed to widely varying environmental regimes.

For long-lived, iteroparous fishes, early-life stages are characterized by high mortality. Thus, the degree to which individuals can respond plastically to variation in ecological factors can have significant effects on individual survival and recruitment rates, which is important for both commercially harvested as well as populations of conservation concern.

Lake sturgeon (*Acipenser fulvescens*) are long-lived, iteroparous fish with high fecundity but low annual recruitment due to high mortality during early life stages. Eggs are widely dispersed throughout the water column where they adhere to the stream substrate and remain until hatch with no parental care provided. Newly-hatched lake sturgeon larvae lack many prominent structures necessary for movement, sensory perception, and resource acquisition (e.g. tail fin, eyes, mouth) and as a consequence immediately burrow in gravel and generally remain in the stream substrate until yolk-sac absorption is complete. Therefore, larval dispersal is believed to be motivated by the need for exogenous food resources. However, there is considerable plasticity in the timing of emergence, which may be due to the wide array of environmental conditions that individuals experience. Early developmental conditions can have a long-term impact

on an individual's fitness and can be important in determining transitional timing and survival through later life stages. Several ecological factors can influence early-life stage phenotypes and behaviors. Predation risk can influence growth rates during early life stages. Additionally, conspecific larval density can affect the timing and size at transition through life-history stages. However, individuals that experience delayed growth due to environmental effects can show compensatory growth by increasing growth and development following an interval of decreased food availability or low temperatures.

Ecological factors experienced at a given ontogenetic stage can also influence trait expression during subsequent stages. On the upper Black River in Michigan, the timing and location of spawning events of Lake sturgeon coincide with differences in several environmental variables including temperature. In some years, two distinct spawning events can result in "early" or "late" spawning females that corresponds to (on average) an 8°C difference in spawning temperature, and some spawning locations are inaccessible to late-spawning groups. Crossman (2008) observed that early spawning females, when water temperatures are typically lower, produced offspring that had longer incubation times, larger body size, and larger yolk-sac reserves at hatch compared with offspring of late spawning females, which could influence the timing of emergence from the substrate of post-hatch larvae.

In this study, we exposed larval lake sturgeon to several ecological and environmental factors and quantified the effects on the length of time between hatch and emergence from the substrate and growth. We tested the primary hypothesis that plasticity in time until emergence and larval growth is influenced by food availability, presence of predators and conspecific density. We predicted that individuals exposed to chemosensory predator cues or high conspecific densities would emerge to drift in a shorter time period and a smaller size compared to individuals supplied with a constant exogenous food resource or controls. We also tested whether emergence time and larval growth are affected by 1) conditions reflected in early/late spawning differences(incubation temperature and substrate) 2) family effects, and 3) deviance in temperature post-hatching, but before larval emergence.

Methods

Experimental Design and Sampling

Stream environments are inherently complex due to the large number of abiotic (i.e., temperature, flow, substrate) and biotic (i.e., food availability, predators, competitors) factors that can affect growth, behavior, and survival. To simplify the study system, we tested the effects of several abiotic and biotic variables on larval emergence time in an experimental setting. All work was conducted at the Black Lake stream-side research facility located on the upper Black River, Cheboygan Co., Michigan.

Eggs from two early-spawning females were fertilized with sperm from two early-spawning males to produce full-sib families. Fertilized eggs from each family were divided into two groups and incubated in trays at water temperatures typical of thermal regimes experienced by eggs in natural stream systems deposited by early-spawning (10°C) or late-spawning (18°C) adults (Forsythe 2010). Temperatures were maintained through the incubation period via a re-circulating tank using heaters and chillers. Hereafter, the 10°C and 18°C treatments are referred to as "cold" and "warm"

respectively. The 18°C incubation regime was ecologically deviant from 'status quo' conditions typically experienced by eggs from the early-spawning females used in the experiment, but represent conditions that eggs from late-spawning females typically experience.

At hatch, all larvae were anesthetized in a 20 mg/mL solution of tricaine methanesulfonate (MS-222) and photographed (Olympus E-420) for later analysis with an image analysis program (ImageJ 1.44). A ruler was included in all photographs to allow accurate estimation of larval body size (total length and total body area) and yolk sac area (a measure of endogenous energy reserves).

