# The Lateral Line System of *Pristella maxillaris*

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## Introduction

The lateral line system, present in aquatic amphibians and over 30,000 fish species, is a mechanosensory system—a sensory structure that responds to mechanical changes such as water movement and pressure fluctuations—and is crucial for behaviors such as prey and predator detection, navigation, and intraspecific (within-species) interactions (Bleckmann & Zelick, 2019; Faucher et al., 2010; Webb, 2011). The lateral line system consists of neuromast receptors—clusters of sensory hair cells—that detect water flow along the head, trunk, and tail (Webb, 2011).

Neuromasts are sensory organs found within the skin's epithelial layer. They contain two main cell types: sensory hair cells, which detect movement, and nonsensory support cells (Webb, 2011). In bony fish such as *Pristella maxillaris*, there are two classes of neuromasts: canal neuromasts, which are located in fluid-filled canals and detect changes in water acceleration, and superficial neuromasts, which are found on the skin surface and detect water velocity (Webb, 2011). The combined input from these neuromasts helps fish sense their environment and guide their behavior.

*Pristella maxillaris*, known as the X-ray fish due to their translucent body, is a shoaling, or grouping, fish inhabiting the Amazon, Orinoco, and Guianas Rivers (Bian et al., 2019). Due to morphological diversification and mutations, the coloration of *Pristella maxillaris* varies from wild-type black-gray to mutant I silver-white or mutant II transparent (Bian et al., 2019). Furthermore, genetic modification has produced fluorescent varieties known as Glo Pristellas. While research exists on their growth, development, and coloration, limited studies have examined the lateral line system of *Pristella maxillaris*, particularly the fluorescent Glo Pristella.

The experiment examined Glo Pristellas, a genetically modified *Pristella maxillaris* that expresses fluorescent proteins. The standard lateral line examination protocol involves fluorescent staining and imaging, which may pose unique challenges with the fluorescent variant. Furthermore, variation in the lateral line system across species, based on habitat and behavior, suggests potential differences in Glo Pristellas compared to their unmodified counterparts.The experiment uses fluorescence staining techniques to observe the lateral line system of the Glo Pristellas, aiming to identify the external anatomy and the lateral line system along the head and body. Due to their shoaling behavior, it is hypothesized that Glo Pristellas will exhibit a high density of neuromasts on their heads, as observed through fluorescence staining.

## Methods

The following procedure was followed based on the "Lab 5 – Fish Lateral Line System Student Protocol" provided by Dr. Schwalbe and *Lateral Line Structure* by JF Webb (Schwalbe, 2024; Webb, 2011).

### *Apparatus preparation*

Before obtaining the fish, the apparatus was prepared. Prior to the lab, the digital camera was mounted on a dissecting microscope, which was connected to a monitor via an HDMI cable and to a laptop via a USB cable to produce the visual output. Therefore, the laptop was turned on, and the CaptaVision+ software was opened. Once the camera was on and connected, the microscope's field of view was visible on the monitor. After testing the camera, the apparatus was prepared for the experiment.

### *Preparing the fish*

First, the fish was selected from Dr. Schwalbe's Lab, Lillard 176. For this experiment, a *Pristella maxillaris*, commonly known as Glo Pristellas, was selected. Using a net, the fish was carefully removed from the fish tank and transferred to a 200 mL beaker of conditioned tap water.

Once back in the lab room, the fish was transferred, using a net, into a 100 mL beaker containing 4-di-2-ASP for 5 minutes. This solution was orange and served as the fluorescence stain that was imaged later in the experiment. After 5 minutes of staining, the fish was transferred, using gloved hands to prevent staining the net, to a beaker containing 100 mL of MS222. This solution was a muscle relaxant that humanely euthanized the fish; therefore, it was important to take precautions and prevent skin contact. After five minutes and verification by Dr. Schwalbe that the fish was no longer moving or moving its gills, the fish was transferred, using gloved hands, to a sylgard-lined petri dish prepared with 1:1 conditioned tap water and MS222.

Using a ruler placed beneath the petri dish, the total length, from the mouth to the edge of the caudal fin, was measured and recorded in Excel. The fish was then pinned in the middle of the petri dish using insect pins. While the pin placement varied across views, the first view imaged was the lateral view. Therefore, the fish was pinned with its mouth facing left and its left side facing upward. When pinning, it was important to take precautions to avoid piercing the body and disrupting neuromasts. The fins tore easily; therefore, pins were placed on the edge of the body and at an angle to prevent obstructing the camera view.

