Establishment of lepidotrichia branching networks in Zebrafish caudal fins

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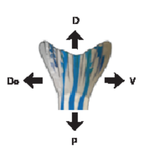
Motivation

The unpaired caudal fin of zebrafish *Danio rerio* can fully regenerate within approximately 2 weeks following a surgical amputation [1] (Fig.1). While size and shape are almost identically restored even after multiple rounds of amputation [2], a distal shift of ray bifurcation points can be observed in the regenerated fin [3].



Main anatomical axis:
distal (D), proximal (P), dorsal (Do), ventral (V).

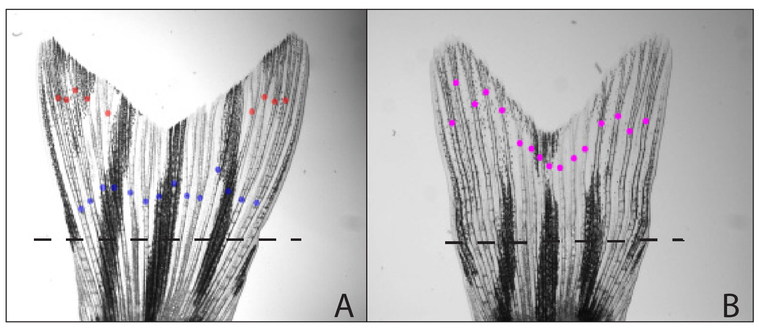


Fig. 1: Primary (A, blue dots) and secondary (A, red dots) ray bifurcations of an adult wild-type caudal fin are distalized (B, pink dots) following a surgical amputation (dashed lines: amputation plane).

The positioning of the ray bifurcation points could not be explained so far. Furthermore there are contradictory results presented in the literature dealing with sonic hedgehog expressing cells that split into two domains preceeding the branching event [3]. Therefore we want to approach this open question by taking the hydrodynamic environment into account for the first time.

Research Question

The fully established branching network of an adult caudal fin is established during early development as the fish experiences a different hydrodynamic regime (low Reynolds numbers (Re)) compared to an adult fish.

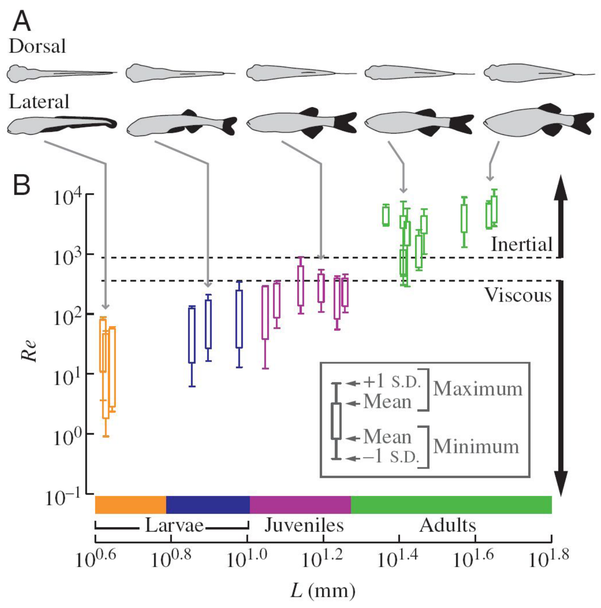


Fig. 2: This image illustrates a hydrodynamic transition from laminar to turbulent flow conditions that a zebrafish undergoes during ontogenetic development [4].

Therefore the ray branching network during epimorphic regeneration of an adult fish is also established in a different Re-regime compared to early ontogeny. The distalization of bifurcation positions can also lead to flexibility changes that we will measure and compare to hydrodynamic experiments (PIV) and computational simulations.

