Introduction: Maternal effects lie at the interface between genetic and environmental sources of phenotypic variation within populations, and have critical implications for evolutionary responses to natural selection[1]. Maternal effects arise when the mother's environment or phenotype (rather than her genes) affects the phenotypes of her offspring. The underlying mechanisms of these maternal effects may range from cellular and epigenetic modifications to changes in maternal behavior. Classic examples of maternal effects involve the level of maternal provisioning to offspring, as well as the choice of nest microhabitat in many oviparous taxa[2].

Many factors involved in nest-site choice contribute to fitness of offspring[3]. In painted turtles (*Chrysemys picta)*, for example, nest microhabitat influences patterns of embryo development, sex determination, and morphology of offspring[4,5]. Moreover, because hatchling *C. picta* over-winter in their natal nest, nest location may influence survival over winter or during dispersal to water the following spring[6]. Although, previous studies show relationships among nest-site choice, offspring morphology and survival, the mediators of these relationships are poorly understood[7,8]. **I will use a cross-fostering experiment to quantify the relative contributions of maternal identity (unmeasured maternal or genetic factors), yolk provisioning, nesting behavior, shade cover, and nest location to variation in phenotypic development and offspring survival across three early-life stages (embryo, over-winter, and dispersal stages).**

My experimental design will test the hypothesis that different maternal factors will influence offspring phenotypes and survival across early life stages. Specifically, I predict that (1) nest site microhabitat and egg provisioning will contribute to variation in egg survival and offspring size; (2) shade cover will significantly affect over winter survival due to its thermal effects on nests[6]; (3) nest distance to water will negatively correlate with dispersal success of offspring.

Research Plan: To test these predictions, I will study *C. picta* at a field site in northern Idaho (Round Lake State Park). Monitoring of nesting activity will follow established protocols at this site. After females have nested, I will excavate each nest to count, weigh, and mark each egg (clutch size ~15 eggs). Eggs will be cross-fostered among nests to disentangle the effects of clutch and nest location and to mitigate variation due to genetics. The experimental design (Fig. 1A) will consist of 15 blocks of three nests each (45 nests total). Two eggs from each female will be cross-fostered among three nests within a block, resulting in six eggs in each nest.

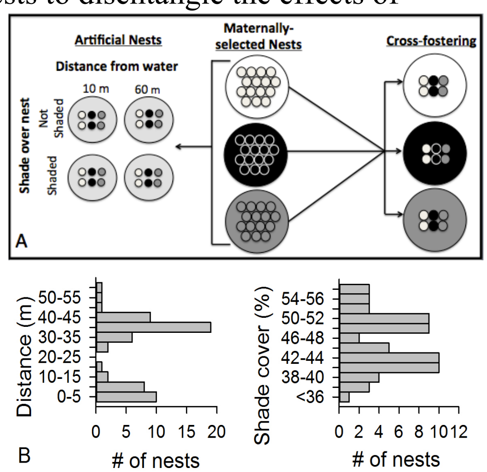

Fig. 1. A) Experimental design showing one of 15 blocks. Eggs from each nest are color-coded to illustrate how they will be allocated among nests. B) Range of nest distance to water and shade cover at the field site. Manipulations to artificial nests will capture the natural range of micro-habitats chosen by females.

Eight additional eggs from each nest will be randomly assigned to one of four artificially constructed nests per block (each with 6 eggs). Shade cover and distance to water will be manipulated for each artificial nest. These nests will be placed near (10 m) or far (60 m) from water, and half of them will be under shade cloth (mounted on 1 m stilts) to capture the extremes of nest conditions (Fig. 1B). The remaining 1-2 eggs from each nest will be frozen and later assessed for yolk steroids (radio-immunoassays) and energy content (bomb calorimetry). Temperature and humidity loggers will be placed in each nest, covered with soil, and protected from predators with hardwire cloth. Nest shade cover and solar transmittance will be quantified with hemispherical photographs.

