Analysis of *Polypterus* fish fins and their control

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Introduction

Fish can move their fins in many directions in very subtle and intricate patterns. Each fin ray has a set of independent muscles associated with it that allow it to stiffen and bend in 3 dimensions. *Polypterus senegalus* is an amphibious fish that has recently shown that in terrestrial environments, it can use two distinct fin-assisted gaits to walk on land. Unlike most terrestrial vertebrates it can use both the medial and lateral side of its fin to support its weight (Standen et al 2014). However the mechanism that it uses to control the fin during this unique flipping is unknown. Through Dr. Standen's past research, it has been observed that the pectoral fins of *Polypterus* have unique and not previously described 'hooks' at the base of their fin rays, the function of which is unknown. Through histochemical methods, this project proposes to describe the way these unique 'hooks' are attached to the fins of *Polypterus senegalus*, and to predict how they help with movement. It is hypothesized that these hooks are attached to muscles, that they help support and control the fin during walking, and that they hooks are present only on the paired fins of *Polypterus senegalus* and not on other species of aquatic fishes. This project is the first description of *Polypterus* pectoral fin ray 'hooks' and so contributes to the evolutionary and comparative biomechanic understanding of how fins can be used to walk in a terrestrial environment.

Methodology

Standard histological methods were used, to clear and stain the tissues of a diverse group of five different species of fish (Song and Parenti 1995). The unique fin ray anatomy of *Polypterus* was described and compared with a variety of ray-finned fishes to assess their anatomical novelty. Light microscopy and dissection scope were used to properly draw anatomical features. Descriptions were used to develop hypotheses of the biomechanical function of the unique hooks found at the base of the *Polypterus* rays.

Table 1 - Timeline of the staining and clearing procedure.

Step		Species' name: *Zebrafish*	*Trout*	*Goldfish*	*Polypterus*
Fixation (10% formalin)		5 d	6 d	6 d	10 d
Wash (distilled H_20)		4 d	4 d	4 d	4 d
Cartilage staining (Alcian blue dye)		3 d	3 d	3 d	3 d
Rehydration (ethanol)	95% (1)	2.5 h	2.5 h	2.5 h	3 h
	95% (2)	8 h	8 h	8 h	2 d
	75%	10 h	10 h	10 h	4 h
	50%	8 h	8 h	8 h	18 h
	30%	24 h	24 h	24 h	8 h
Muscle digestion (trypsin)		21 d	31 d	21 d	16 d
Wash out enzyme (0.5% KOH)		4 h	3 h	4 h	3 h
Bone staining (Alizarin red solution)		24 h	24 h	24 h	24 h
Destaining and bleaching (0.5% KOH and bleach solution)		0.5 h	1 h	0.5 h	1 h
Dehydration (ethanol)	30%	0.75 h	8 h	0.75 h	8 h
	50%	6.5 h	18 h	6.5 h	18 h
	70%	8 d	21 h	8 d	21 h
Storage (glycerin and 0.5% KOH)	30% and 70% respectively	2 d	1 d	2 d	1 d
	70% and 30% respectively	2 d	1 d	2 d	1 d

Results

As was hypothesized, hooks were observed attached to the base of the fin rays of the *Polypterus* species, while they were not seen in any of the other species that were observed (refer to the images to the right). They were seen located superimposed over the first segment of each ray. Figure 4.2 (taken by Trina Du) shows the ideal positions of hooks, in a grown fish. In the samples observed in this project, however, they appear to be displaced, perhaps due to aggression between individuals in the tank, due to the fact that these fish are younger and so do not yet have completely developed structures, or due to displacement during the clearing process.

It is possible that these hooks at the base of *Polypterus* fin rays bear the tension that is applied to these fins when the fish push them against the ground while walking. Because of the presence of these ossified hooks, the base of the fin rays are thicker and thus might be able to contribute more mechanical support when weight is loaded onto the fin during walking. It is also possible that the importance of the role of the hooks during walking correlates positively with the age of the fish, since they are more pronounced in larger fish; further research and analysis must be done to prove this hypothesis.

Figure 1 - Goldfish right pectoral fin

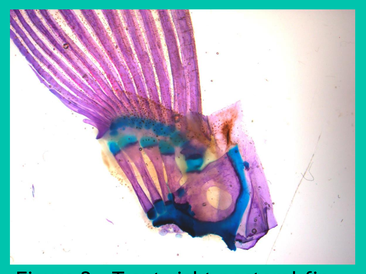

Figure 2 - Trout right pectoral fin

Figure 3 - Zebrafish right pectoral fin

Figure 4.1 - Young *Polypterus* right pectoral fin

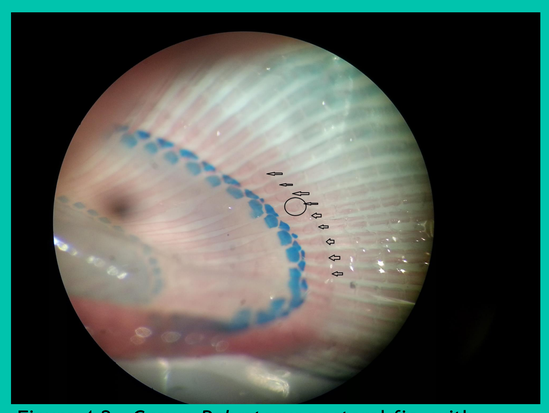

Figure 4.2 - Grown *Polypterus* pectoral fin, with labeled hooks

Conclusion

In conclusion, hooks were observed at the base of *Polypterus* fin rays, and none were seen on any of the other fish fin rays, as was expected. These ossified hooks contribute to the walking ability of the *Polypterus* by providing more strength at the base of the fin rays, allowing them to be more stable when force is applied to them during walking. How the hooks are attached to the base of the rays and when they develop in the fish's life cycle is unknown; further research looking at a wider range of fin ages will help clarify these pieces of information.

First and foremost, I would like to thank God for giving me the opportunity to participate in this research. I would also like to thank UROP for providing me with the funding and resources to complete my research. Lastly, special thanks to Dr. Emily Standen for taking time out of her very busy schedule to teach me about the biomechanics of *Polypterus* fins!

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