After newly hatched larvae were photographed, individuals from each family and incubation treatment (cold and warm) (N=400) were randomly assigned to one of four 12ft by 2ft fiberglass raceways and placed individually into 16oz plastic cups (Fig. 1). All cups contained gravel that simulated stream substrate, which allowed larvae to burrow into the interstitial spaces. Substrate length and depth were measured and used as variables quantifying characteristics of the substrate in each cup. The cups had two, 2.5cm by 8cm sections removed on opposite sides of the cup and replaced with mesh screen to allow constant flow of water through the cups but which precluded movements of larvae out of the cups. Stream water at natural (ambient) temperature was pumped into the raceways at a constant flow-through velocity of 0.03m/sec ± 0.003 measured by timing the flow of dye between two set points (the first row of cups to the last). Water depth was maintained in the raceways at depths approximately 1cm below the top of the cups to prevent larval dispersal. Water temperature was monitored hourly using a model 550A YSI meter throughout the time the larvae were in the cups to estimate the cumulative thermal units (CTU). A photoperiod of 9L:15D was set to simulate a longer night regime because larvae emerge to drift more frequently during night time hours..

The raceways were used to examine the effects of four variables on timing of emergence. Larvae in the first raceway were used to simulate the effects of conspecific density. Two larvae were added to each cup as opposed to a single larva per cup in all other raceways. Larvae in the second raceway were provided a small amount of food in each cup (approx. 100µL of solution containing brine shrimp nauplii; mean = 746, SD = 235) twice a day (08:00, 20:00) simulating a substrate environment with available food sources allowing for exogenous feeding. In the third raceway, predators of lake sturgeon larvae (Crossman 2008) including 14 rusty crayfish (*Orconectes rusticus*) and 85 odonate larvae (including *caliopterygidae, caliopteryx, gomphidae, cordulagastridae, aeshnidae*) were maintained in cages at the upper end of the raceway. All water pumped through the raceway passed through the cages resulting in chemosensory predator cues defusing throughout the raceway simulating the effects of predators. Larvae in the fourth raceway served as a control where a single larva was placed in each cup and no food or chemosensory predator cues were included.

All cups were monitored for emerged larvae using a flashlight at 03:00, 08:00, 20:00, and regularly during the day each day. The time of emergence (in days) was recorded. Upon emergence from the substrate, larvae were anaesthetized and photographed to obtain measures of body size and yolk sac. Differences in body size from the time of entry into the cup and emergence from the gravel provided a measure of growth during the time in the substrate. Differences in yolk sac area from time of entry to emergence provided a measure of the use of endogenous reserves.

Measures of Environmental Deviance

We estimated two measures of thermal deviance to test whether the degree of deviation in conditions at or near the time of emergence relative to previous conditions during the entire period in the gravel would stimulate emergence. The first measure of deviance (ΔTE:TW; Eq. 1) was calculated as the difference in the mean daily temperature for the 24 hr period prior to emergence (TE) compared to the mean daily temperature over the entire duration in the substrate excluding the last 24 hours (TW). A positive value in the deviance in mean daily temperatures indicates that the mean temperature during the 24 hours immediately prior to emergence from the gravel was greater than the mean daily temperature over the entire period in the gravel.

$$[\text{Eq. 1}]\ \Delta TE{:}TW = \frac{\sum_{i=1}^{24} (EmergTemp_i)}{24} - \frac{\sum_{j=1}^{N} (OverallTemp_j)}{N}$$

where *i* is the hourly temperature for the 24 hours prior to emergence, *j* is the average temperature for one 24 hour period and *N* is the number of days the larva was in the cup excluding the last 24 hours.

The second measure (Δ RTE:RTW; Eq. 2) was calculated as the difference between the minimum and maximum temperature in the 24 hours prior to emergence (RTE) and the average difference between the daily minimum and maximum temperature over the entire duration spent in the substrate excluding the last 24 hours (RTW). For this measure of deviance in daily ranges (min-max) in temperature, a positive value would indicate that the range in temperature min-max during the 24 hours immediately prior to emergence from the gravel was greater than the mean daily temperature range over the entire period in the gravel.

$$[\text{Eq. 2}]\ \Delta RTE{:}RTW = (MaxTemp_i - MinTemp_i) - \frac{\sum_{j=1}^{N} (OverallMaxTemp_j - OverallMinTemp_j)}{N}$$

where *i* is the temperature for the 24 hours prior to emergence, *j* is the temperature for one 24 hour period and *N* is the number of days larva was in cup excluding the last 24 hours.

Deviance was also measured between the incubation temperature and the temperature of the raceway when the larvae were first placed into the raceway treatments. Temperature deviations were measured as both the deviance for the mean daily temperature at placement as well as the hourly temperature at placement. Temperature data are provided in Fig. 2.

