
*Diaptomus sanguineus*, a small, freshwater copepod is typically a resident of temporary ponds where its ability to produce diapausing eggs permits it to survive annual dry periods. Some populations, however, are occasionally found in permanent ponds where the timing of diapause seems to be adjusted by selection to provide an escape from seasonally intense fish predation. Spring diapause permits the copepods to avoid intense summer predation by allowing dormant eggs to remain safely on pond sediments where they are unlikely to be eaten, and if they were to be consumed, they would survive passage through the guts of the fish. Past experiments have led researchers to believe that the mean date of switch from production of immediate hatching eggs to diapausing eggs came 1.3 copepod generations before the mean date of onset of fish predation, which was very close to the mean switch date predicted by and ESS (Evolutionary Stable Strategy) model of the dynamics of a copepod population exposed to annual predation. This model allows researchers to assume that copepods who switch too early suffer from diminished representation in the following seasons because other individuals who have not yet switched are producing immediate hatching eggs, that in turn mature and produce diapausing eggs of their own. Late switchers also experience reduced fitness because of heavy losses due to intense predation. The best switching time is a function of the timing of onset of fish predation, which means that selection on diapausing eggs varies annually as does fish predation. In the Rhode Island ponds under investigation, pond depth is an important factor in the variation of timing and fish predation intensity, because when the water is shallow, the fish and the copepods are together, which can result in early and intense predation. On the other hand, when the water is deep, fish and copepods are spread out, allowing for less intense predation. Such a variation in response must imply the existence of heritability of genetic variation in traits.
related to the production of diapausing eggs. This study was undertaken to document that diapause timing in *D. sanguineus* is a heritable trait.

In order to investigate diapause timing, *D. sanguineus* living in Bullhead Pond and Little Bullhead Pond in Rhode Island were studied. Both contained fish at the start of the study in 1978, but Little Bullhead dried in a severe drought in 1981 that killed all the fish. Fish were not reintroduced when it refilled and by 1983 the timing of copepod diapause shifted to later in the season as a response to selection resulting from the absence of fish. In 1985, Little Bullhead Pond was divided into quarters with a solid vinyl curtain, and in 1986 fish were reintroduced to two of the quadrants. The ponds were sampled for 11 years, during which sampling trips were made weekly when the copepods were active in the water column, and monthly at other times of the year. To determine what eggs the females carried, and indirectly the mean of the timing of switch, live eggs were held in the lab for 3 weeks. Immediate eggs hatched within a few days, while eggs that had not hatched were assumed to be diapausing. During reproduction, about 24 ovigerous females were isolated in tissue culture plates on each date. In each year the mean and standard deviation of the timing of the switch to diapause were estimated from the fraction of individuals switching between each pair of consecutive sampling dates. The density of planktivorous fish was used as an indicator of the magnitude of predation on copepods. In the spring, females are especially visible because the carry dark egg clutches and are therefore consumed at a greater rate, causing an increase in the sex ratio, from which it is possible to estimate the timing and intensity of the seasonal onset of predation using the timing and slope of a plot of sex ratio against time. Fish density is a function of both the total number of fish and the volume of water in the pond. Females make either immediate eggs of diapausing eggs depending upon the environment to which they are exposed, and although day length and temperature have been found to be important cues used by the copepods in determining egg type, only photoperiod was manipulated in the experiments. It was found that copepods reared from eggs in the lab at eight-hr day length made immediate eggs, while 16 hr light made diapausing
eggs. At intermediate photoperiods some made immediate while others made diapausing eggs. Heritability of photoperiod sensitivity was calculated by rearing a wild population of the copepods at a constant intermediate photoperiod and temperature, and rearing the resulting immediate eggs under the same conditions and the parents, then recording the fraction that made immediate eggs for comparison. Photoperiod sensitivity is heritable if the daughters making immediate eggs exceeds significantly the proportion of mothers making immediate eggs, or if full-sib sisters resemble each other in photoperiod response more than they resemble the total original parent population.

