Lateral Line System of a Juvenile Guppy: *Poecilia reticulata*

Amanda Grassel
Lake Forest College
Lake Forest, Illinois 60045

Introduction

Aquatic environments can involve water that is rapidly flowing and highly turbulent or completely still–often a combination of both. For the animals that live in such environments, successfully navigating through water is key to survival. Most species of fish and other aquatic animals do this through the use of a unique sensory system: the lateral line. This system allows animals to detect water disturbances and pressure fluctuations (hydrodynamic stimuli) that occur at the surface of a body of water, such as an insect landing on it, or throughout it (Bleckmann & Zelick, 2009; Mogdans, 2018). The lateral line system is composed of neuromasts, with superficial and canal variations, that allow fish to receive this hydrodynamic sensory input imperative to their survival (Bleckmann & Zelick, 2009). These neuromasts are the receptive sensory structures located along the head, trunk, and tail fins of fish (Bleckmann & Zelick, 2009; Mogdans, 2018).

Superficial neuromasts are located on the surface of the skin and are primarily responsible for detecting velocity. In contrast, canal neuromasts are found within small canal pores and function as detectors of water acceleration by responding to differences in the pressure gradients between adjacent canal pores (Mogdans, 2018). Previous research has shown that both neuromast types vary across and within species, as well as across an individual organism's lifetime. For instance, eight species of *Forsterygion* display different numbers and groupings of neuromasts with distinct lateral line traits unique to each species, despite being closely related (Nakae et al., 2012).

Additionally, neuromast variations in zebrafish at different ages support the migratory theory of development of the lateral line system in which a group of migratory cells deposits neuromasts along the fish (Wellenreuther et al., 2010). These migratory cells, the primordium, are generated near the ear and migrate around the face and along the body axis (Wellenreuther et al., 2010). As such, adolescent fish have few trunk neuromasts and will develop increasing numbers of them as they reach maturity.

In order to evaluate the lateral line system in fish, *Poecilia reticulata* (guppies) provide unique advantages over many other model species. Guppies display extreme phenotypic and genetic variability, allowing for great insights into intraspecies variability of the lateral line system (Brenden, 2006). It is relatively unknown if the neuromast developmental pattern of guppies is similar to that of most other bony fish. It is hypothesized that juvenile guppies will display neuromast patterning of the head and trunk similar to that of other related juvenile fish.

Methods

This experiment allows for quantification of the dependent variable, the number of neuromasts, on the head and trunk region. The age of the guppy is subject to experimental manipulation, with a juvenile being selected for this experiment. The following experimental procedure was derived from Professor Margot Schwalbe's Spring 2022 "Lab 3--Fish Lateral Line System Student Protocol" for the Neuroscience 301: Neuron to Brain Lab (Schwalbe, 2015).

One juvenile guppy was extracted from a large tank and was placed in a beaker of 100-200 mL of conditioned tap water. The fish was then scooped out with gloved hands, as was done for all transferring, and transferred into 100 mL of 4-di-2-ASP for 5 minutes. Characterization of neuromasts in both live and dead fish has been historically done through macroscopic visualization with and without the use of stains such as Methylene blue (Topic Popovic et al., 2011). While this is often sufficient for large specimens, other techniques must be employed to visualize the neuromasts of juvenile guppies. The cationic mitochondrial dye 4-Di-2-ASP has been extensively used with a fluorescence microscope for neuromast staining of live larval, juvenile, and small-sized specimens (Topic Popovic et al., 2011).

Following the five-minute period, the fish was transferred to a beaker with 100 mL of MS222. The efficacy of this widely used anesthetic for fish is largely dependent on a wide range of environmental factors and biological factors (Brenden, 2006). In order to account for response variability, the anesthesia effect was monitored according to the activity of the fish. In accordance with the literature, the subject was removed from solution once it stopped moving. Additionally, high concentration anesthesia for a few minutes is ideal for the terminal experimental procedure performed here. A petri dish lined with sylgard was filled with a 1:1 ratio of conditioned tap water and MS222. The fish was then transferred to the dish where the total length of the fish was measured. Next, insect pins were used to secure the fish for imaging in lateral, ventral, and dorsal orientations. Care was taken to ensure the body was not impaled and that pins did not obstruct the view of the body.

Neuromast visualization was done using fluorescent microscopy at 6.7X with the CaptaVision+ camera software. The subject was imaged in both natural light and with fluorescence. Following completion of all imaging, the subject was disposed of. Neuromasts were quantified by counting the visually apparent fluorescing neuromasts.

Results

The use of fluorescent microscopy revealed the specific locations of the neuromasts on the head and trunk regions of a juvenile guppy. There were distinct collections of neuromasts on the head region, notably around the eyes and operculum. Most neuromasts of the head appeared relatively large and oval-shaped, while those along the trunk and infraorbital region were small and circular.

