

Development of behavioural assays related to physiological function in

Danio rerio

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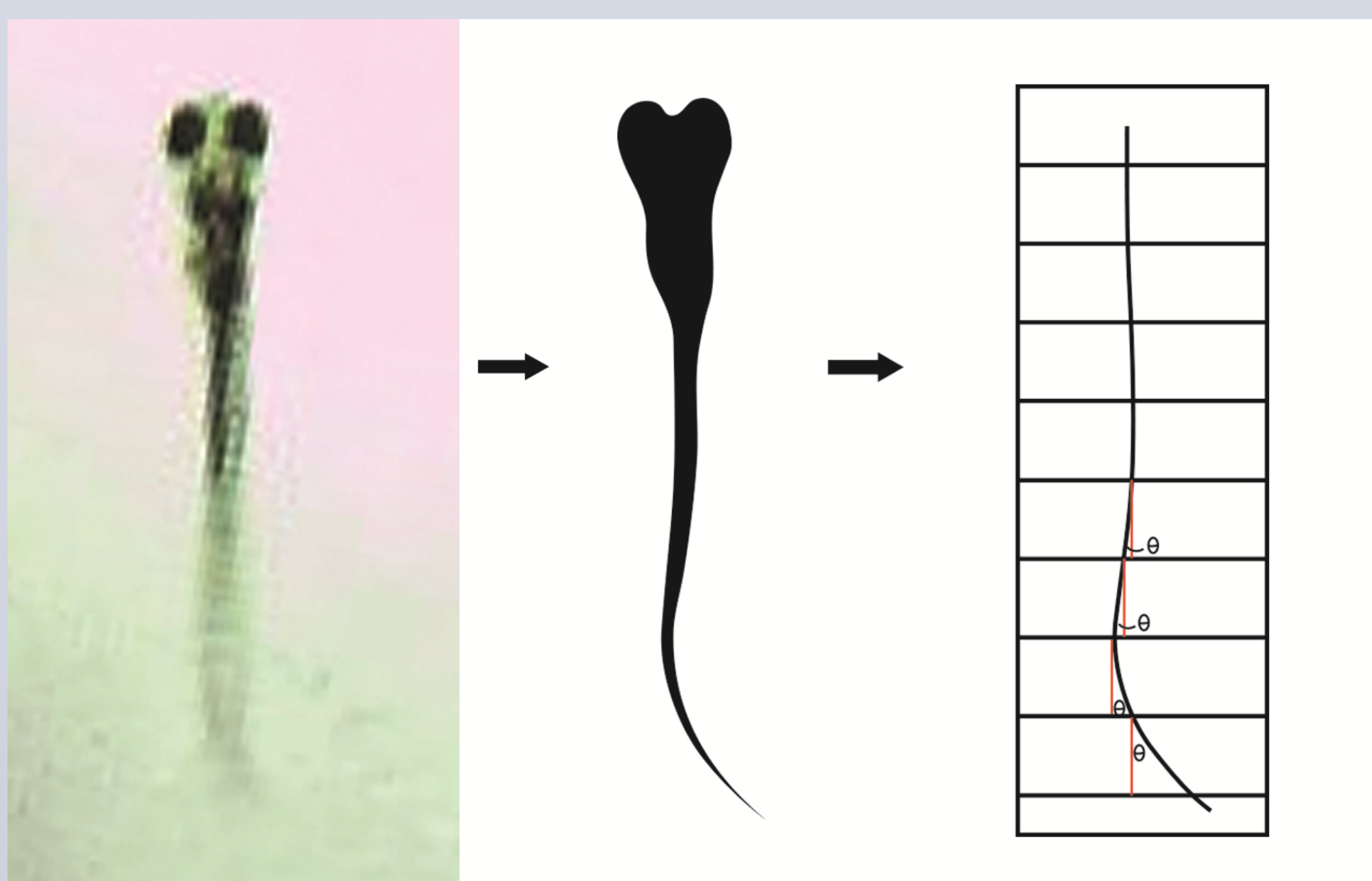
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Introduction

In this study, we implemented computer algorithms to analyze high speed footage of swimming larval zebrafish in order to extract pertinent features of the zebrafish's innate motor operations in an automated fashion. These features include measures of swimming activity such as distance travelled, speed, as well as kinematic measures such as the angles of body curvature during swimming stroke and frequency of body curvature. With the development of automated analysis of these movement-related metrics, we will be able to discern behavioural differences between wild-type zebrafish with transgenic zebrafish models of neurobiological diseases in future studies.