Fish density varied by an order of magnitude over the study, but the timing of diapause changed only subtly between any two years. The standard deviation of the switch changed between years in some cases substantially, for reasons that appear to be related to water depth and the disturbance of long dormant eggs in the sediments. When fish density was high in a year and hence early and intense predation selected against late switching genotypes, the timing of diapause came earlier in the following year. When fish density was low in a year, and late and not intense predation selected for late switchers, the timing of diapause advanced in time between the previous and following year. This demonstrated a negative covariance. For the copepods from each of the four quadrants in Little Bullhead Pond, the fraction of offspring of mothers making immediate eggs was higher than that for the original parent population as a whole at the same photoperiod.

The result that there is heritability of diapause response in *D. sanguineus* is consistent with the conclusion that changes in diapause timing in the field represent selection responses to fluctuations in predation intensity and timing. Since only photoperiod was used as a diapause cue in this experiment, even though temperature plays a very important role, the copepods may have been deprived of important environmental cues in the laboratory and may therefore have been constrained from expressing the full range of response in diapause timing present in the field. Genotype-by-environment interactions can also make laboratory heritability studies difficult to interpret, so this study can only be completely applicable to
field results for this particular population of copepods. For *D. sanguineus*, selection response and selection intensity of diapause timing varies both in sign and magnitude around a central value of zero, but a mean response of zero does not imply that selection has not acted. Temporal variation in selection in Little Bullhead Pond may contribute to the maintenance of heritable variation, which is provided by the year-to-year changes in the timing and intensity of the onset of fish predation.


The common snapping turtle *Chelydra serpentina* is usually encountered in most bodies of freshwater. Eggs of snapping turtles are variable in size and size and locomotor performance of hatchlings are affected by moisture conditions experienced by eggs during incubation. Hatchlings are thought to experience considerable mortality during the first few months of life. Turtle embryos in species that lay flexible-shelled eggs frequently hatch at a larger size when they are incubated in relatively warm, dry surroundings. Temperature and availability of water affect metabolic rate and developmental time and are therefore the primary factors determining hatching size, as is egg size which varies considerably among species. The ecological and evolutionary importance of variation in body size of hatching turtles is unknown. The prevailing view is that “bigger is better” when it comes to hatching size. Although it is possible that larger hatchlings may reach reproductive maturity faster and maybe even exhibit greater fecundity than smaller turtles, most speculation about significance of body size of baby turtles has focused elsewhere. Several studies have shown that larger hatchlings may be less susceptible to predation because they may run and swim faster than smaller hatchlings, may grow faster, and be less susceptible to predation because they are more aggressive than smaller hatchlings in obtaining food. Larger hatchlings may also be more difficult for predators to capture or swallow. The objective of this study was to estimate the strength and form of natural selection on size of hatchling turtles during a critical phase of
their life history, migration from the nest to an aquatic site, and to evaluate the hypothesis that “bigger is better” for juvenile turtles. This was done by manipulating hatchling turtles to increase phenotypic variance in body size and locomotor performance in order to evaluate the potential for an evolutionary response to natural selection on body size.

Eggs of *C. serpentina* from 17 fresh water nests were collected from a National Wildlife Refuge area in Illinois in June of 1990. Fertile eggs from each clutch were weighed, assigned to egg containers with moistened vermiculite whose water potential was calibrated at -150 kPa and -950 kPa, then placed in an incubator at 27.5 C. Containers were rehydrated twice weekly and rotated daily to expose all eggs to a similar thermal environment. Date of hatching, weight, carapace length, carapace width at mid-body, and plastron length were recorded. Approximately 2 weeks after hatching, locomotor performance was evaluated by tapping the hatchlings on the tail with a metal probe to induce movement and speed was timed with a stopwatch. Turtles were marked and released into the parental population. To recapture the hatchlings a drift fence was put up and 18 lidless containers were implanted into the sand at 3m intervals. After recapture turtles were released at the edge of the river and the experiment was terminated when two successive monitoring periods failed to produce a hatchling turtle. The statistical analyses of natural selection on the turtles were based on a multivariate extension of the univariate regression method. The strength and form of natural selection on incubation treatment, family, egg size, body size, and locomotor performance were determined using multiple quadratic regression procedures. Heritability of body mass was calculated separately for each hydric environment.