Fluorescent imaging of the lateral aspects of four species of fish revealed that the head-region neuromast patterning on juvenile bony fish was quite similar across species (Figure 1). All four fish displayed distinct collections of neuromasts around the eye and operculum with only slight variations in the relative sizes and numbers of neuromasts. The juvenile *Poecilia* most closely resembles juvenile *Oryzias* in neuromast patterning of the head region. Interestingly, the trunk neuromasts appear to follow a somewhat similar pattern of development, but with more variation in location. *Poecilia* displayed three distinct rows of trunk neuromasts: superior, midline, and inferior. More variation was seen with the other species as the trunk neuromasts are not organized in straight rows along the longitudinal body plane.

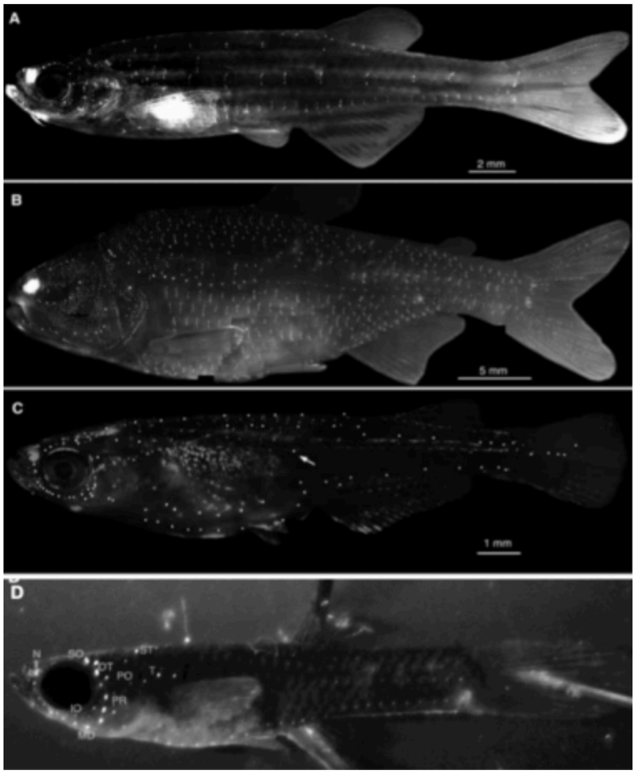

Figure 1. Fluorescent imaging of the lateral aspect of the juvenile guppy. A-C were sourced from Sapède et al. (2002) and display fluorescent imaging of juvenile *Danio* (A), *Astyanax* (B), and *Oryzias* (C) fish. The distribution of neuromasts in Poecilia (D) were classified and labeled according to anatomical location on the lateral aspect. The head region consisted of the: N, naris; OT, otic; PR, preopercular; PO, postoptic; SO, supraorbital; MD, mandibular; IO, infraorbital; and ST, supratemporal. The trunk region consisted of the: T, trunk.

Fluorescent imaging of the dorsal aspect of the juvenile guppy revealed clear neuromast collections primarily along the supraorbital border of the eye and the supratemporal region (Figure 2). Very few trunk neuromasts were visible. Fluorescent imaging of the ventral aspect of the juvenile guppy revealed two distinct rows of trunk neuromasts on either side of the ventral and anal fins (Figure 3). The neuromasts of the head were primarily along the infraorbital border of the eye and the mandibular region.

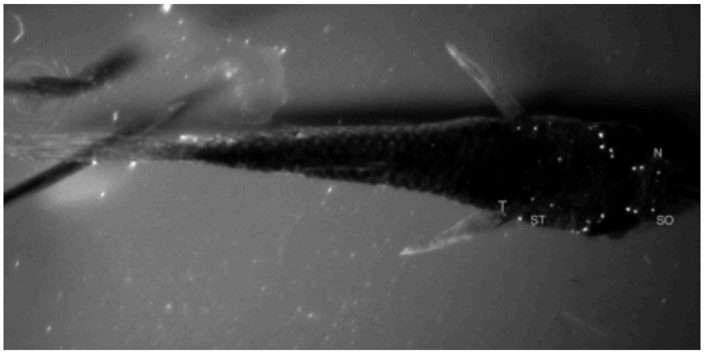

Figure 2. Fluorescent imaging of the dorsal aspect of the juvenile guppy. The distribution of neuromasts were classified and labeled according to anatomical location on the dorsal aspect. The head region consisted of the: N, naris; SO, supraorbital; and ST, supratemporal. The trunk region consisted of the: T, trunk.

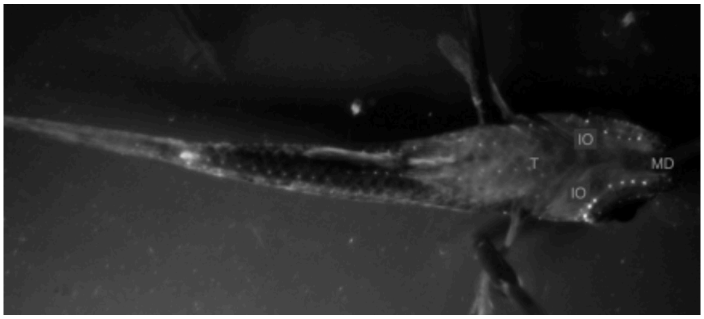

Figure 3. Fluorescent imaging of the ventral aspect of the juvenile guppy. The distribution of neuromasts were classified and labeled according to anatomical location on the lateral aspect. The head region consisted of the: IO, infraorbital and MD, mandibular. The trunk region consisted of the: T, trunk.