Principle of *in-vivo* measurements

In order to quantify bending stiffness profiles across the caudal fin surface we have developed a custom built setup (Fig. 3) that can measure in the 100 µN range. The main aim was to determine stiffness profiles along the proximal-distal axis as indicated below (Fig. 4). Using piezo-positioning stages we were able to perform precise indentations deflecting the fin a certain distance (Fig. 3 C). As the pin was mounted onto an elastic substrate containing two linear strain gages, an electronic readout (mV) could be converted into a force measurement. Finally the setup was calibrated and benchmarked using cantilevers with known properties. For the *in-vivo* measurements adult zebrafish were anesthetized and placed in a custom fabricated fixation device and positioned inside a basin containing adjusted dilutions of anaesthetics (MS-222, Tricaine methanesulfonate).

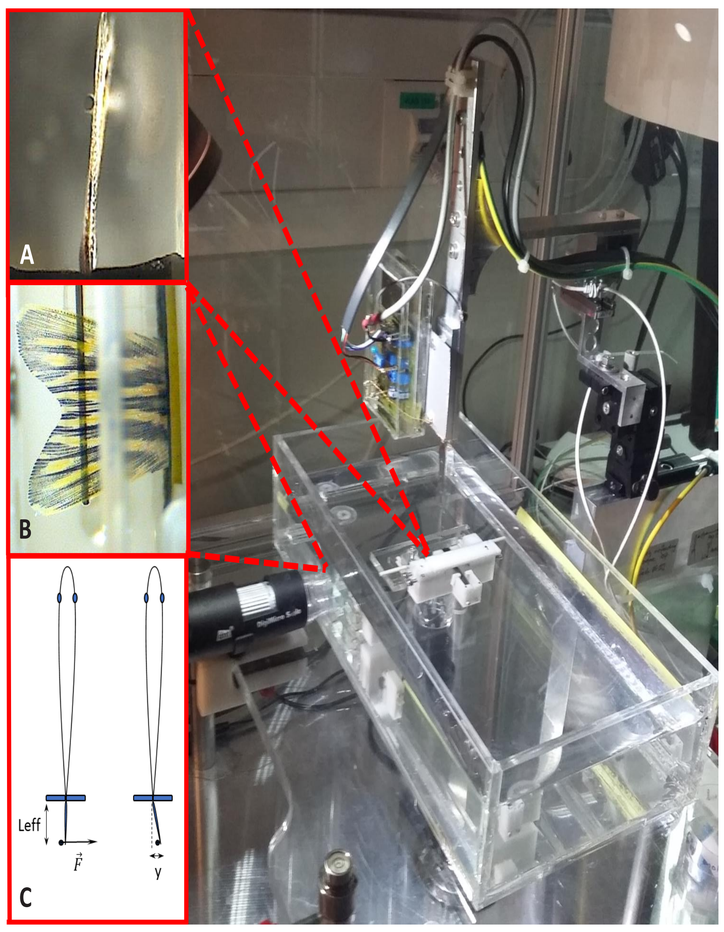


Fig. 3: Two camera views are shown from below the basin (A) and from the side (B), indicating the position of the indentation pin. A further illustration (C) shows some relevant parameters that were used to calculate bending stiffnes values.

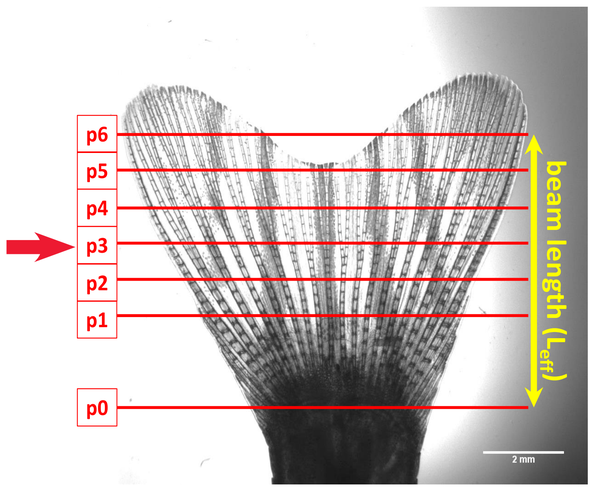


Fig. 4: Stiffness measurements were performed along a line at 6 distinct positions (p1-p6). The resulting effective length resulting as a distance from the fixation point (p0) was used to compute the bending stiffness (EI) as a first approximation [5].