Incubation conditions significantly modified the phenotypes of hatchling snapping turtles. Turtles hatching from eggs incubated on wet substrates were larger and took longer to hatch than dry incubation hatchlings. Neither hatchling success nor locomotor performance was influenced by water potential, nor was locomotor performance related to size of the hatchling turtles. All measurements of size were significantly and positively intercorrelated. Relatively larger hatchlings had increased survivorship over
smaller hatchlings. Estimates of the heritability of body mass from both hydric environments were variable. Heritability of body mass in hatchlings from the wet environment was moderate in magnitude, but was significantly greater than zero, however, body mass was not significantly heritable in hatchlings from the dry treatment.

Hydric conditions proved to have a tremendous influence on body size of hatchlings emerging from flexible-shelled eggs and incubation of eggs in differing water potentials allows experimental modification and increase in variance of body size of hatchling turtles independent of the influence of egg size or genetic maternal effects. The results of this study maintain that larger body size means greater fitness because larger turtles may reach reproductive maturity sooner and be more fecund than smaller siblings, and may also be less susceptible to predation or starvation. The results of this experiment indicate that larger hatchlings may have a survival advantage over smaller hatchlings during migration from the nest to an aquatic habitat, which is consistent with the “bigger is better” hypothesis. Significant but moderate directional selection was detected for overall size and mass, plastron length, and a tradeoff between hatchling mass and width. Disproportionate survivorship of larger and heavier hatchling turtles cannot be attributed to their improved locomotor performance or to dehydration effects of water potential on smaller individuals. The absence of significant selection among families suggest that clutch size and perhaps genetic similarity, may have little impact on survivorship of hatchling turtles in this population apart from the minimal effects of those factors on offspring size. Increased fitness of larger turtles may be related to size-selective predation, to behavioral differences, or to decreased susceptibility to ambient conditions in the field.

There is no information about the ecological and evolutionary maternal effects on body size of hatchling turtles, but the experimental manipulation of body size in this study should minimize the impart of maternal effects for predictions of the evolution of this trait. This study provides the first experimental evidence for the ecological significance of variation in size of hatchling turtles in the wild, and shows that
experimental manipulation of phenotypic variation is an extremely useful technique for detecting and understand the strength and form of selection on a given trait. These manipulations provide useful insight into the ecological and evolutionary impact of variation in body size during a critical and poorly understood period in the early life history of common snapping turtles.


In this experiment, the relationship between phenotypic plasticity and selection were studied in tadpoles of the chorus frog *Pseudacris triseriata* in order to test whether selection acts in conflicting directions in environments characterized by different predation regimes. These frogs inhabit small ponds which offer the necessary conditions to favor plasticity, such as waterborne chemical signals which provide an indication of environmental conditions. Previous research has established that this species has flexible behavioral and morphological responses to predation, so morphological and behavioral responses to two distinct environments with the direction of selection acting on plastic traits of tadpoles were compared. Direct measurement of selection in the separate environments are necessary to determine if the same traits that exhibit plasticity are under divergent selection. This direct measurement of selection can help test whether plasticity is an adaptation currently maintained by variability in the environment.

Eggs were collected from at least 50 females in early April 1995 from a large population of *P. triseriata* in Tinkle’s Marsh in southeastern Michigan. Three kinds of data sets were used to compare the direction of phenotypic change due to plasticity with the directions of change due to selection. These data sets are plasticity, selection in the absence of predators, and selection by *Anax*. Morphological plasticity in the tadpoles exposed to non lethal dragonfly predators was measured. Ponds were established in black tanks filled with well water and stocked with three well-mixed collections of zooplankton and
phytoplankton from nearby ponds. The tanks were covered to prevent unwanted colonization by insects and amphibians. Each tank contained three floating cages which were empty in the predator free treatment and contained a single *Anax* larva in the predator treatment which served to present the perception of predation risk. Tadpoles were introduced to each tank and behavioral responses were observed and time spent resting, swimming, and feeding were recorded. Morphology was also measured and length and depth of body and the maximum depth of the tail fin and tail muscle at the base of the tail were recorded. The significance of plastic responses to predators was tested by comparing the morphology of tadpoles from the no-predator and caged-predator tanks after 32 days, using mixed-model multivariate ANOVA.

