

Revisiting Morgan: Spatial Control of Fin Growth in *Danio rerio* (zebrafish)

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Objective/Background:

Over a century ago, Thomas Hunt Morgan (1902) studied the physiology of the regeneration of teleost caudal (tail) fins. Through his experimentation, Morgan observed that:

- cuts that are more proximal to the base have a faster growth rates
- fin tissue regenerates faster when neighbouring tissue extends distally from the cut, not when it is either the same level or proximal from the cut

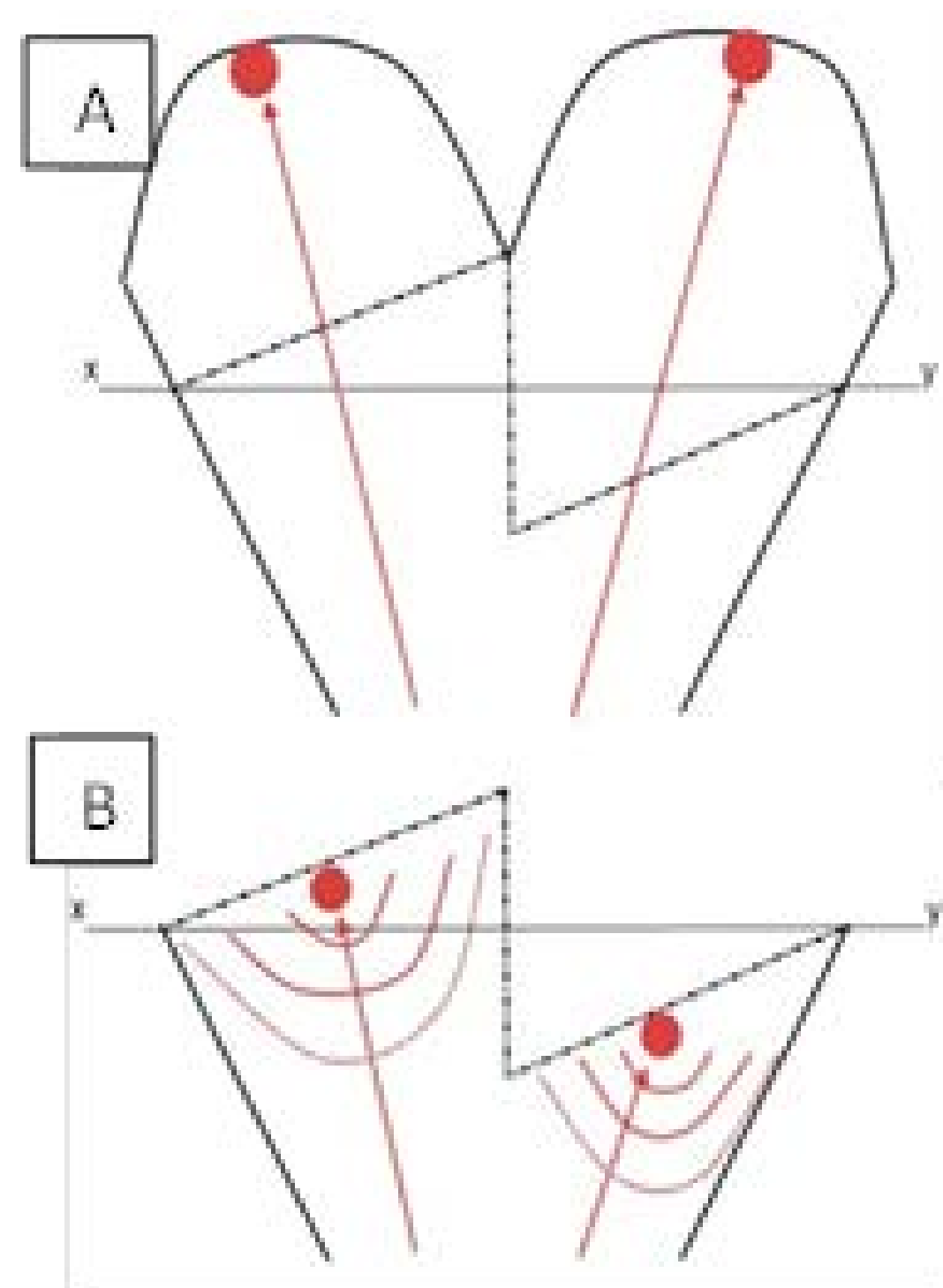


Figure 1. Our hypothesis on the movement of growth morphogen in the caudal fin. (A) Shows the complete fin and (B) shows the cut invented by TH Morgan that was used in the regeneration experiment.