In order to obtain a stiffness profile across the fin surface we use [5]:

$$EI = \frac{FL^3}{3y}$$

where F is the measured force, L the effective beam length and y the indentation distance.

Results

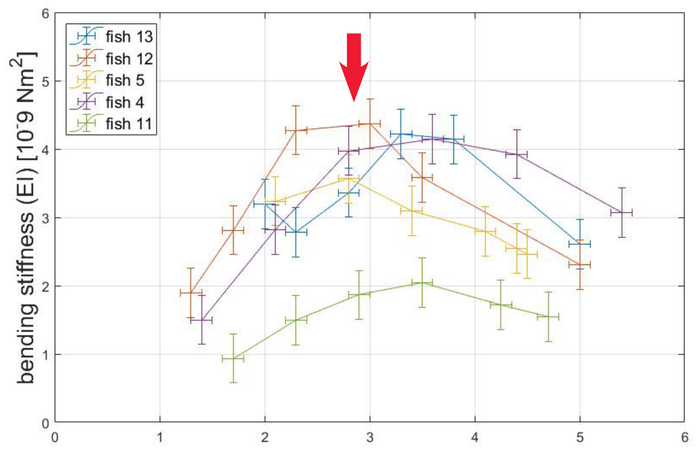


Fig. 5: Bending stiffness profiles are shown for 5 different fish at 6 distinct positions. Error bars for effective beam length correspond to SDs (± 0.1mm), error bars for stiffness are obtained from errors on force, indentation distances and effective beam lengths using error propagation (± 0.4 x 10^{-9} Nm^2).

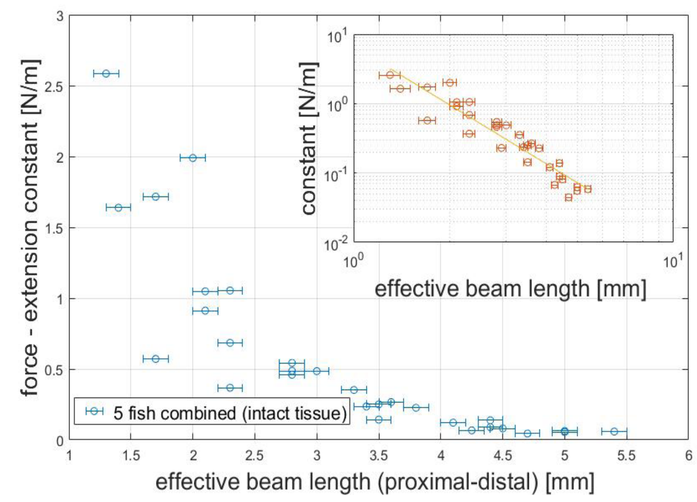


Fig. 6: Dependence of the bending stiffness given by the slope of a force indentation curve on beam length (error bars as in Fig. 5 (± 0.1mm), slope uncertainties are not visible due to their low values (< 0.004 N/m)). The inset shows the same data on a double logarithmic scale. A power-law dependence of the bending stiffness on the effective beam length with an exponent of -3.0(2) can be seen.

Summary & Outlook

As a proof of principle we were able to quantify bending stiffness profiles of adult caudal fins for the first time *in-vivo*. We were able to detect flexibility changes in all fish fins that morphologically correlate with the first bifurcation planes as indicated by red arrows (Fig. 4 & 5).

We are now in the process of measuring regenerated fins and are also planning to measure flexibility profiles during regeneration and ontogenetic development.

References

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