Methodology

After a 24 hour separation period, wild-type strains of *Danio rerio* were allowed to breed. Fertilized eggs were collected 1 hour after the onset of the light cycle, where they were subsequently bleached and stored in embryo medium buffered at pH 6.6-6.8, at a temperature of 25-28°C. Four days post fertilization, larval zebrafish activity footage is captured by a high-speed camera attached to a stereomicroscope, capturing images at 30fps. Locomotive function was assessed using ImageJ. Video footages are then converted into images frame-by-frame, where ImageJ is then used to assess speed and distance traveled. To determine body curvature angles, images are processed to give monochromatic profiles of larval zebrafish. Midlines of these profiles are then determined, where body angles are then calculated at various intervals:



Results

On average, we were able to discriminate between two distinct methods in which larval zebrafish move. Slow, crawling-like motions were characterized by tail locomotion initiated at the trunk of its body, with body curvature angles ranging from 6° to 37° from the midline.

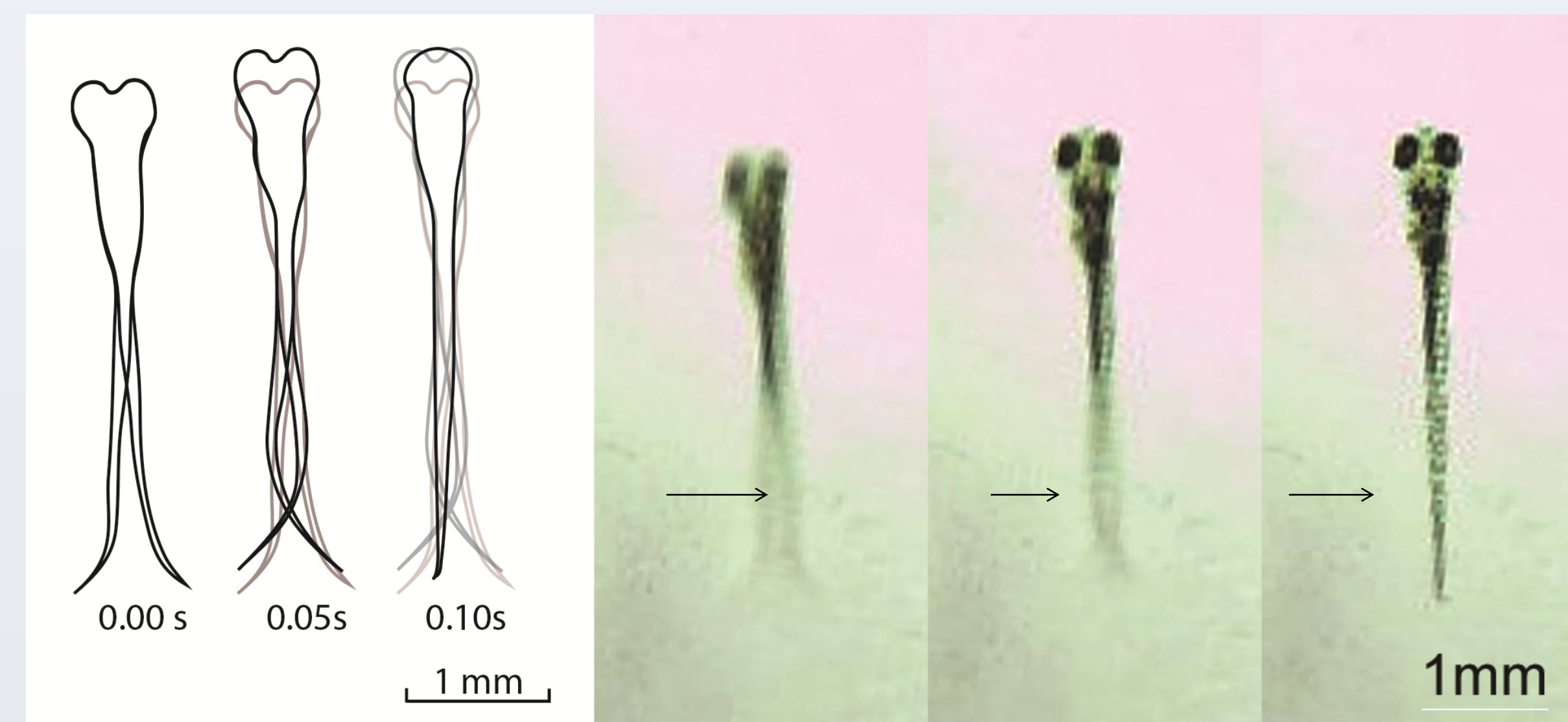


Figure 1: Vector images of linear larval Zebrafish movement at 0.05s intervals (left) extracted from captured video footage (right).