### *Imaging the fish*

Three views of the fish were imaged: lateral, dorsal, and ventral. After the fish was secured to the petri dish with insect pins, the petri dish was placed under the microscope. For the lateral view, the fish was pinned with its mouth facing left and its left side facing upward, as previously described, with the pins placed along the edge of the body. For the dorsal and ventral views, the pins were placed in a "V" formation to create a supportive structure that prevented the fish from moving while avoiding pinning through the fish.

Picture information was recorded in Excel for record-keeping and easy retrieval. Images were captured in both bright and fluorescent light for all views, as outlined below.

First, a fiber-optic light or a bright light was used. The microscope was focused, and the fish was observed by moving it around underneath the microscope. The external anatomy was identified, using the bright light to examine the eyes, gills, scales, fin rays, and tail. Images were taken to record the anatomy.

Second, the fluorescent light was used. To prepare the microscope, the provided yellow filter was placed underneath the microscope objective lens. With the Glo Pristellas used in the experiment, it was challenging to observe the fluorescent neuromasts due to their color. Therefore, a bright light was kept on in the background to help with visualization. The exposure was set to 3 seconds. The microscope was focused, and the fish was observed by moving it around underneath the microscope. Important lateral line structures in each view included head canals, trunk canals, and clusters of superficial neuromasts. Images were taken to record the anatomy and lateral line structures. When finished with fluorescence imaging, immediately turn off the light, do not look directly into it, and remove the yellow filter.

The fish was re-pinned for each new view, and imaging was repeated with both bright and fluorescent light. Images were saved to the computer, then used for data analysis. Picture information was recorded in Excel for proper record keeping and easy image retrieval.

### *Clean-up*

Once all images were obtained and properly organized, the bright field and fluorescence lights were turned off. For disposal of the fish, it was unpinned and placed in the provided Ziplock bag for proper disposal by Dr. Schwalbe. The 4-di-2-ASP and MS222

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solutions were disposed of in the provided containers, not in the sink. Lastly, the station was wiped completely with 70% ethanol, including personal devices, to prevent transfer of MS222 out of the lab.

*Data analysis*

For this experiment, no quantitative data were collected. As a result, data analysis involved manually stitching images of the fish together for full-length views. This was done in PowerPoint, where multiple images were uploaded and properly aligned to create a cohesive image of the fish rather than individual pieces. While CaptaVision+ offers a stitching feature, some views remained difficult to obtain with it.

## Results

*Results summary*

Fluorescent staining and imaging were performed for the lateral line system, including the canal and superficial neuromasts, of the Glo Pristellas variant of the *Pristella maxillaris*. The distribution of the lateral line system along the body, from rostral to caudal, was examined in lateral, dorsal, and ventral views. When examining the distribution of the lateral line system, as exhibited in Figures 2, 3, and 4, a high density of canal neuromasts was observed in the naris, infraorbital, postotic, and mandibular regions surrounding the eyes, nares, and mandible.

Neuromasts were less prominent in the ventral view of the fish than in the lateral and dorsal views. In the trunk, neuromasts were less dense, but superficial neuromasts were visible along the lateral view. Neuromast visibility was lower in the tail and fins. Based on fluorescent staining and imaging, the lateral line system of *Pristella maxillaris* is widely distributed throughout the body, with a high density towards the rostral, dorsal end.

*Tables and figures*

| Lateral Line Distribution, Neuromast Abbreviations | Lateral Line Distribution, Neuromast Abbreviations |
|---|---|
| **Infraorbital** | IO |
| **Mandibular** | MD |
| **Naris** | N |
| **Otic** | OT |
| **Preopercular** | PR |
| **Postotic** | PO |
| **Supraorbital** | SO |
| **Supratemporal** | ST |
| **Trunk** | T |

**Table 1. Lateral line distribution and neuromast abbreviations.** The table above lists the neuromasts labeled in the following pictures of the *Pristella maxillaris,* along with the abbreviations used to denote them. In summary, the definitions for the abbreviations are as follows: IO, infraorbital; MD, mandibular; N, naris; OT, otic; PR, preopercular; PO, postotic; SO, supraorbital; ST, supratemporal; and T, trunk.