Morgan saw faster growth at point “x” compared to point “y” (Fig 1). A conceptual model explaining Morgan’s results in terms of morphogens involved in fin outgrowth is shown in the figure above. In this model, there are two zones in the distal parts of the fin lobes from which growth factors are produced. These growth factors are assumed to move in a polar fashion towards proximal parts of the fin.

Our objective is to recreate Morgan’s experiment to test the hypothesis.

Method:

A total of 20 wild type zebrafish, 5 fish in each batch for each type of amputation. Four fish in each batch were suitable and survived the entire process.

- Batch 1 was used to test the growth rate of the central rays.
- Batch 2 was the original Morgan amputation.
- Batches 3 and 4 are fish that were used as controls to examine regular growth rate.

Amputations were performed under anesthesia, as outlined in Dr. Marie-Andrée Akimenko’s protocol. Following amputation the fins were imaged repeatedly to obtain time-lapse data. The pictures were taken three, four, and five days post amputation and then every three days until day twenty.

The images were analysed using custom software designed in the lab. First, I measured the fin size including length, width, and area. Next, I digitized the rays of the fins. To keep consistent for measuring growth rate, I selected a segment junction for each ray that was clearly visible in each image. The end result was a series of digitized fins from which I could obtain information on ray lengths over time. Each ray was standardized against its length just after amputation in order to obtain comparable linear growth rates.

Results/Discussion:

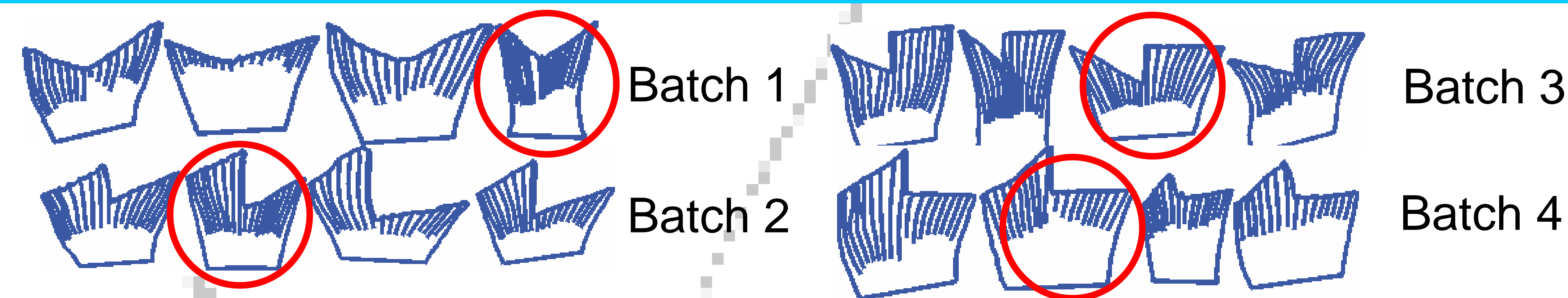
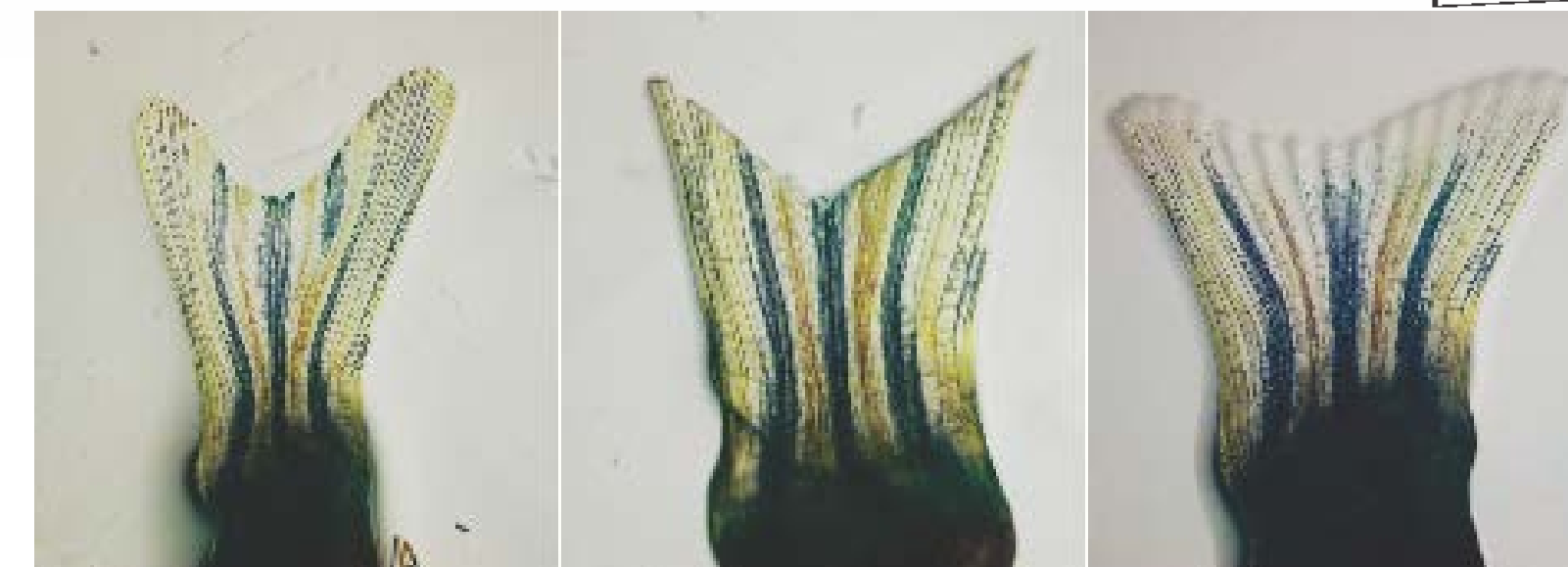


