Searching for a physiological basis for age-specific alarm response in creek chub (*Semotilus atromaculatus)*

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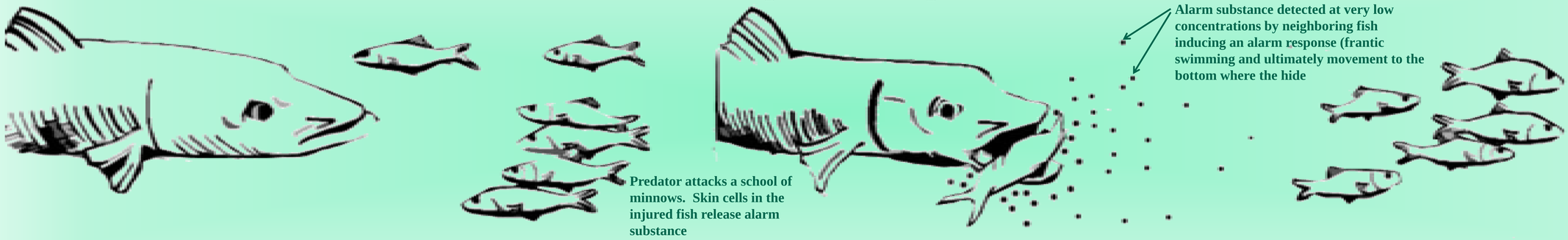
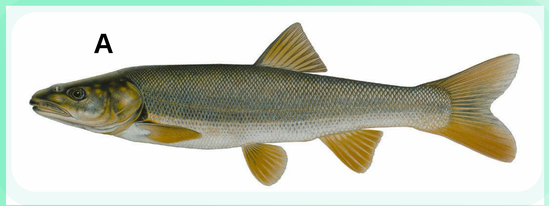
Chemicals warn fish of a predator's presence



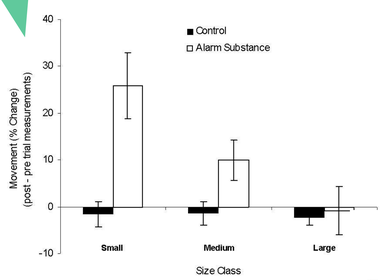
Evidence of nuanced response to alarm chemical

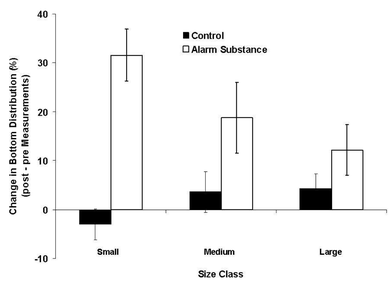
- All species of the Family Cyprinidae (minnows) produce a pheromone known as the Schreckstoff substance that is released when skin tissue is damaged, such as during predation (Smith 1992).

- Most minnow species do not grow to large sizes (<10 cm); consequently they tend to be susceptible to predation throughout life.
- There are a few minnow species, however, (e.g., pikeminnows, A and creek chub, B) that can grow to sizes greater than 1 m in length. In fact, these species are important fish predators in many rivers of North America.



- Earlier, students in Dr. Lonzarich's lab conducted an experiment examining the sensitivity to alarm pheromones of small and large creek chub (*Semotilus atromaculatus*). Our hypothesis - that the alarm response in chub would diminish with size as individuals grew from prey into predator – was strongly supported by our experimental data.

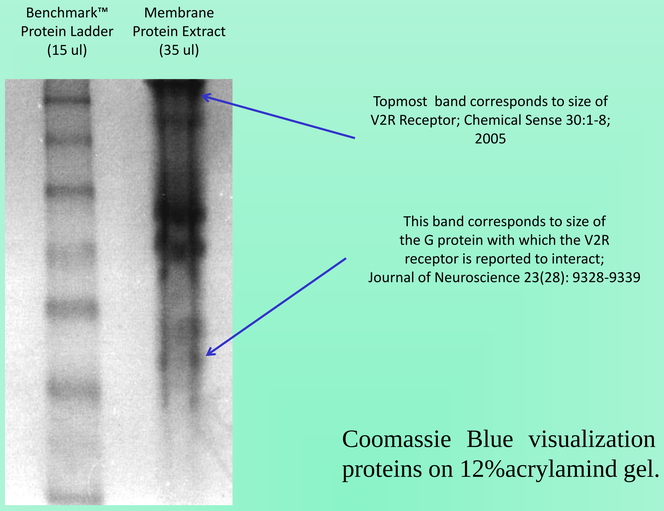




A physiological basis for variability in the alarm response?

Introduction

The V2R protein, is a G-protein coupled receptor, which we hypothesize mediates the effects of the alarm substance has a reported molecular weight of ~100KDa in other species of fish. To date, there are no antibodies against V2R receptor commercially available in the creek chub. Thus identification of the protein was achieved in these initial studies, based on size. These studies will be repeated in adult fish.



Coomassie Blue visualization of membrane proteins on 12%acrylamind gel.

Methods

The olfactory epithelium of juvenile creek chub were rapidly dissected and stored at -80C until use. Membrane proteins were extracted from the OE by dependent phase partition using FOCUS™ Membrane Protein Extraction Kit (G-Biosciences, St. Louis, MO). Briefly 3 OE (~100 mg tissue) was sonicated for 60 seconds on ice in membrane extraction buffer. Samples were incubated briefly at 37C, and then centrifuged the suspension into two phases which contained hydrophilic and hydrophobic membrane proteins. 35ul of the extracted protein and Laemmli sample buffer (final volume 20%) was loaded on a 12% acrylamide gel and electrophoresed for 3 hours at 160mV. The gel was stained with Coomassie Blue and membrane proteins visualized.

Conclusion

The V2R receptor has been cloned in a number of species, but not in our research model, the creek chub. Therefore, at this time, we are able to detect the V2R receptor visually, via reported V2R molecular weights in other species. Following extraction of membrane proteins, a band was detected via Coomassie staining at ~ 95kDa (reported molecular weights 94-116 KDa; Sivotti et al., 2005, Chemical Senses 30:1-8). Interestingly, a ~30 KDa band was also detected. This is consistent with the molecular weight of the G-protein with which the V2R interacts (Hansen et al. 2003, Journal of Neuroscience 23(28): 9328-9339). Therefore we have concluded that this receptor is present in the juvenile creek chub. Future studies will examine the presence of the receptor in adults.

Acknowledgments

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