Quantification of the neuromasts revealed that, in the lateral and dorsal positioning, the subject had more neuromasts on the head than on the trunk (Figure 4). Interestingly, when in the ventral position, there was an equal number of neuromasts on both the head and trunk region. In the lateral position, 46 neuromasts were counted on the head and 38 on the trunk. In the dorsal position, 25 were on the head and 9 on the trunk. And in the ventral position, 36 were on each the head and trunk. The greatest number of visible neuromasts were those present around the eyes, mandible, and ventral trunk.

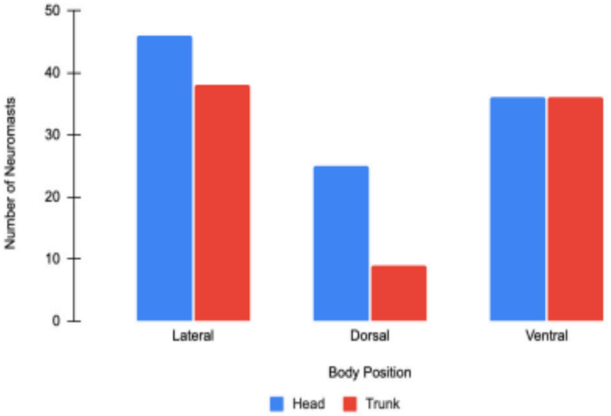

Figure 4. Distribution of neuromasts in the juvenile guppy. Superficial and canal neuromasts were counted on the head and trunk regions from fluorescent imaging of lateral, dorsal, and ventral body positions.

Discussion

The present study assessed the developmental progression of the lateral line system in a juvenile *Poecilia reticulata* by quantifying the head and trunk neuromasts as well as through cross-species comparisons. These findings suggest that juvenile guppies are developmentally similar to at least three other species in respect to their lateral line system, as all were found to have similar neuromasts distributions, especially on the head. Additionally, these preliminary findings provide support for the migratory theory of neuromast development as there were more head than trunk neuromasts in the lateral and dorsal orientations.

This study has contributed to the literature by providing support for the current theory of lateral line development in another species. Understanding the development of the lateral line system in a variety of species is key to its applications for human use. One such application is the development of an artificial lateral line for enhanced underwater vehicles and robots (Gui Jie et al., 2014). Additionally, this research contributes to a much-desired understanding of why different species develop morphologically different lateral lines (Modgans and Bleckmann, 2012). Further research is needed in order to better understand this fundamental system so that it can be applied for future endeavors.

References

Bleckmann, H., and Zelick, R. (2009). Lateral Line System of Fish. *Integrative Zoology*. 4(1), 13-25. doi:10.1111/j.1749-4877.2008.00131.

Breden, F. (2006). Guppies. *Current Biology*, 16(20), R865-R866.

Gui Jie, L., Yao Gong, H., Yan, R., Fang Gao, W. (2014). Bionic Research and its Application of Artificial Lateral Line System. *Applied Mechanics and Materials*. 602-605, 1503-1507.

Mogdans, J. (2018). Sensory Ecology of the Fish Lateral-Line System: Morphological and Physiological Adaptations for the Perception of Hydrodynamic Stimuli. *J Fish Biology*. 95, 53–72.

Nakae, M., Asaoka, M., Wada, H., and Sasaki, K. (2012). Fluorescent Dye Staining of Neuromasts in Live Fishes: An Aid to Systematic Studies. *Ichthyological Research*. 59, 286–290.

Sapède, D., Gompel, N., Dambly-Chaudière, C., and Ghysen, A. (2002) Cell migration in the Postembryonic Development of the Fish Lateral Line. *Development*. 129(3), 605–615.

Schwalbe, M. (2015). Lab 3 – Fish Lateral Line System Student Protocol, Spring 2022. *Lake Forest College.*

Topic Popovic, N., Strunjak-Perovic, I., Coz-Rakovac, R., Barisic, J., Jadan, M., Persin Berakovic, A., and Sauerborn Klobucar, R. (2011). Tricaine Methan-Sulfonate (MS-222) Application in Fish Anaesthesia. *Journal of Applied Ichthyology.*

Wellenreuther, Maren, Brock, M., Montgomery, J., and Clements, K. D. (2010). Comparative Morphology of the Mechanosensory Lateral Line System in a Clade of New Zealand Triplefin Fishes. *Brain, Behavior and Evolution*. 75(4), 292-308.