Functionality of the spotted salamander egg mass polymorphism

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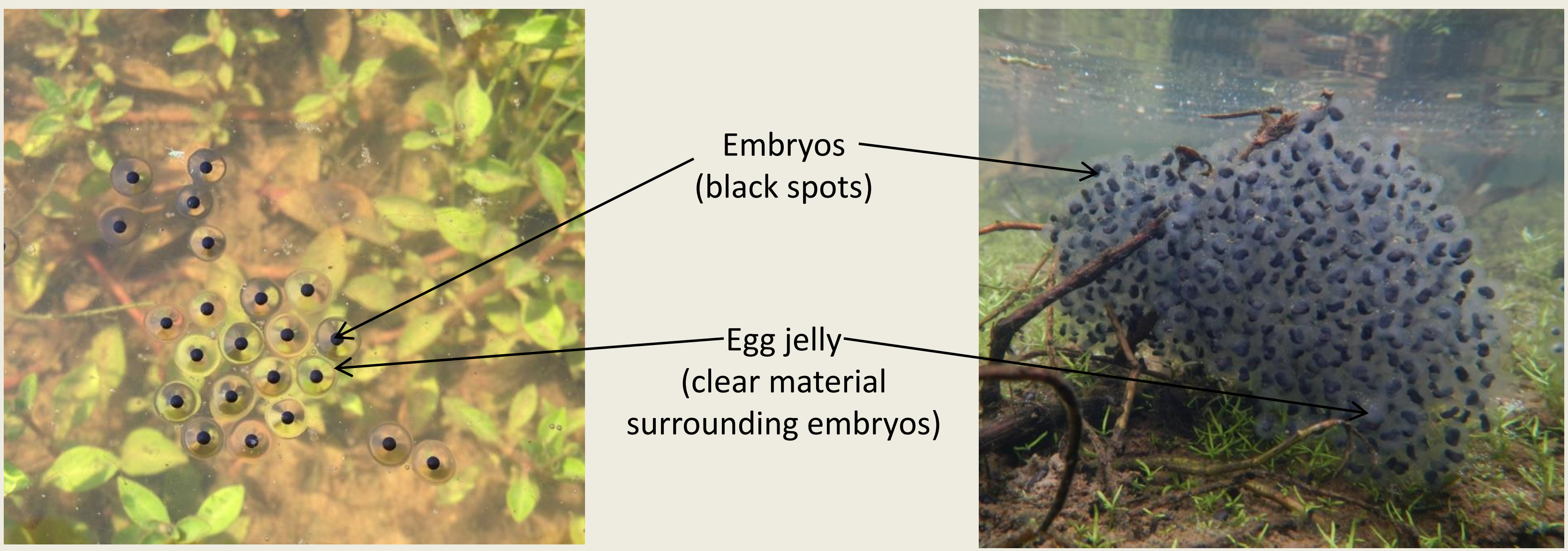
Amphibian eggs

- During reproduction amphibians produce jelly layers that surround embryos
- Jellies have a clear appearance with embryos clearly visible inside



Narrowmouth toad,
Gastrophryne carolinensis

Southern leopard frog,
Rana sphenocephala

Functions of egg jellies

- Attach eggs to structures and each other (forming egg masses)
- Enhance entry of conspecific sperm and prevent entry of heterospecific sperm
- Mediate interactions between embyros and the environment by protecting from:
  - Predators
  - Desiccation
  - Contaminants
  - Pathogens
  - Temperature
  - Ultraviolet light

Spotted salamander,
Ambystoma maculatum

- Range includes much of eastern North America
- Exhibits a unique polymorphism throughout its range where most egg masses have either a clear or white appearance
- A third intermediate or “gray” morph is locally uncommon or completely absent from populations
- Only one other amphibian (*Mantidactylus depressiceps*) is known to produce white egg masses, and no others produce multiple color morphs.

Why does this polymorphism occur?