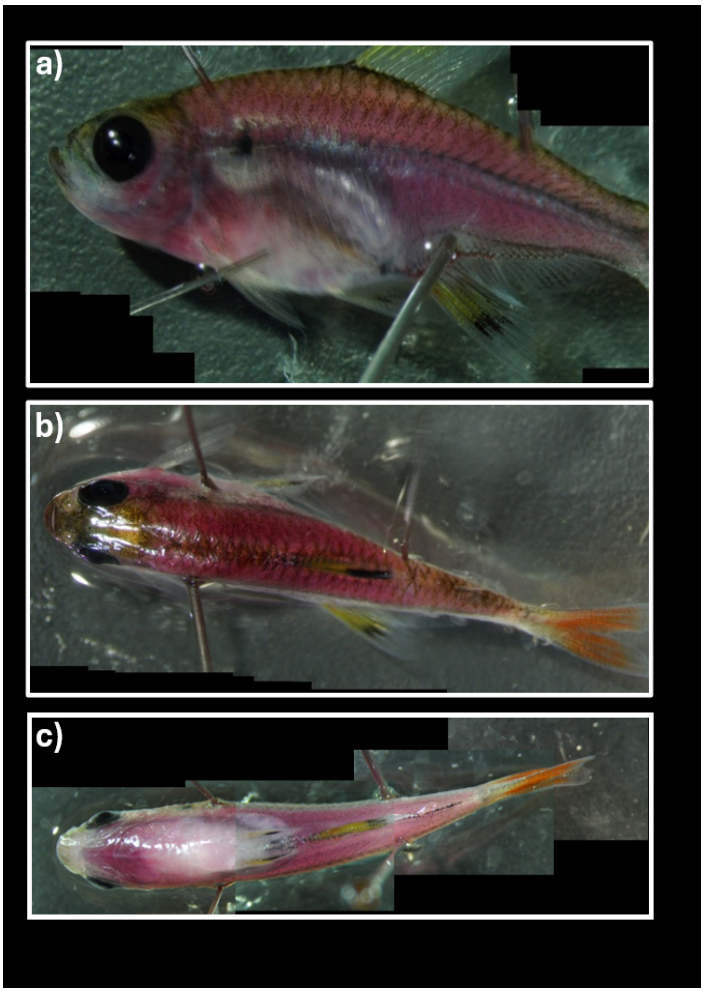


**Figure 1.** Bright light view of *Pristella maxillaris*. The figure above shows the bright-light lateral, dorsal, and ventral views of *Pristella maxillaris* (Glo Pristellas) examined in the experiment. The fish was placed in a petri dish of 1:1 conditioned tap water and MS222 and pinned using insect pins. The lateral line system was not visible in the bright field. Figure 1a) is the lateral view of *Pristella maxillaris*. The image was stitched using CaptaVision+ and a microscope at 6.3X magnification. Figure 1b) is the dorsal view of *Pristella maxillaris*. The image was stitched using CaptaVision+ and a microscope at 6.3X magnification. Figure 1c) is the ventral view of *Pristella maxillaris*. Stitching was performed manually on PowerPoint.

## Discussion

This experiment hypothesized that Glo Pristellas would exhibit a high density of neuromasts on their head, potentially helpful with their shoaling behavior. Using fluorescence staining, the experiment visualized the lateral line system and confirmed a significant concentration of canal neuromasts in the naris, infraorbital, postotic, and mandibular regions, as shown in Figures 3 and 4, supporting the hypothesis.

Glo Pristellas are a social shoaling fish that potentially rely heavily on their lateral line system. This aligns with research by Faucher et al., which examined the importance of the lateral line system for accurate shoaling behavior in *Hemigrammus bleheri (firehead tetras)*. The experiment concluded that fish deprived of the lateral line system cannot maintain a shoal, causing them to move further apart rather than closer, indicating the importance of the lateral line system in shoaling behavior (Faucher et al., 2010). The observed neuromast density in Glo Pristellas may support their social interactions.

