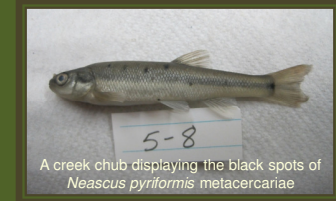


# Histology of Alarm Substance Cells in Relation to Parasite Load and Fish Size for Creek Chub (*Semotilus atromaculatus*)



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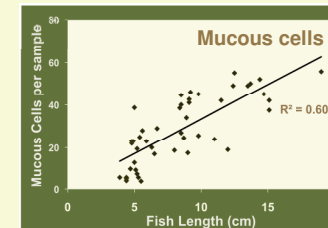
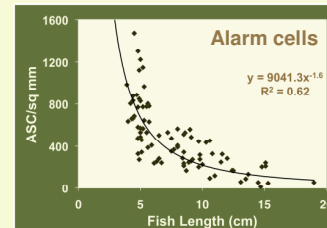
## INTRODUCTION

It is generally thought that alarm substance cells (ASC) in fish epidermis evolved as a means of reducing predation risks via the release of a chemical substance these cells hold. It has been recently hypothesized, however, that the evolution and presence of ASC in fish may have more of a relationship with parasitism than predation. The goal of our study was to determine if an increase infestation of the black spot parasite (*Neascus pyriformis*) in creek chub (*Semotilus atromaculatus*) also correlates with an increase in the density of ASC. Additionally, we wanted to determine whether there was a correlation between epidermal surface area and ASC density. To our knowledge, this is the first study to field test this recently developed parasitism hypothesis concerning the evolutionary origins of ASC in freshwater fish.

## METHODS

- 103 creek chub were collected from seven Wisconsin streams as well as from Minnesota and Stevens Point museum archives.
- We extracted a section of epidermis from the nape of the fish and subjected the samples to a dehydration and fixation process.
- The tissue sections were stained using varying concentrations of ethanol, Schiffs' reagent, and Eosin Y counter stain to color the alarm substance cells.
- Slides were imaged by randomly selecting one tissue section per slide under 20x magnification.
- From the image, we counted the number of alarm cells present and extracted surface area and thickness measurements.

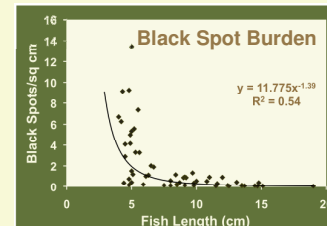
## RESULTS



### Epithelial Cells and Fish Size

Graphs to the left indicate that alarm cells were less numerous in large fish while epithelial mucous cells were more numerous in large fish.

These patterns suggest that different factors are responsible for the production of the two cell types.

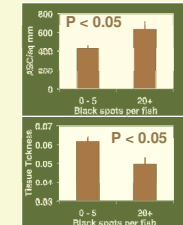
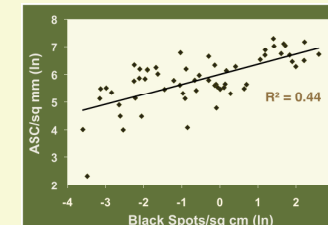


### Black Spot Burden and Fish Size

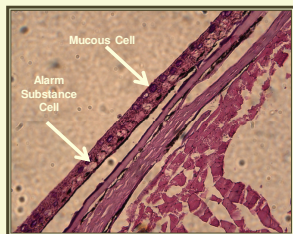
The graph above indicates that large fish possessed relatively fewer black spots than small fish. This relationship is nearly identical to the ASC-Size relationship.

### Black Spots and Alarm Cells

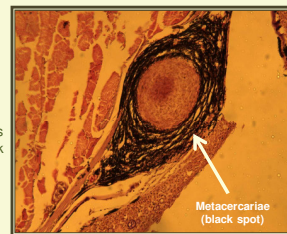
ASC density was positively related to black spot parasite density (graph left). Fish with heavy black spot burden had thinner epidermal layer (mm) and contained 50% more ASC than fish with low burden (graphs on right)



## HISTOLOGY



ASCs and mucous cells in the creek chub epidermis.



Cross section of *Neascus pyriformis* metacercariae (20x magnification).

## ACKNOWLEDGMENTS

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## SUMMARY

We identified two patterns in the distribution of epidermal ASC never before documented in wild fish. They are:

- 1) that ASC densities decline with fish size but
- 2) increase in fish with relatively higher black spot infestations.

## SUPPORT FOR IMMUNITY HYPOTHESIS?

Two related lines of evidence:

- 1) Size relationships for black spot and ASC are nearly identical. We discount the alternative explanation that ASC distribution is based on predation because predation risk does not likely vary continuously with size (Mittelbach 1981).
- 2) Our results are comparable to the experimental findings of Chivers et al (2007); fish with heavier parasite burden contained more ASC.

Future work will focus on measuring more fish to determine whether these findings can be generalized to other populations and species.

## LITERATURE CITED

- Chivers, DP et al. 2007. Epidermal 'alarm substance' cells of fishes maintained by non-alarm functions: possible defense against pathogens, parasites and UVB radiation. Proc. R. Soc. B. 274: 2611-2619.
- Mittelbach, G. 1981. Foraging efficiency and body size: A study of optimal diet and habitat use by Bluegills. Ecology 62:1370-1386.