- Color is genetically determined by a single gene
  - Clear morphs contain a water soluble protein
  - White morphs contain a hydrophobic protein
- These proteins are produced in the female’s oviductal wall cells
- Previous work shows that:
  - Development of embryos does not differ between high/low light or pH conditions (Ruth et al. 1993)
  - The proteins in white morphs may prevent other animals from feeding on the eggs (Petranka et al. 1998)

Why does this polymorphism occur?

- Because the difference in morphs is due to the presence or absence of a protein, the presence of this protein would likely not be without a function
- The proportion of white egg masses was negatively correlated with dissolved nutrient concentrations in natural ponds from Louisiana to Pennsylvania (Ruth et al. 1993)

Hypothesis:

White morphs are advantageous in low nutrient conditions

Objectives:

1. Determine if egg mass morph affects larval size in high and low nutrient conditions
2. Determine if the proportion of white egg masses in ponds relates to dissolved nutrient levels

Study design

- Conduct two mesocosm experiments using both morphs in high and low nutrient conditions:
  - Experiment 1: Does size of hatchlings vary between morphs in pools with different nutrient levels?
  - Experiment 2: If so, do these differences persist throughout the larval period?
- Collect field data from ponds at the University of Mississippi Field Station to determine the natural distribution of the morphs

How to set different nutrient levels?

- Use leaf litter – it leaches nutrients into the water while maintaining realism
- Experiment 1:
  - Egg masses are caged to prevent physical interaction with the leaf litter
  - High nutrients: 1 kg hardwood leaf litter
  - Low nutrients: No leaf litter
- Experiment 2:
  - Set treatments 2 months prior to larvae addition
    - High nutrients: 2 kg leaf litter
    - Low nutrients: 0.25 kg leaf litter
  - Replace all leaf just before larvae addition with 0.5 kg in all pools to maintain equivalent structural complexity

Methods: Experiment 1

- Collect egg masses the night after they are laid (all on the same night) so all are the same age
- Raise egg masses in mesocosms (wading pools)
- 2 × 2 factorial design
  - Nutrient level: high/low
  - Color morph: white/clear
- 6 egg masses per treatment
- 1 egg mass = 1 replicate
- Blocked by pool; 3 egg masses per pool

The experimental mesocosms about one month after the end of the experiment

Methods: Experiment 1

- Collect individuals daily as they hatch
- Determine
  - Size (total length)
  - Time to hatching
- 1 egg mass = 1 replicate
- Average all individuals from each egg mass

Hatchling

Embryos developing in a clear egg mass

Methods: Experiment 2

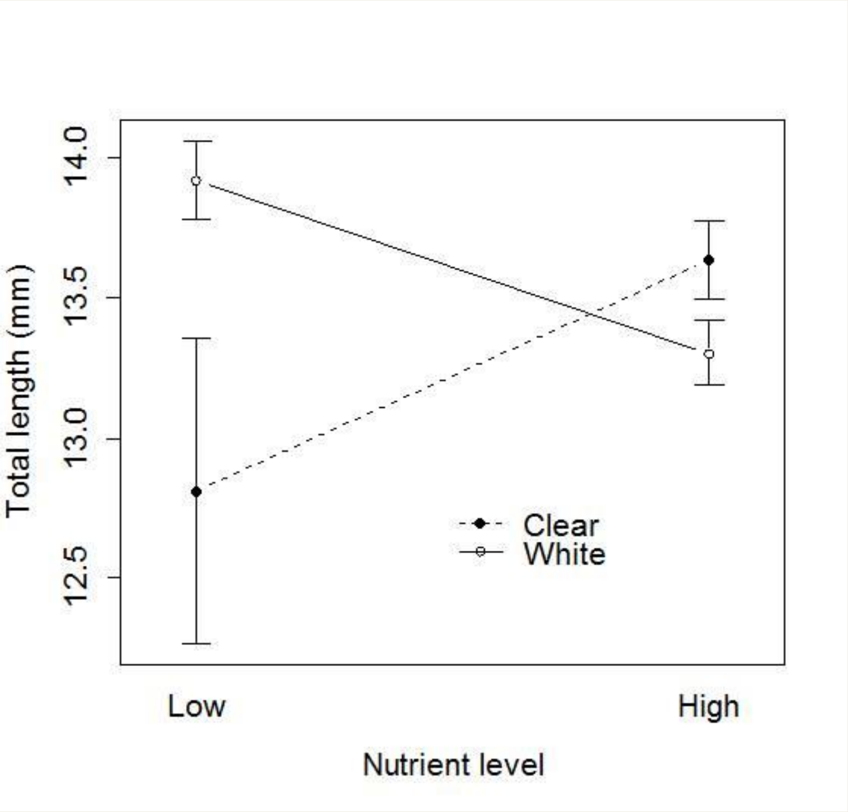
- Raise larvae from Experiment 1 in separate mesocosms with high/low nutrient levels
- Treatments and egg morphs are matched between experiments 1 and 2
- 7 larvae per pool (1 from each egg mass in Experiment 1 + a randomly selected individual)
- Collect larvae at the end of the larval period (but prior to metamorphosis) to determine size
- Also determine resource abundance (food – zooplankton, insects)

