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Comparison of Muscle Development in *Moenkhausia Sanctaefilomenae* and *Danio Rerio*

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Abstract

Previous research has shown that larval redeye tetra *Moenkhausia sanctaefilomenae* have large craniofacial skeletons compared to equivalent zebrafish *Danio rerio*. In order to understand the further development of the craniofacial region, the development of the craniofacial musculature in both species were examined at different stages using whole mount immunohistochemistry. In addition, Alcian blue staining was used to observe their craniofacial cartilage to better understand the anatomy and identify the individual muscles. Unexpectedly, the muscle development was found to be more robust and intense in three day old *D. rerio* compared to equivalent *M. sanctaefilomenae* specimens. Differences were also observed in regard to the temporal and spatial patterns of muscle formation between the two species. Owing to their larger craniofacial skeletons, it was expected that *M. sanctaefilomenae* would likewise exhibit larger muscle corresponding with their large skeleton development. However, it was seen that the muscle development does not seem to coincide with the skeletal development.

Introduction

D. rerio is a well-studied organism whose development is well known in the scientific community. To better understand anatomical and developmental differences between groups of fishes, the development of the craniofacial features of *D. rerio* were compared to those of the redeye tetra *M. sanctaefilomenae.* Figure 1 shows the skeletal structures of *D. rerio* and *M. sanctaefilomenae* at different developmental stages. It is readily observable that *M. sanctaefilomenae* has a larger skeletal frame than that of *D. rerio.* Based upon these observations, it stands to reason that *M. sanctaefilomenae* would produce craniofacial muscle that is large enough to support their large skeletal frame. Therefore, this experiment was designed compare muscle development between *D. rerio* and *M. sanctaefilomenae.*

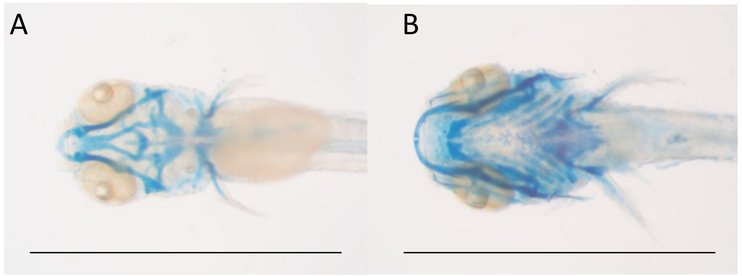
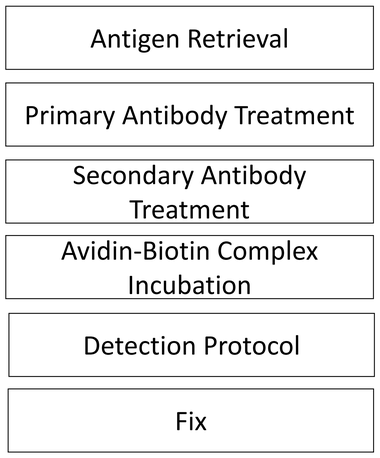


Figure 1: Skeletal staining in both *D. rerio* (A) and *M. sanctaefilomenae* (B) at 120 hours. These two specimens have been stained and oriented into ventral view to observe their cartilage (in blue) and their bone (in purple). The scale bar is 2mm in length. Comparing these two, it appears that *D. rerio* has a smaller craniofacial region than *M. sanctaefilomenae.*

Methods and Materials

To observe the developing muscle, immunohistochemistry was implemented (Figure 2), followed by the ABC method for detection (Figure 3). The antibody used (MF20) is directed against myosin heavy chain, and it is known to be reactive among a wide range of vertebrates. The specimens were pooled to ensure consistency between groups during the staining protocol. If there were any differences between the two specimens it would be due to the fact that there are anatomical differences between them, and not because of the staining procedure.



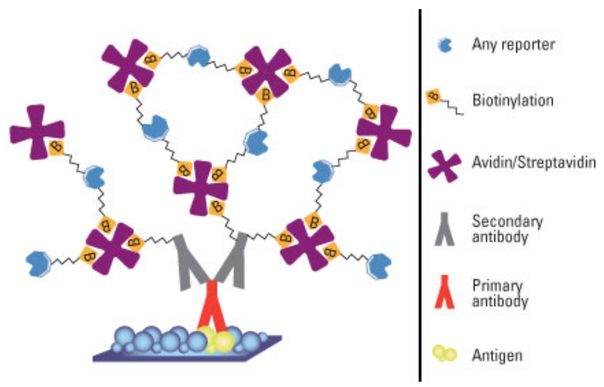


Figure 3: ABC Method. Figure from Thermo Fischer.

Figure 2: Immunohistochemistry protocol. Primary antibody, MF 20, was deposited to the DSHB by Fischman, D.A. (DSHB Hybridoma Product MF 20) and secondary antibody was supplied by Vector Labs.