Eggs will be carefully removed shortly before hatching and taken to the lab for the rest of incubation. Because eggs will be marked, I will identify each egg and hatchling in relation to clutch of origin. I will measure and photograph hatchlings for identification, and return them to their nests prior to winter. This protocol has been successful in previous studies[7]. Overwinter survival of hatchlings will be quantified when nests are excavated the following spring. At this point, hatchlings will be identified, measured, and placed back in their nests. A 100-m drift fence with pit traps along the length of the creek at the base of the nesting area will capture hatchlings as they disperse to water. Pit traps will be checked three times daily over one month after the first hatchling is captured. Recaptured individuals will be identified and measured; those not captured will be assumed dead[10].

Linear mixed models will quantify the relative contributions of maternal identity (random effect), egg size, yolk quality, nest location, nest microhabitat, and their interactions on offspring phenotypes and survival at each life-history stage. Logistic models will assess the relationship between each variable and survival at each life stage (embryo, overwinter, and dispersal stage).

Intellectual Merit: This cross-fostering design coupled with manipulations of nest microhabitat will decouple maternal and environmental variables that are naturally confounded. Specifically, I will determine the relative contributions of maternal nesting behavior, nest site location, yolk quality, and nest microhabitat to variation in phenotypes and fitness under ecologically-meaningful conditions. Because embryonic stages are sensitive to abiotic conditions and predation during hatchling dispersal from nests is high, my focus on three early life stages will provide an unmatched evaluation of the consequences of maternal nesting behavior and egg provisioning. This project also has practical applications. Because turtles are an imperiled vertebrate group, *C. picta* can serve as a model for understanding factors that contribute to variation in egg or hatchling survival, which will inform conservation efforts.

Broader Impacts: In addition to standard broader impacts (e.g., undergraduate research, publications, presentations at scientific meetings), we will be working with the Science in Motion program at Auburn University, which is an Alabama state-funded initiative that provides equipment, lesson plans, and training to STEM educators around the state. Many schools targeted by this program serve minority groups and have relatively low competence in STEM fields. Through this program, we will develop a teaching kit with a lesson on the scientific method, a core curriculum requirement in Alabama, that will push beyond logistical barriers of gaining field experience by bringing field activities into the high school classroom. This teaching kit will include (1) a short video documentary of our time in the field, and (2) a set of multiple (~5) nests (made of rigid plaster) with artificial eggs covered with soil. Each nest will be assigned a set of conditions (soil type, shade cover, etc.) that are encountered in the field. Student activities will involve most of the procedures described above (e.g., excavating nests, measuring nest depth, weighing eggs). Students will compare data collected, make ecological observations, generate hypotheses, and discuss experimental designs to test those hypotheses. This project will improve competencies in areas of observation, data collection, and data analysis, which meets several requirements in Alabama core curriculum standards. Working with Science in Motion will also provide an invaluable opportunity to train teachers in ways that will allow continued use of our project over time, and reach many students beyond the timeframe of my research.

Citations: [1]Wade 1998. In: *Maternal Effects as Adaptations* (Moussaeu & Fox, eds.) p 5-21, [2]Angilletta et al. 2009 *Ecology* 90:2933-2939, [3]*Refsnider* & Janzen 2010. *Ann Rev Ecol Evol Syst* 41:39-57,[4] Janzen & Morjan 2002. *J Herpetol* 36:308-311, [5]Mitchell et al. 2013. *Proc R Soc B* 280:1-7,[6]Weisrock & Janzen 1999. *Funct Ecol* 13:94-101, [7]Warner & Mitchell 2013. *Oecologia* 172:679-688,[8]Mitchell et al. 2013. *Ecology* 94:336-345, [9]Casale et al. 2015 *Aq Conserv* 25:551-561, [10]Janzen et al. 2000 Ecology 81(8):2290-2304