Frequency of body curvature for one full period (defined as one full tail shift from the mid-line to one side and then the other, back to the midline) can occur between 0.05-0.10s to generate average speeds of 1.80mm/s, with max speeds of 5.53mm/s (see Figure 2 for localized speeds of swimming paths).

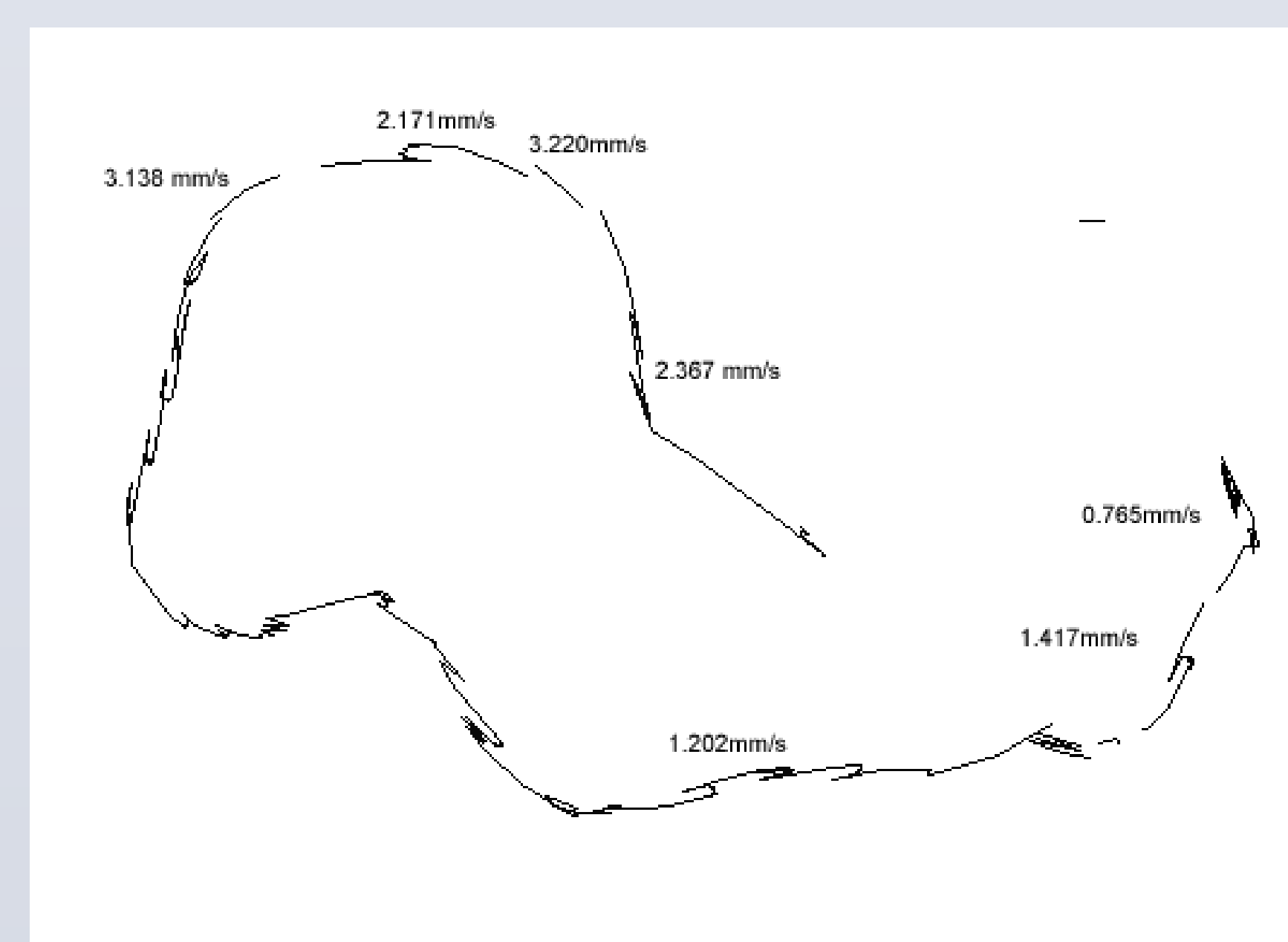


Figure 2: Recorded path (16mm) of movement by Image J, with average speed indicated adjacent to path.

We were also able to observe instances of rapid swimming behaviour. However, to record these movements with sufficient resolution, a camera with faster capture frame rates would be required. We were able to evoke a similar rapid swimming motion by fixing larval zebrafish in 15% agarose, and stimulating an escape response.