Results and Conclusion

Negative controls, in which the primary antibody was excluded, revealed no staining pattern (Data not shown).
Overall, the muscles in *M. sanctaefilomenae* were broader in appearance indicating that *M. sanctaefilomenae* requires thick bands of muscle for their larger craniofacial skeletal frame. However, there was a notable difference in the intensity of stain indicating that there is less muscle development in *M. sanctaefilomenae. D. rerio* has more mature muscle development than the comparable *M. sanctaefilomenae*. This is an interesting observation because *M. sanctaefilomenae* has a larger craniofacial skeletal frame than *D. rerio,* it is expected to have the corresponding muscle to support the skeleton however; the muscle appears to not yet be fully developed. It is unknown why the muscle in *M. sanctaefilomenae* is less reactive to the primary antibody and not fully developed at the 72 and 120-hour development stage. In the future, other experiments will be designed to answer the question of when muscle starts to develop in *M. sanctaefilomenae,* and why it forms at such a late developmental stage in comparison to *D. rerio*. Later stages will be examined to further detail the progression of muscle development.

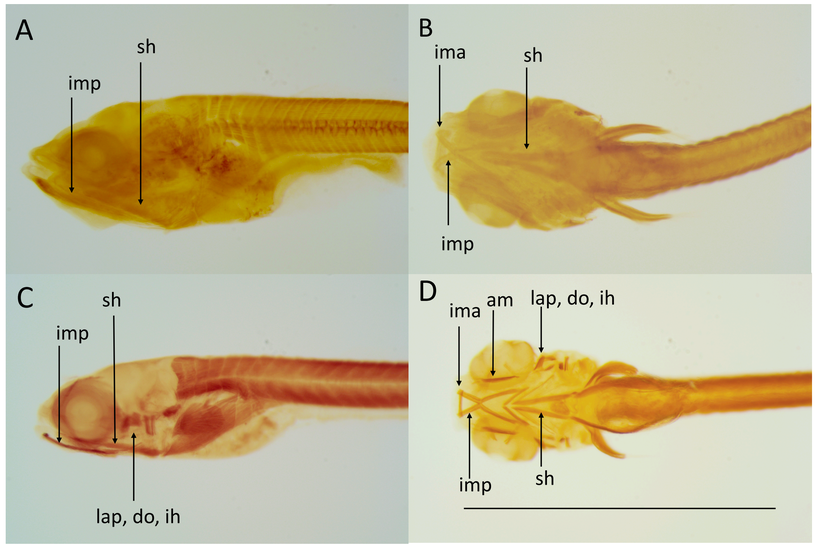


Figure 4: Immunohistochemistry staining in *D. rerio* and *M. sanctaefilomenae* at 120 hours. The scale present is 2mm in length. Names and functions of the muscles seen are found in the table below. Image (A) *M. sanctaefilomenae* oriented in a lateral view. Muscles imp and ih are visible, but not stained as intensely as seen in image (C). Despite the low intensity staining, the muscle appears to be broader and longer in *M. sanctaefilomenae* compared to *D. rerio* in image (C). Image (B) *M. sanctaefilomenae* is oriented in a ventral view. Muscles ima, imp and sh are visible, but are not heavily stained. While the muscle intensity is lacking, the size of the muscle is again longer and broader than the muscle in *D. rerio* in Image (D). *D. rerio* in Image (D) has heavily stained muscles. Muscles appear to be narrower and shorter compared to the muscles seen in *M. sanctaefilomenae.* The am, lap, do, ih muscles are visible in *D. rerio* and not visible in *M. sanctaefilomenae.* Muscle names based on Schilling and Kimmel (1997) and Stiassny (2000).

Muscle Function

Abbreviation	Name	Function
lap	Lavator arcus palatini	Abduction of suspensoria
do	Dilatator operculi	Abduction of the opercle
ih	Interhyoideus	Elevation/protraction of the hyoid bar
ima	Intermandibularis anterior	Stabilizes the mandible
imp	Intermandibularis posterior	Elevation/protraction of the hyoid bar
sh	Sternohyoideus	Depression of the hyoid; abduction of the suspensoria
am	Adductor Mandibulae	Adducts the mandible

Citations

Schilling, Thomas F, and Charles B Kimmel. "Musculoskeletal Patterning In the Pharyngeal Segments of The Zebrafish Embryo." *Development*, no. 124, 1997, pp. 2945–2960.

Stiassny, M.L.J. 2000. Gross Functional Anatomy: Muscular System. Chapter 7. In: G. Bullock and T.E. Bunton (eds). *The Handbook of Experimental Animals; Laboratory Fish*. Academic Press, London. pp. 119-128.

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