AN INVESTIGATION OF THE MECHANO-SENSORY SYSTEM ON THE BODY OF FOUR MEMBERS OF THE NORTH AMERICAN CYPRINID GENUS *NOTROPIS* (TELEOSTEI: CYPRINIDAE)

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

An Investigation of the Mechano-sensory System on the Body of Three Members of the North American Cyprinid Genus *Notropis* (Teleostei: Cyprinidae)

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Scanning electron microscopy and light microscopy will be used to investigate the distribution of superficial neuromasts on the surface of the body in three species of the North American minnow genus *Notropis.* This will include two species with elevated lateral line scales (*N. buchanani* and *N. volucellus*) and one with non-elevated lateral line scales (*N. sabinae*). Previous research investigations of the mechano-sensory system in fishes have shown that lateral line scales (those bearing the lateral line canal) support higher numbers of superficial neuromasts than non-lateral line canal bearing scales. I hypothesize that the elevated lateral line scales of *N. buchanani* and *N. volucellus* may provide a greater surface area for the mechano-sensory system and these elevated scales will have significantly higher numbers of superficial neuromasts than both adjacent non-lateral line scales and also the non-elevated lateral-line scales *N. sabinae*. This study will represent the first to investigate the mechano-sensory system of the body in multiple members of North American cyprinid genus *Notropis*. The results may have broader implications for understanding the phylogenetic relationships of *Notropis* through the study of a morphological character system that is well known to exhibit phylogenetically informative

patterns. The three species of *Notropis* that I have chosen to study, though potentially unrelated, are ecologically different (including both pelagic and benthic species) and the differences in the abundance and distribution of superficial neuromasts that I expect to document may offer insight into the link between anatomical diversity in the mechano-sensory system and ecology/behavior.

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NOMENCLATURE

LLS	Lateral-line Scales
SEM	Scanning Electron Microscopy

CHAPTER I

INTRODUCTION

The Ghost Shiner, *Notropis buchanani,* is a small, semi-transparent North American minnow that is native to the Mississippi River basin and the Gulf slope drainages of the US, from the Rio Grande to the Calcasieu River (Page and Burr, 2011). The rivers it inhabits are often larger order streams that exhibit high turbidity. Within these systems, *N. buchanani* inhabits warm, slower moving pools where they breed over sand and fine gravel (Edwards, 1997). *Notropis buchanani* is one of only a hand full of North American minnows that exhibit elevated lateral-line canal bearing scales, meaning scales that are taller than they are wide (Page and Burr, 2011). Elevated lateral-line bearing scales (LLS) may enhance the mechanosensory system by providing a greater surface area for superficial neuromasts, which are part of the lateral line mechanosensory system.

The lateral line mechanosensory system of fishes is comprised of a series of neuromast organs, located on the surface of the body or housed inside of canals (Webb, 2013). The sensory canals are organized on the head (cephalic) and body and are open to the external environment via a series of pores. The sensory canals house large neuromasts, referred to as canal neuromasts, which are comprised of multiple hair cells that detect the direction and velocity of water flowing through the canal (Webb, 2013). Superficial neuromasts are typically smaller than canal neuromasts and are located on the surface of the skin. The lateral-line scales of many fishes can exhibit a higher concentration of superficial neuromasts compared to non-lateral line scales.

Though the abundance and distribution of superficial neuromasts on the surface of the body has been relatively well studied in some groups of fishes (e.g., Asaoka et al. 2012), the abundance and distribution of superficial neuromasts in members of the Cyprinidae (carps, minnows and relatives) has been the subject of relatively few studies (e.g., Beckman et al., 2010) and there are currently only a hand full of studies available for the North American members of this group, all of which are focused on the head region only (e.g., Reno, 1966, 1969).

To provide additional information on the mechanosensory system of North American members of the Cyprinidae, I investigated the abundance and distribution of superficial neuromasts on the body of three members of the North American cyprinid genus *Notropis*. Using a combination of light and scanning electron microscopy (SEM), I investigate whether the elevated lateral-line scales of *N. buchanani* and a suspected close relative (*N. volucellus*), exhibit higher numbers of superficial neuromasts than the non-elevated lateral-line scales of *N. sabinae*.