Mesocosms at the end of the experiment

Methods: Pond surveys

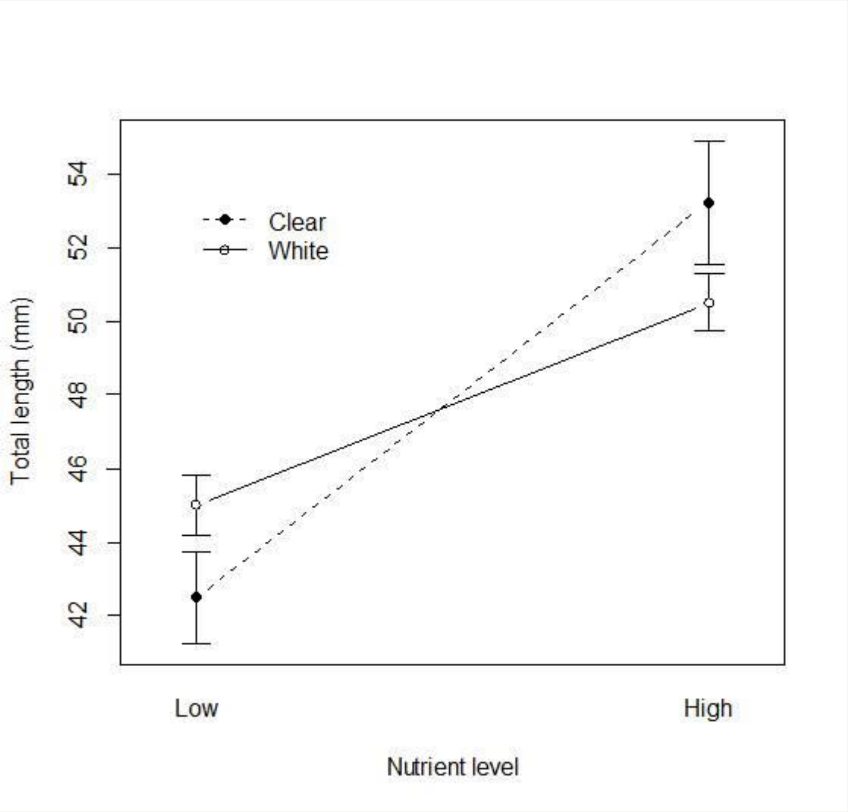
- Survey 56 fishless ponds at the University of Mississippi Field Station (UMFS)
- Count the number of white and clear egg masses in each pond
- Measure pond conductivity, which is an indication of dissolved nutrient levels

Results: Experiment 1



- Significant color morph × nutrient level interaction
- Hatchlings from white morphs were larger in low nutrient conditions
- High nutrient pools had significantly higher conductivity