Figure 2. All of the cuts that were analyzed, separated into batches. All 16 successful regenerated fish are shown. From each batch, a model fish was selected (circled) that (1) had a successful amputation of the desired shape and (2) showed typical results of the group.

Batch 1 Fish 4

We expected to see a large amount of growth in the centre rays. We see the fins obtaining their pre-amputation shape quite early in the process, which is true across all batches. Three days post-amputation the growth rates of the centre rays are faster than that of the lateral rays. After this early period, the fin has regained its bilobe shape and the growth rates slow down.



Batch 2 Fish 2

In Batch 2, the “Morgan” cut, we see one day where the results match Morgan’s original observation. On Day 5, the dorsal lateral ray (with neighbouring tissue above) has a faster growth rate than the ventral lateral ray (with neighbouring tissue below). However, this only occurs again on Day 8 and the growth rates are not mirrored in the standardized ray lengths.



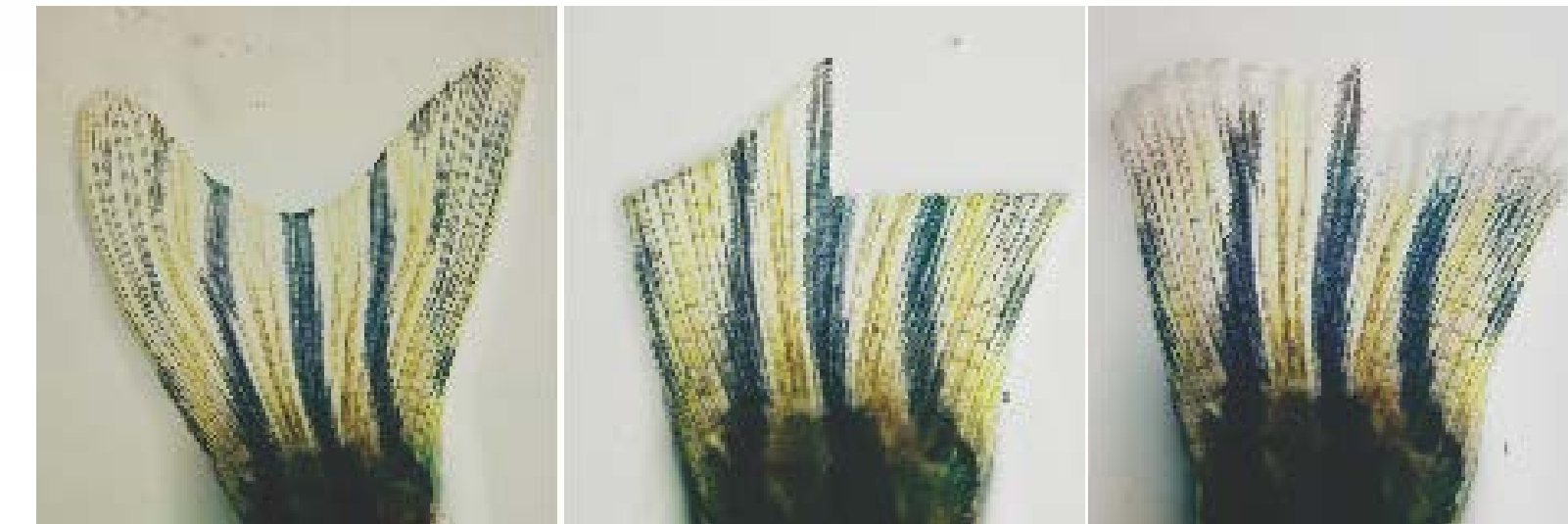
Batch 3 Fish 3

For this batch, we would have expected the dorsal lateral ray to grow slower than the ventral lateral ray. This is not the case; we do not see a difference between the lateral rays.