CHAPTER II

MATERIALS AND METHODS

Specimens used in this study were obtained from the Biodiversity Research and Teaching Collections at Texas A&M University (TCWC). Additional specimens used for Scanning Electron Microscopy (SEM) and fluorescent staining of superficial neuromasts were also collected from the Brazos River (Grimes Co., TX) and maintained in 10-gallon aquaria for a short period of time prior to study. Observations and photographs of specimens were made using a ZEISS SteREO Discovery V20 microscope equipped with a ZEISS axiocam MRc5 digital camera. Scale height and number of superficial neuromasts present on the scale surface for each scale along the course of the lateral-line canal was recorded for five individuals of each species. Scale height was obtained from digital images using the software program FIJI (Schindelin 2012). The number of superficial neuromasts on the surface of lateral line scales were recorded using the aforementioned microscope. All images were processed in Adobe Illustrator and Photoshop. Illustrations were prepared from digital images in Adobe Illustrator.

SEM

Specimens were chemically dried following the process outlined by Ellis & Pendleton (2007), mounted on aluminum stubs and coated with gold using a Ted Pella Cressington 108 auto sputter coater. The coated specimens were photographed using a TESCAN Vega 3 environmental Scanning Electron Microscope (SEM). Images were processed in Adobe Illustrator and Photoshop.

Fluorescent Staining of Neuromasts

Superficial neuromasts were stained with 4-Di-2-Asp following the protocol of Nakae et al. (2012). Stained individuals were observed and photographed using a ZEISS SteREO Discovery V20 microscope fitted with a NightSea Stereo Microscope Fluorescence Adapter system. Images were processed in Adobe Illustrator and Photoshop.

Material Examined

The following specimens were obtained from the Biodiversity Research and Teaching Collection at Texas A&M University (TCWC), College Station and used in this study. *Notropis buchanani,* TCWC 17154.09, 5, 26.2- 31.5 mm SL, *Notropis sabinae*, TCWC 16899.2, 5, 32.9-41.2 mm SL, *Notropis volucellus* TCWC 17153.09, 5, 37.5- 44 mm SL.

CHAPTER III

RESULTS

Lateral-line Scales

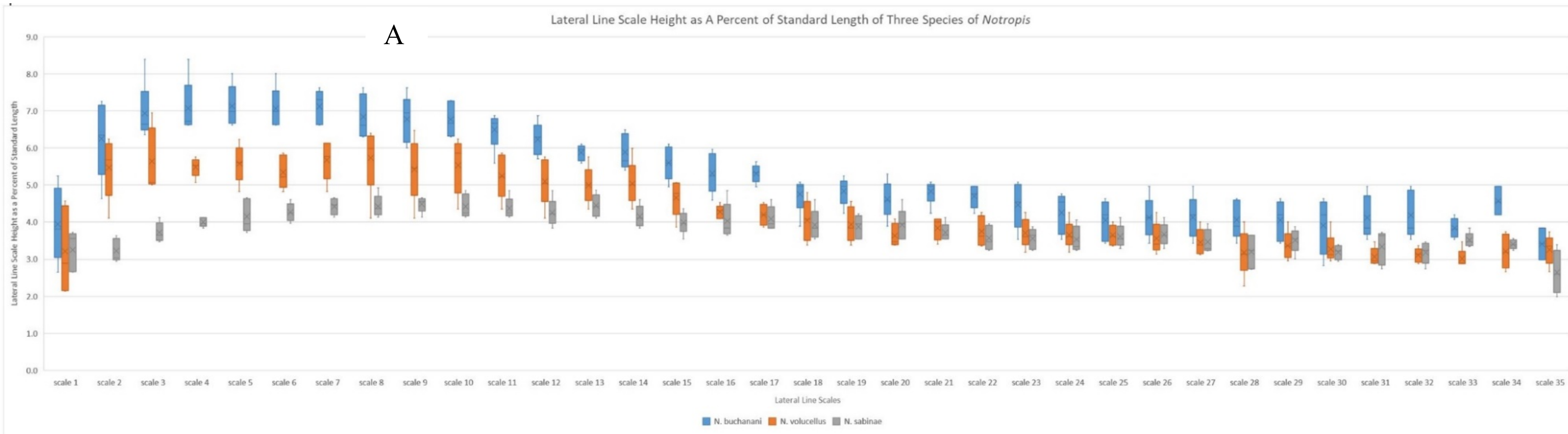
The number of lateral line scales ranged from 32–35 (modally 34) in *Notropis buchanani*, 34–35 (modally 35) in *Notropis* sabinae, and all specimens of *Notropis volucellus* examined exhibited 35 lateral-line scales. The height of the lateral line scales ranged from 8.4– 2.8% SL for *N. buchanani*, 7.0– 2.1% SL for *N. volucellus* and 4.9– 2.0% SL for *N. sabinae* (Fig. 1B).