Fig1 Diagram of raceways testing the treatment effects of food (F), control (C), predator (P) and conspecific density (D). Water entered the raceway at the arrows and drained at the solid rectangles. The rectangle at (A) represents the enclosure that contained predators of lake sturgeon larvae. The test area represented by the dots consisted of seventeen rows of five 16 oz plastic cups housing one (food; control; predator) or two larvae (density). Each cup contained gravel substrate and had two slits of mesh screening to allow a constant flow through of water.

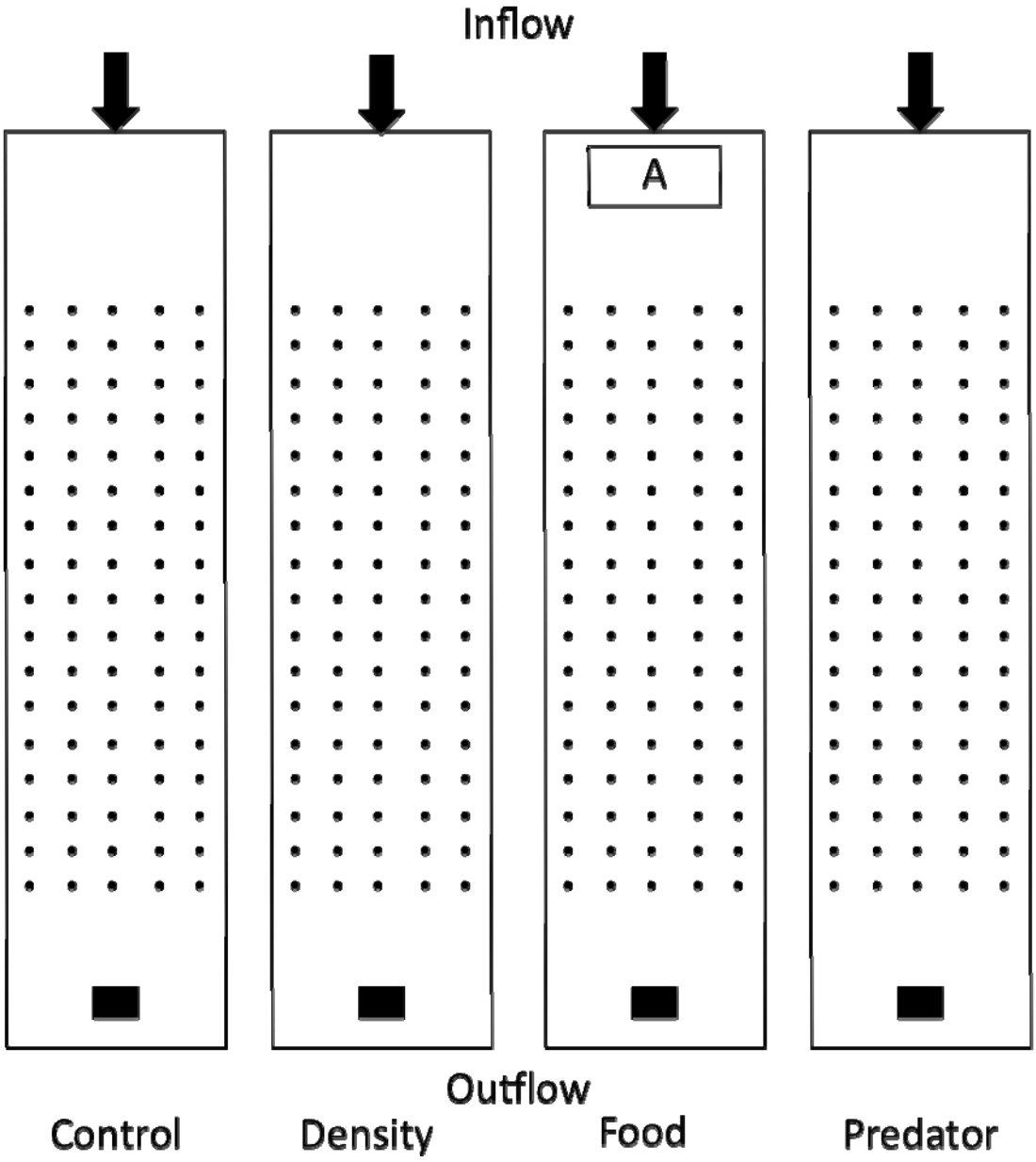

Fig 2 Raceway water temperature (°C ±SD) in relation to the first, last and mean day the larvae were entered into the cups of the raceway treatments (W/I: warm in; C/I: cold in) and the first, last and mean day the larvae emerged from the substrate (W/O: warm out; C/O: cold out)