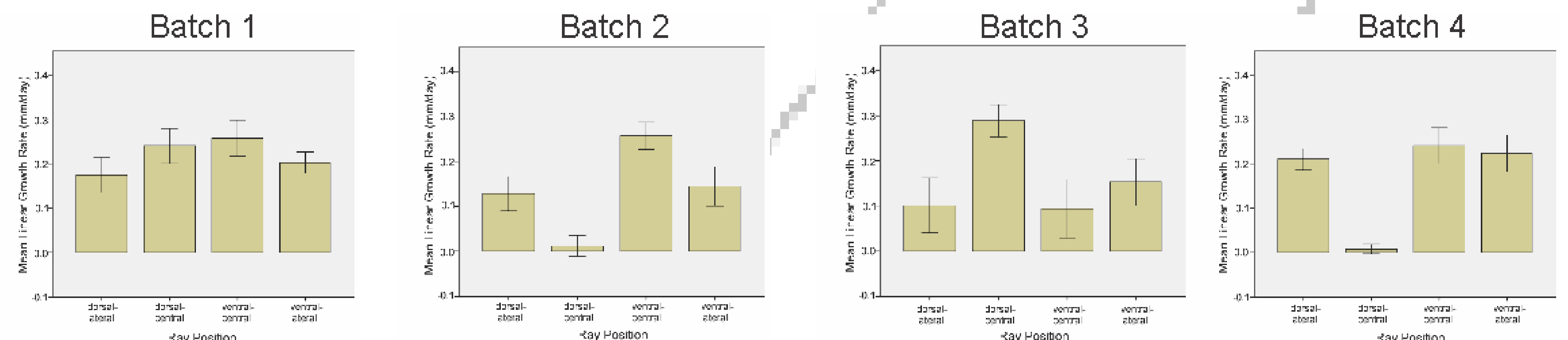
Batch 4 Fish 2

For this batch, we would have expected the dorsal lateral ray to grow faster than the ventral lateral ray. This is not the case; we do not see a difference between the lateral rays.



All Fish – Average Linear Growth

The lateral rays and the central rays were the most important rays for us to examine. I averaged the data for linear growth within the batches (n = 4 fish) and presented it with ± 2 standard error. Day 3 is presented because it appeared to yield the most significant information. Most of the error bars overlap, and therefore very little significant data can be obtained from this data set.



Further Research

Unfortunately, there are a number of problems with this set of data, particularly with the fish that were used. There were difficulties in obtaining the fish for the experiment and as a result the ones I was given had some abnormalities. Future directions for this project should include a repeat of these cuts with more focus on the very early days of regeneration, where most of the differences tend to occur.

References and Acknowledgements:

Morgan, T.H. (1902) Further Experiments on the Regeneration of the Tail of Fishes. *Development Genes and Evolution*, 14(3-4), 539-561.

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