Superficial Neuromasts on Lateral-line Scales

In *N. buchanani,* superficial neuromasts are organized in a vertical row (referred to as a stitch; Webb, 2013) on the anteriormost portion of each lateral line scale (Fig. 2B). Florescence staining revealed the location of the vertical row of superficial neuromasts to be posterior to or directly in line with the position of the closest canal neuromast, located inside of the lateral-line canal. The number of superficial neuromasts per vertical row ranged from 7–27 per lateral-line scale. (Fig. 1A). Lateral-line scales located on the anterior part of the body (scales 2–13) typically exhibited higher numbers of superficial neuromasts per row than those scales located on the posterior part of the body (scales 11–34/35). The anteriormost lateral-line scale (scale 1) exhibits a shorter vertical row of superficial neuromasts (~10) compared to subsequent scales on the anterior part of the body. Each horizontal row of superficial neuromasts transcends the lateral line canal located at the center of the scale, separating each row into an upper and lower part. The number of superficial neuromasts located dorsal or ventral to the lateral line canal is approximately equal on each lateral line scale, excluding the anteriormost scale, on which superficial neuromasts are only located dorsal to the canal (Fig. 2A). SEM revealed the superficial neuromasts on the surface of lateral-line scales to be proximally diamond shaped, with the long axis of the organ located predominantly along the rostro-caudal body axis (Fig. 2C). Though it was not possible to clearly observe the organization of hair cells within each neuromast (due to preservation), based on the orientation of the long axis of the organ, the axis of sensitively of the hair cells is likely also rostro-caudal. A small number of superficial neuromasts per lateral-line scale were also organized with the long axis along the dorso-ventral body axis, suggesting that the axis of sensitivity of the hair cells would also fall along this axis.

The organization of superficial neuromasts on the lateral-line scales of *N. volucellus* is similar to that reported above for *N. buchanani*, with 7–26 superficial neuromasts per vertical row. The number of superficial neuromasts present on the lateral-line scales of *N. sabinae* is much lower compared to that of *N. buchanani* and *N. volucellus.* In *N. sabinae* the 0 –8 superficial neuromasts per lateral-line scale are not organized into vertical rows but are instead organized as two discrete clusters of 2–3 organs, one above and one below the lateral-line canal, on the anteriormost portion of each scale.

A



B

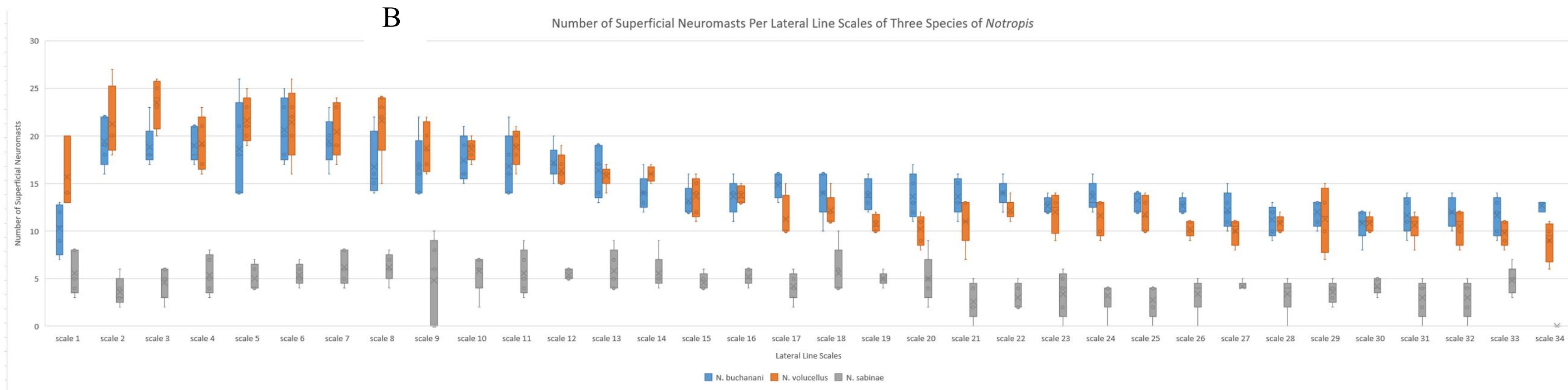


Figure 1. A, Bar graph of lateral line scale height as a percent of standard length for *N. buchanani, N. volucellus* and *N. sabinae*. B, Number of superficial neuromasts per lateral line scale for *N. buchanani, N. volucellus* and *N. sabinae*.