Given the limited research on the lateral line systems of *Pristella*

*maxillaris*, this experiment offers valuable insights into new fish species. Specifically, it introduces experiments regarding the relationship between the color variations, such as translucence and fluorescence, and lateral line function. In particular, it would be interesting to conduct further research investigating how fluorescence affects lateral line distribution and shoaling behavior under varying light conditions in Glo Pristellas.

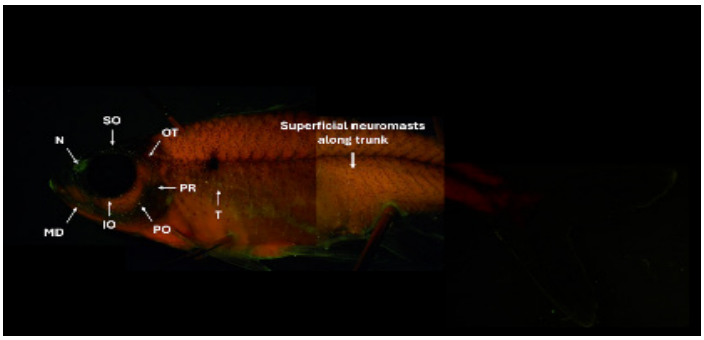


**Figure 2.** Lateral distribution of the lateral line system in *Pristella maxillaris.* The image above is the fluorescent light lateral view of the *Pristella maxillaris* (Glo Pristellas) examined in the experiment. The fish was placed in a petri dish of 1:1 conditioned tap water and MS222 and pinned using insect pins. The image was captured using CaptaVision+ and a microscope at 6.3X magnification. Stitching was performed manually on PowerPoint. The lateral line system was visible throughout the body in the fluorescence view, as labeled in the image above. Not observed in the lateral view were ST, supratemporal, which are expected across the top of the head behind the eyes.

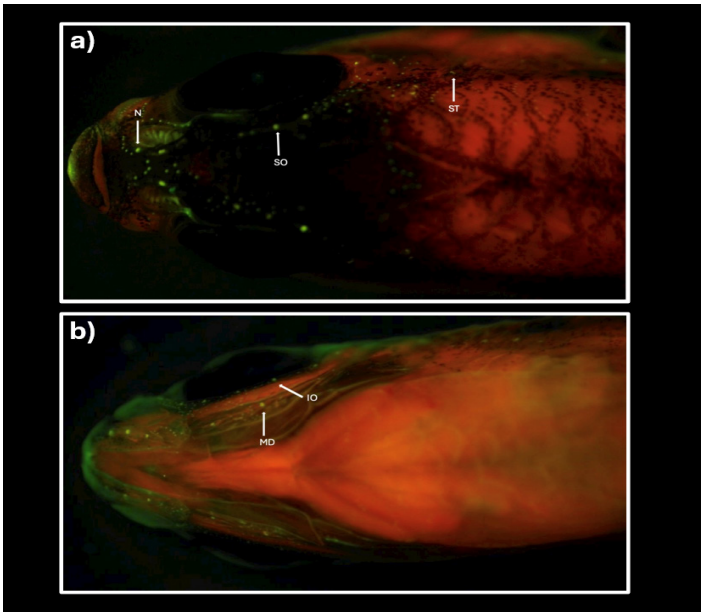


**Figure 3.** Distribution of the lateral line system in *Pristella maxillaris*. The figure above is the fluorescent light dorsal and ventral views of the *Pristella maxillaris* (Glo Pristellas) examined in the experiment. The fish was placed in a petri dish of 1:1 conditioned tap water and MS222 and pinned using insect pins. Figure 3a) is the dorsal view of *Pristella maxillaris*. The image was captured using CaptaVision+ and a microscope at 10X magnification. Several neuromasts were visible on the dorsal view of the head. Figure 3b) is the ventral view of *Pristella maxillaris*. The image was captured using CaptaVision+ and a microscope at 8X magnification. In the ventral view, the lateral line system was not as visible compared to lateral and dorsal views, though several neuromasts were still visible towards the rostral end of the fish, such as the mandibular and infraorbital neuromasts.