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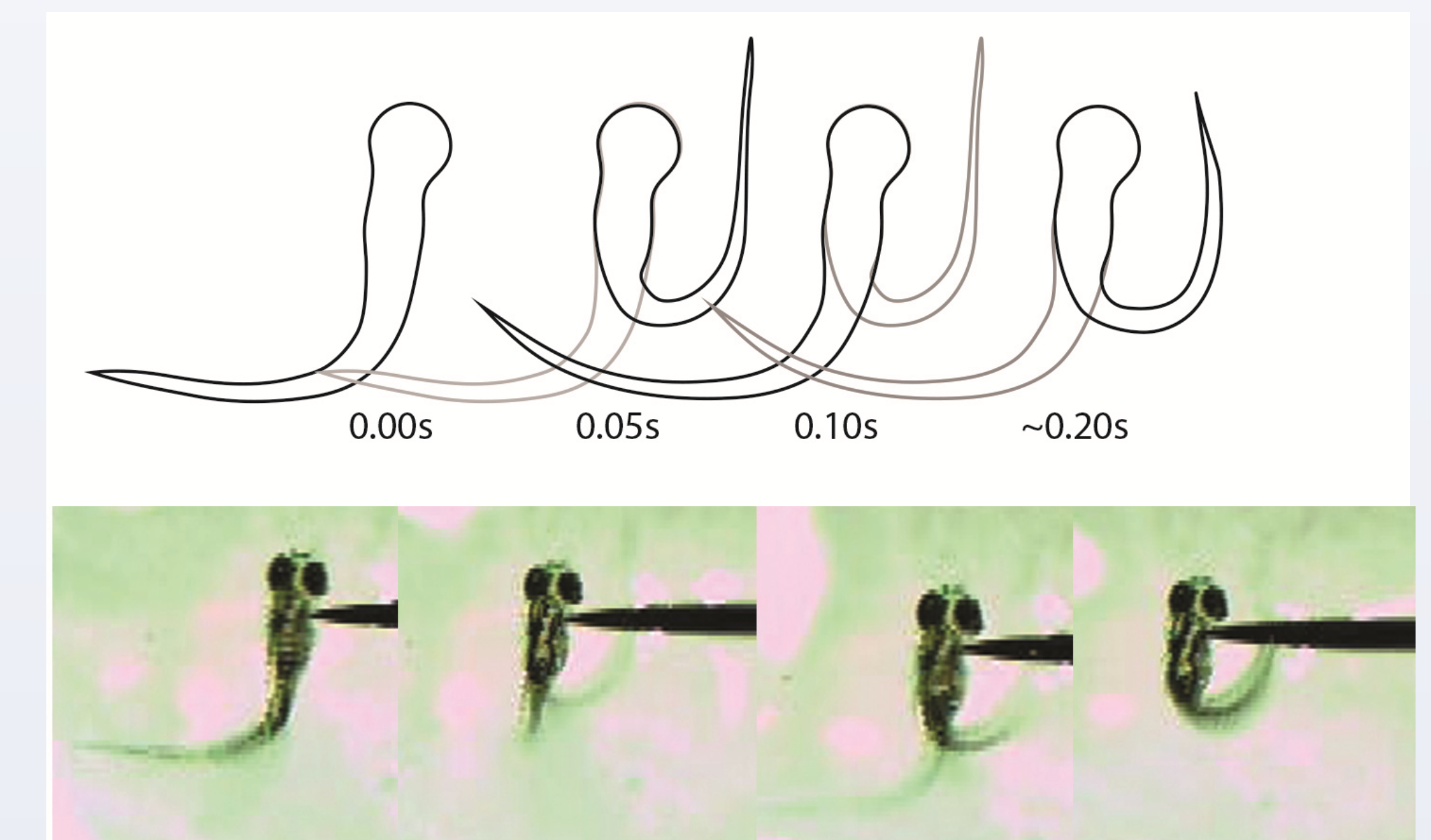


Figure 3: Vector images (top) of simulated escape response in larval zebrafish embedded in 15% agarose. Images were extracted from video footage frames at 0.05s intervals (bottom).

Here, larval zebrafish were treated with dilute Tricaine buffered to pH 6.5-7.5, and were embedded in 15% agarose gel, where its tail was allowed to move freely. Average body curvature angles were approximately 50°, ranging from 6° to 88° from the midline. It was also noted that the fastest tail cycle period observed was around 0.01-0.05s. Cameras with a higher resolution and capture frame rate would be required in order to quantify the cycle period more accurately.

Conclusion

In summary, key aspects that were noted in zebrafish locomotive functions were the scale of body-tail movement as a function of their speed. Faster speeds entailed larger body curvature angles and full tail movement, while slower speeds and shorter distances travelled were observed to be a result of localized and minimalized tail curvatures. Immobilization of larval zebrafish to stimulate an escape response allowed us to measure larger body curvature values, in addition to the higher frequency in which they are observed.

Acknowledgments

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