Results: Experiment 2



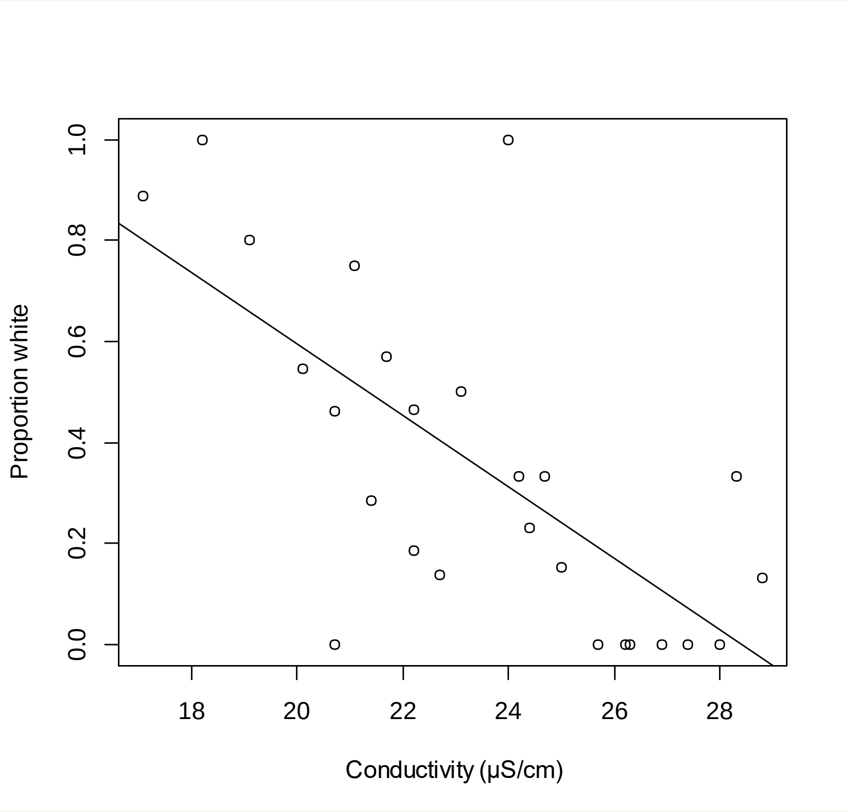
This figure of larvae total length is representative of the pattern and results seen in three other body size measurements: snout-vent length, head length, and head width.

- The significant color morph × nutrient level interaction persisted for 4 of 5 body size measurements
- The interaction was not significant for mass, which is more flexible and responsive to resource availability
- Larvae from white morphs were larger than those from clear morphs in low nutrient pools
- Both morphs were larger in high nutrient pools likely because of greater food abundances

Results: Experiment 2

- Larval size significantly covaried with survival; larvae were smaller in pools with more survivors
- Survival was significantly higher among larvae from white morphs, but did not vary with nutrient level
- Larval head size (width and length) was analyzed with snout-vent length as a covariate to determine if it varied independently of body size, which it did not
  - Head size can be an indication of feeding ability
- Conductivity was higher in high nutrient pools; other pool conditions did not vary between treatments

Results: Pond surveys



- 26 of 55 ponds surveyed had egg masses: 193 clear, 72 white, 0 intermediate
- There was a correlation (r = -0.702) between the proportion of white egg masses found in a pond and the pond's conductivity
  - Higher proportions of white egg masses were found in ponds with lower conductivity
- The proportion of white morphs did not correlate with any other variables

Conclusions

- The spotted salamander egg mass polymorphism may be an adaptation to ponds with varying nutrient levels
  - Larvae from white morphs are larger in low nutrient conditions
- Differences in the abundances of the two morphs at both local and regional scales may be maintained by differential performance of the morphs in the conditions at individual sites
- Over the long-term, potential fitness differences may select for one morph over the other
  - This could explain why some breeding sites and populations contain predominately or only one of the two morphs

Conclusions

The correlation between the proportion of white morphs and conductivity at UMFS indicates that:

- Pond conditions affect local fitness of the morphs

  and/or

- Females select ponds to breed in that match their phenotype

- Also, differences among ponds can be maintained at small spatial scales (< 1 km^2 at UMFS) and among sites that are very close to each other (< 5 m apart)

Significance

- Habitat management can help maintain conditions suitable to one of the morphs if it is locally more abundant than the other
- Changes in habitat quality may affect abundances, persistence, or distributions of species
- Polymorphisms can be adaptations to, and maintained by, environmental heterogeneity
- Supports the idea that protein substitutions are major changes that typically have functions

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