Natural selection in the absence of free predators was estimated by examining the relationship between phenotype and performance in the plasticity experiment. Fitness measures for this analysis included both survival and growth rate. Natural selection on morphology in environments containing dragonflies was estimated by exposing tadpoles to free-ranging *Anax* and determining whether the predators killed a nonrandom sample of individuals in short-term predation trials. Selection on behavior was estimated by measuring activity just prior to beginning the predation trials, and determining whether survival during the trial was related to the average activity level of the tadpoles. At the end of the plasticity experiment, 120 tadpoles were collected from each tank and divided into 12 sets of 10 tadpoles. Four sets chosen at random were placed into tubs with no predators and eight sets were placed into tubs with one *Anax*. Dragonflies were fed a single tadpole and confined to small cages for 24 hours to allow the tadpoles to become aware of the predators. The behavior of the tadpoles was observed the day after they were introduced to the tubs, then the dragonflies were released from their cages and predation trials were run overnight. Survivors were collected and selection on morphology was estimated by regressing the mean of each morphological trait within a tub against the number of tadpoles surviving in the tub.
There were differences in behavior and tail shape between the tadpoles in the *Anax* and no *Anax* environments reflecting significant phenotypic plasticity. Tadpoles with predators spent less time actively swimming or feeding, and more time resting, than the tadpoles in the no-predator tanks. No differences were revealed in size-specific body depth and width, but a significant increase of relative tail muscle depth, muscle width, and tail fin depth were witnessed in presence of predators. In the absence of predation there was significant selection against tadpoles showing traits characteristic of high-predation environments. Mass was significantly higher in tanks that had tadpoles with narrow tail muscle which implies that individuals with wide tail muscles grew slowly regardless of treatment. In the presence of predators, when a population of tadpoles suffered high mortality the surviving tadpoles were morphologically distinct from the surviving unselected tadpoles. *Anax* did not impose selection on tadpole behavior since there was no relationship between survival during the predation trial and proportion of tadpoles actively feeding or swimming just before the dragonflies were released. Shifts in average phenotype due to selection in the presence and absence of predators were in opposite directions for all five measures of tail and body shape, as expected if selection acts to maintain plasticity.

Natural selection on body and tail shape in the *Pseudacris triseriata* tadpoles occurred in the presence and absence of predators, and the selection regime in environments lacking free predators was strongly divergent from that in ponds with dragonflies preying on tadpoles. Selection during predation trials reflected differences among individuals in traits that enhance ability to escape or avoid detection by predators. *Anax* tended to kill tadpoles that had deep, wide bodies, shallow tail fins, and narrow tail muscles, which may influence a tadpole’s ability to accelerate suddenly and swim rapidly. Selection in the absence of predation mortality was predominantly in the opposite direction from selection in the presence of free dragonflies. Tadpoles with deep wide bodies, shallow tail fins, and narrow and shallow tail muscles tended to grow faster in the absence of predators. The overall pattern was consistent with the notion that traits useful for predator escape are disfavored when predators are absent. Activity level was not under
selection in the predation trials, which leads researches to believe that activity is less important than morphology in escaping a predator’s attack, although movement frequency and speed certainly affect initial detection by predators. The results of this study strengthen the case that phenotypic plasticity is maintained by selection favoring different phenotypes in different environments, presumably because selective optima vary among environments. This study helps in beginning to build a picture of how phenotypic plasticity is favored and maintained in environments characterized by spatial or temporal variability.