In addition, this experiment has broader scientific implications. An experiment on *Danio rerio (zebrafish)* lateral line systems conducted by Young et al. revealed that stormwater containing toxicants damages neuromasts, leading to fewer neuromasts with fewer hair cells (Young et al., 2018). This indicates the potential of the lateral line system as an environmental health indicator. Additionally, Holmgren et al. used zebrafish lateral line hair cells, which are structurally and functionally analogous to cochlear hair cells, to examine damage caused by noise, aging, and ototoxic drugs (Holmgren & Sheets, 2021). This underscores the value of the lateral line system as a model for developing therapies for human hearing loss.

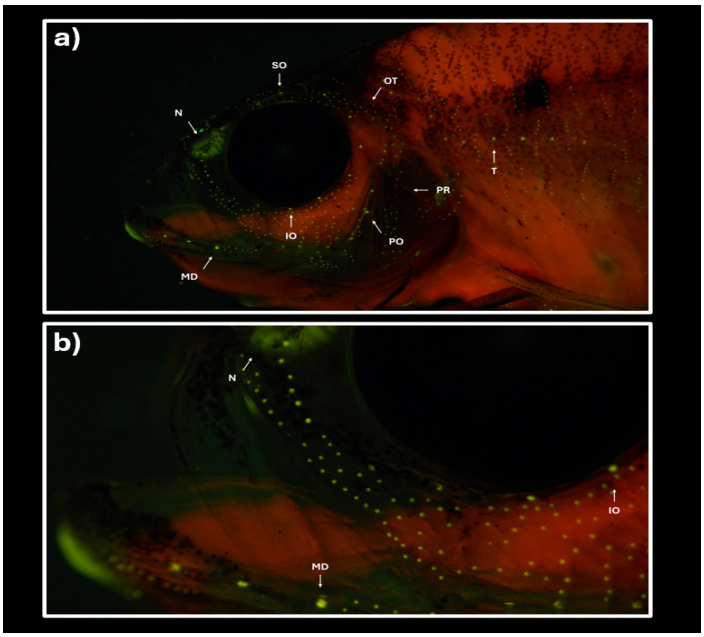


**Figure 4.** Close-up of the lateral head distribution of the lateral line system in *Pristella maxillaris*. The image above is the fluorescent light lateral view of the *Pristella maxillaris* (Glo Pristellas) examined in the experiment. The fish was placed in a petri dish of 1:1 conditioned tap water and MS222 and pinned using insect pins. The image captured CaptaVision+ and a microscope. Figure 7a) is a close-up of the head of *Pristella maxillaris*. The image was captured using CaptaVision+ and a microscope at 8X magnification. Figure 7b) is a further examination of the infraorbital region. The image was captured using CaptaVision+ and a microscope at 20X magnification. Not observed in the lateral view were ST, supratemporal, which are expected across the top of the head behind the eyes.

Therefore, this experiment validates the hypothesis regarding neuromast distribution in the heads of Glo Pristellas and provides a foundation for future studies on variations in the lateral line system and their broader applications in conservation and human health.

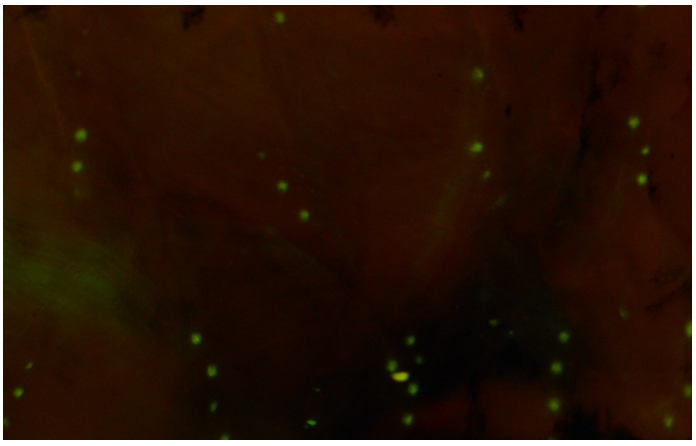

**Figure 5.** Close-up of the lateral trunk distribution of the lateral line system, specifically the superficial neuromasts, in *Pristella maxillaris.* The image above is the fluorescent light lateral view of the *Pristella maxillaris* (Glo Pristellas) examined in the experiment. The fish was placed in a petri dish of 1:1 conditioned tap water and MS222 and pinned using insect pins. The image captured CaptaVision+ and a microscope at 40X magnification.

**References:**

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