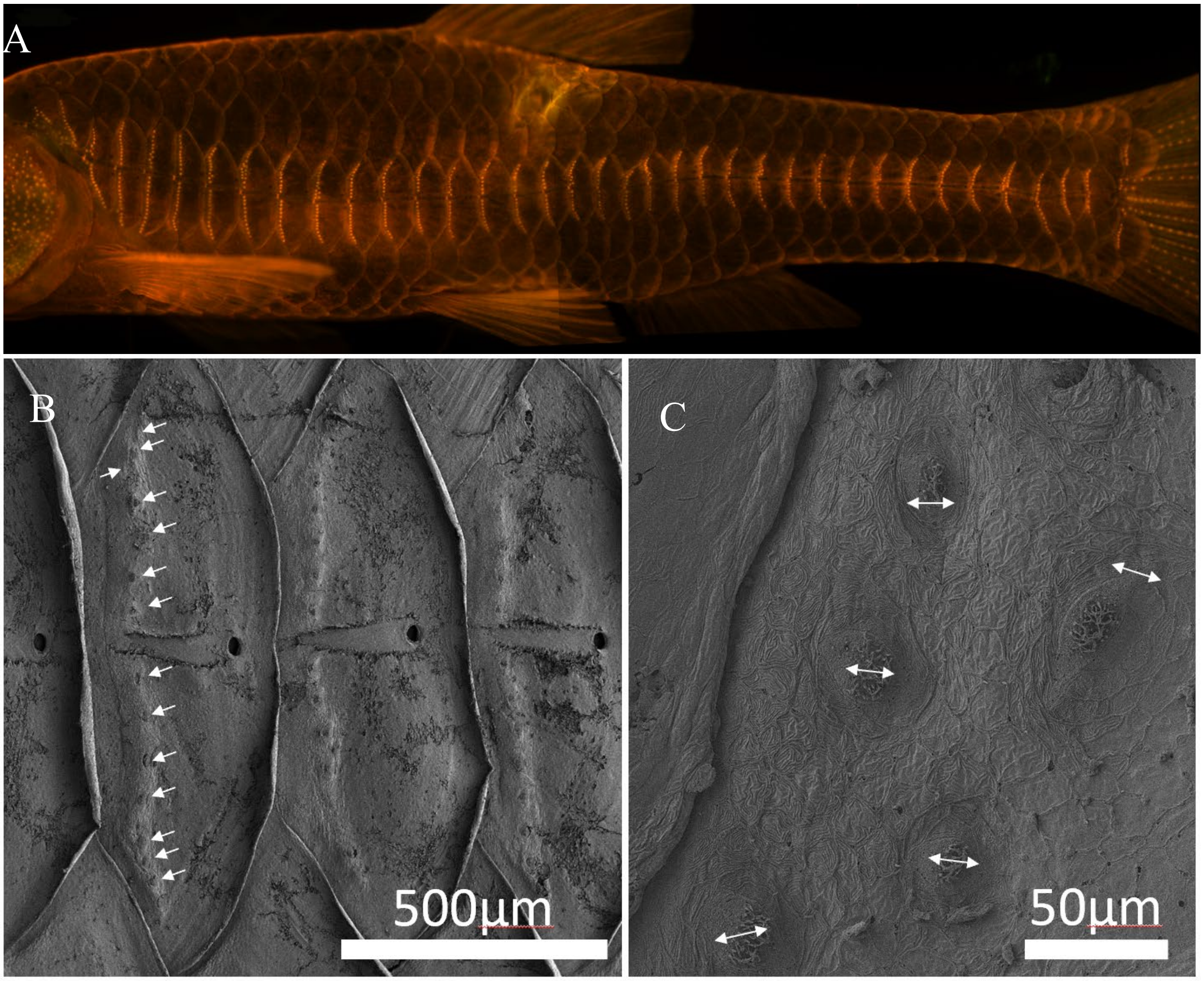


Figure 2 A, *N. buchanani* stained with 4-Di-2-ASP (Nakae et al., 2012). and B, SEM showing the vertical alignment C, *N. volucellus* SEM showing the polarity of each hair cell which are sensitive along the rostro-caudal body axis.

CHAPTER IV

CONCLUSION

The mechano-sensory system of *Notropis* is still relatively understudied. This study provides a detailed account of the lateral-line canal bearing scales and associated superficial neuromasts in *N. buchanani*. Using a combination of SEM and fluorescence microscopy, I have documented that superficial neuromasts are arranged in rows or stitches (sensu Webb, 2014) above and below the canal on all lateral line scales excluding the anteriormost. The number of superficial neuromasts on the elevated lateral line scales of *N. buchanani* and *N. volucellus* is similar and much greater than the number present on the non-elevated lateral line scales of *N. sabinae*. This suggests that elevated lateral-line scales support higher numbers of superficial neuromasts than non-elevated lateral line canal scales. Additional study will be needed to show if this pattern holds when other taxa are examined in a similar manner. This study will provide a basis for future studies on the mechano-sensory systems of North American cyprinids.

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