Towards the development of a testable model for spinal deformities using zebrafish

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Abstract—A wide range of species, from chickens to pigs have been used as model systems for studying spinal deformities. However most of these models focus on the etiology of the problem, and few studies examine the effects of spinal deformities on locomotion. The goal of this study was to create a 3D geometrical description of the zebrafish musculoskeletal system using Micro CT imaging technology as a basis for comparing normal zebrafish and zebrafish with spinal deformities. Specifically, for use in developing a model as a basis for testing the functional consequences of spinal deformities. Large 3D deformities and structural differences were observed in the vertebral column of the scoliotic fish relative to the normal fish. Also, further analysis of the structural differences revealed that the facets of the vertebrae in the deformed spinal sections were fused with rays extending from both the dorsal and anal fins of the zebrafish with spinal deformities, which could translate to changes in the normal function, and could affect the survivability of affected individuals.

Keywords—zebrafish; spinal deformities; model

I. INTRODUCTION

Numerous studies using animal models to investigate spinal deformities in humans have been reported [1]. However, much of this research has focused on studying the etiology, and possible corrective procedures of various spinal deformities. There is little information on how these species are hindered or adapt to their surroundings when such spinal deformities occur.

One of the species used to study spinal deformities, but only briefly explored beyond the point of causation, is fish [2]. Few studies exist describing spinal deformities in fish and how it impacts locomotion. It has been found that fish provide a great model to study the organism-environment interface and the response to natural or anthropogenic stressors [3].

The goal of this study was to compare the 3D musculoskeletal structure of the zebrafish with spinal deformities to a normal zebrafish sans deformities in order to develop a model for spinal deformities. Through researching the species in terms of form and function, insight on the viability of individuals with a spinal deformity could be achieved.

II. METHODS

Through continuation of a previous study, a line of zebrafish with spinal deformities was obtained through crossing a normal zebrafish line with a potential founder fish with abnormal spinal curvatures [4]. One of the founder fish with spinal deformities were selected and imaged using a Micro CT (Ziess, CA). A normal zebrafish (free of spinal deformities) was also selected and scanned using Micro CT. Both fish were euthanized and mounted on the manufacturer provided plate. The normal zebrafish was used as a basis of comparison in order to identify changes in the vertebral column and associated structures in zebrafish with spinal deformities.

The power and voltage used for the CT images was set at 40 kV and 10 Watts. An exposure of 7 seconds was used with 2401 total projects taken of each sample. Using XM Reconstructor software (San Jose, CA) the CT images were constructed and processed. The structure of both zebrafish types were analyzed in XM3D Viewer (San Jose, CA). The image was then transferred to Mimics Software (Plymouth, MI) for further analysis. Straight lines, parallel with the vertebrae were drawn through the center of each vertebra. The angle between intersecting lines of adjacent vertebrae was measured to analyze spinal curvatures.

III. RESULTS

A viable model system, contains a control as a basis of comparison. For this study the control was a normal zebrafish with no apparent spinal abnormalities. Since, there are no formally designated subdivisions of the spine in zebrafish, unlike for humans, the zebrafish spine was split into three regions: i) anterior, which starts from the head and goes to the first bend in the spine; ii) middle which is from the first bend in the spine to the second bend; iii) and posterior which goes from the second bend in the spine to the tail. From analyzing the CT image it was found that the normal zebrafish has a fairly straight spine for the first 7 vertebrae from the head with a 2.3 degree incline from horizontal (Fig. 1a). Following the 7th vertebrae the spine starts to descend posteriorly at an angle of 10 degrees relative to the anterior 7 vertebrae. At the 14th vertebrae, the spine starts to straighten out further and descends at an angle of 5 degrees relative to the first 7 vertebrae. The length of the vertebrae were fairly uniform, averaging 0.71 mm with a standard deviation of 0.06 mm. The measured curvatures were similar to previously found results documented in the Zebrafish Atlas [5]. It was also observed that there is no direct cartilaginous attachment of the dorsal and anal fin to the spine. The normal zebrafish spine can be seen in Figure 1.
The spinal curvatures of the zebrafish with spinal deformities are shown in Figure 2. At the 8th vertebrae from the head the spine starts curving 42 degrees ventrally. Six vertebrae later the spine turns 92 degrees posteriorly for four vertebrae. Afterwards, the spine turns 120 degrees dorsally followed by a 28 degree vertebrae decline in the posterior direction. The degree of curvature is not strictly two dimensional, as the spine curves in a spiral fashion as well. The lateral curvature can be seen in Figure 2c. The length of the vertebrae varied slightly more with a standard deviation of 0.1 mm and an average vertebrae length of 0.62 mm.

A very interesting discovery was the fusion of the dorsal and anal fin’s cartilaginous rays directly with the dorsal facets of the vertebrae, unlike the normal zebrafish. In the CT of the normal zebrafish spine, it is clear that the vertebrae and the two fins are not linked.

There are also abnormalities in the facets on the dorsal side of the vertebrae. Starting with the 5th vertebrae from the head, the dorsal facet starts to curve towards the head of the fish. As they appear to fuse together from vertebrae 9-12. Following the 12th vertebrae the dorsal facet of the vertebrae curve towards the tail of the fish for 3 vertebrae while the ventral facets curve...
towards the head. This is unlike the normal zebrafish where the dorsal facets do not curve but exhibit a slight tilt towards the tail of the fish.

IV. DISCUSSION

One of the more interesting differences noticed in the deformed zebrafish was the fusion between rays emanating from both the dorsal and anal fins in the fish with spinal facets in the region of the spinal deformities. These fusions could lead to an impairment in their normal function and therefore affect their mobility. Fins are instrumental in providing dynamic stability during locomotion, which requires fish to have full control of their shape and area [6]. However, the fusion of the fins with the spine could inhibit control, resulting in either the dynamic instability or limited functional mobility. These fish could struggle to maintain stability when subjected to perturbations from the current in their environment, resulting in an inability to evade predators or capture prey in the natural environment.

The unnatural curvature of the spine, ultimately changes the complete structure of the fish, resulting in an altered center of mass and upsetting the natural buoyancy of the fish [7]. The abnormal spinal sections consist of curves that run ventrally then dorsally, followed by a section with an abnormal decline toward the fish’s tail, changing the center of mass and, consequently, the center of buoyancy from the normal zebrafish. These changes could cause the fish to float differently and consequently hinder the locomotion capability of the fish. Overall, the abnormal structures seen in the deformed zebrafish can translate to changes in normal locomotion. With severe curvatures in the spine and fused fins, the zebrafish’s natural buoyancy, stability, and speed of locomotion could be affected. Although there is only a sample size of one, this is a morphological study that highlights how a zebrafish animal model can be used to study the effects of spinal deformities. With future research in this area additional knowledge can be gained about the relationship between the degree of deformity and function.

References


