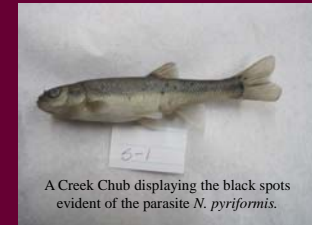


Methodology for Determining ASC Density in Relation to Black spot Parasite Load in Creek Chub



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Introduction

It is generally thought that alarm substance cells (ASC) in the fish epidermis evolved as a means to warn other fish of nearby predation via release of the chemical substance these cells hold. It has been recently hypothesized, that the evolution and presence of ASC in fish may have more of a relationship with parasitism than predation. The goal of our study is to determine if an increased infestation of the black-spot parasite (*Neascus pyriformis*) in creek chub (*Semotilus atromaculatus*) also correlates with an increase in the density of ASC. To our knowledge, this would be the first study to field test this recently developed hypothesis concerning the evolutionary origins in ASC in freshwater fish. We will focus at this time on the methodology involved in collecting our data (collection of field specimen, quantifying external signs of parasitism, preparing the tissues for cross-sectioning using microtoming techniques, mounting the cross-sections onto microscope slides, staining techniques, microscopic imagery, and finally quantifying the density of ASC in the fish epidermis) as well as statistical analysis of our data.



Megan and Kate collecting Creek Chub from Little Niagara in Eau Claire, WI.

Methods



1. We extracted a sample of epidermis from the nape of each creek chub.



2. These extracted samples were dehydrated in a tert-butyl alcohol (TBA) series.



3. Then the samples were embedded in paraffin wax.



4a. The samples were carved out of the wax in the form of a trapezoid.



4b. The samples were placed on a wooden block and secured with melted wax.



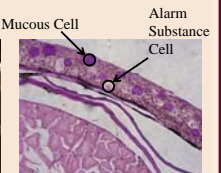
5. We sectioned the samples using microtoming techniques.



6. The sectioned samples were adhered to a slide using Haupt's Gelatin Adhesive and 2% Formalin.



7. We stained the slides using Schiff's Reagent with Eosin Y counter stain to unmistakably color the ASC.



8. An image of a completed slide at 40X magnification showing ASC and mucous cells in the epidermis.

Statistical Analysis

Since we were unsure how ASC varied in fish, specifically creek chub, we were driven to develop a method that would allow us to compare alarm substance cell density to black spots. We did this by randomly selecting a finished slide from each fish and taking thirty random counts of the sections under 20X magnification. We then divided the total number of black spots on the fish by the length. We also compared the total number of ASCs to the number of black spots per fish.

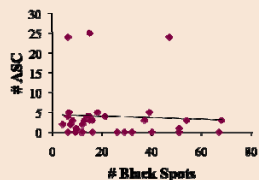


Figure 1: The relationship between the number of black spots and the number of ASC. $R^2=0.003$. No relationship was found.

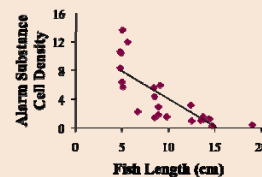


Figure 2: The relationship between ASC density and length. $R^2=0.6035$ and $p=0.004$.

Preliminary Findings

At this point we have 31 fish from 4 streams in northwestern Wisconsin with varying degrees of black spot infestation ranging from minimal to heavy loads. Our analyses have thus far shown no relationship between black spots and alarm substance cell count, there seems to be quite a bit of variability here. However, statistical analyses have shown that ASC densities decrease with length, suggesting that any relationship between ASC densities and parasite burden must also account for this size effect.

Future Goals

We plan on continuing to collect creek chub specimen from streams in northwestern Wisconsin so that we will have a greater amount of data for statistical analyses. We also hope to continue applying the histology knowledge acquired in this project in future projects.

Acknowledgements

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