Adaptation mechanism of the adult zebrafish respiratory organ to endurance training

Matthias Messerli[1, x], Dea Aaldijk[1, x], David Haberthür[1], Helena Röss[1], Carolina García-Poyatos[2], Marcos Sande-Melón[2], Oleksiy-Zakhar Khoma[1], Fluri A. M. Wieland[1], Sarya Fark[1], Valentin Djonov[1*]

[1]Topographic and clinical Anatomy, Institute of Anatomy, University of Bern, 3012 Bern, Switzerland, [2] Developmental Biology and Regeneration, Institute of Anatomy, University of Bern, 3012 Bern, Switzerland, [x] The authors contributed equally to this work.

`{dea.aaldijk, david.haberthuer}@ana.unibe.ch`

Introduction & Methods

Adult zebrafish (*Danio rerio*) were subjected to endurance exercise for 5 weeks to study the adaptation of their respiratory organ. Zebrafish (Tg(*fli1a*:eGFP)^{y7}) [1] at the age of 18 to 24 months underwent a 6-hour training at 5 days/week for a total of 5 weeks with increased swimming speed [2].

Critically-point dried heads of the fishes were imaged on a Bruker SkyScan 1172 high-resolution microtomography machine (Bruker microCT, Kontich, Belgium) with an X-ray source voltage of 50 kV and a current of 167 µA. A set of 3979 projections of 4000 × 2672 pixels was recorded over a 180° sample rotation. The projections were then reconstructed into stacks of images with an isometric voxel size of 1.65 µm. After reconstruction, we manually delineated the gills in CT-Analyser (Bruker, Version 1.17.7.2+) and exported these volumes of interest (VOI) as a set of PNG images for each fish head. These sets of images were then analyzed with a Python script in a Jupyter notebook, which is available online [3].

The data presented here is only a small subset of data acquired in a larger study [4], where we also looked at electron microscopy images to describe the morphology of zebrafish gills in detail.

Results

Micro-computed tomography indicated a significant increase in the gill volume (p=0.048) by 11.8 % from 0.490 mm^3 to 0.549 mm^3. The space-filling complexity dropped significantly (p=0.0088) by 8.2 % from 38.8 % to 35.9 %, i.e. making the gills of the swimmers less compact. The zebrafish respiratory organ—unlike the mammalian lung—has a high plasticity, and after endurance training increases its volume and changes its structure in order to facilitate O_2 uptake.

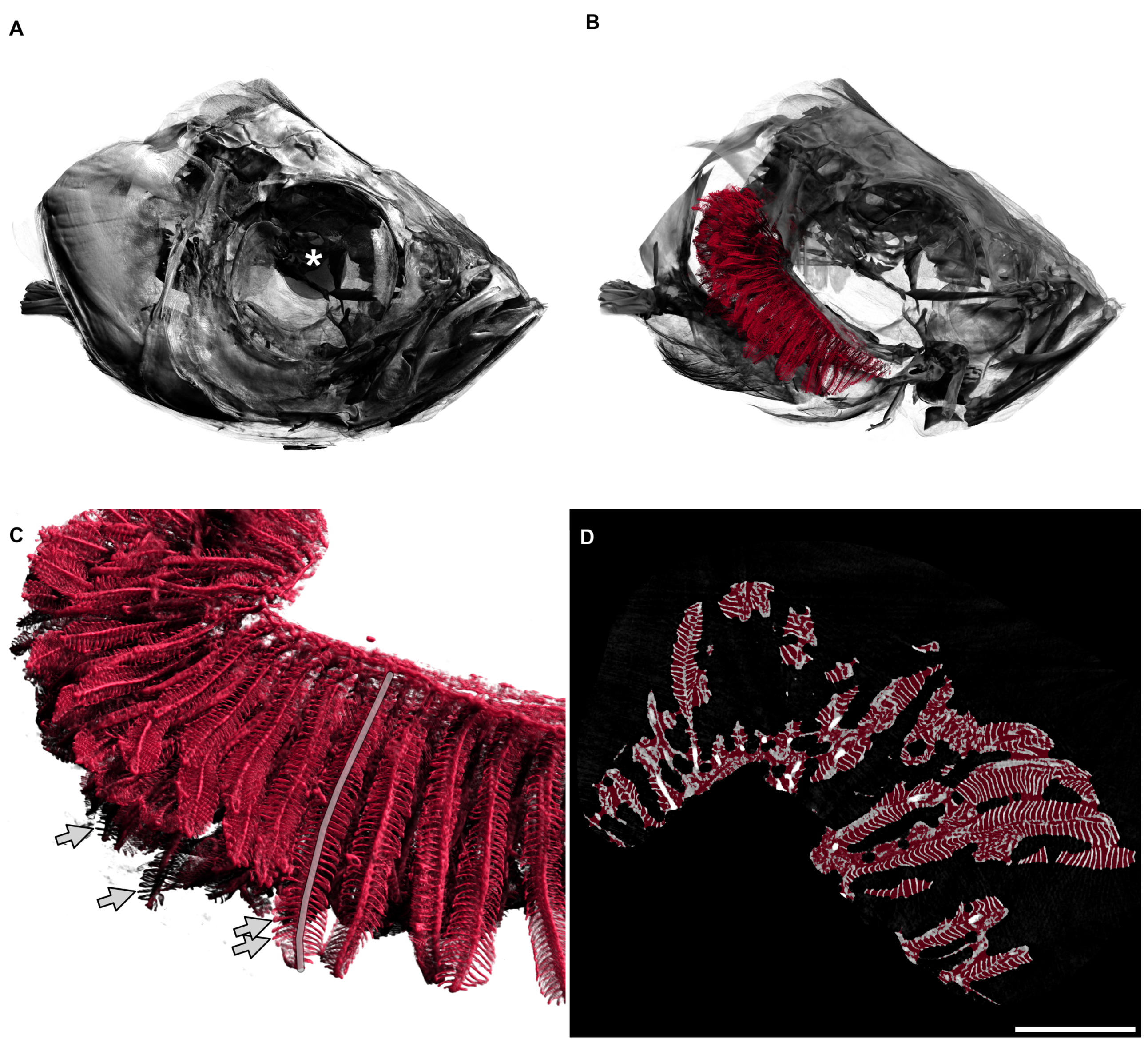

Figure 1: *3D visualization of a tomographic scan of a fish head from the control group. A: Fish head. The diameter of its eye (center marked with a white asterisk) is approximately 0.83 mm. B: The delineated gills in red are shown inside the head of the fish. The primary filaments are mainly pointing to the left of the image (back of fish). C: Detailed view of gills. Secondary filaments are seen as leaf-like structures attached to the primary filaments. The semi-transparent gray line marks one primary filament. Arrows mark the tips of four secondary filaments. D: Two-dimensional view of the gills, e.g. one slice of the tomographic data set where all three-dimensional measurements were based on. The red overlay denotes the estimation of the hull of the gill organ. The filling factor shown in the right panel of Figure 2 has been calculated by dividing the red volume by the white volume. Scale bar 0.5 mm.*

Results (continued)

We present evidence of the long-lasting morphological adaptation of respiratory organ of adult animals to a physiological stimulus. Specifically, we measured an increase in primary filament length (+6.1 %), number of secondary filaments per primary filament (+7.7 %), and total gill volume (+11.8 %) in adult zebrafish after endurance exercise.

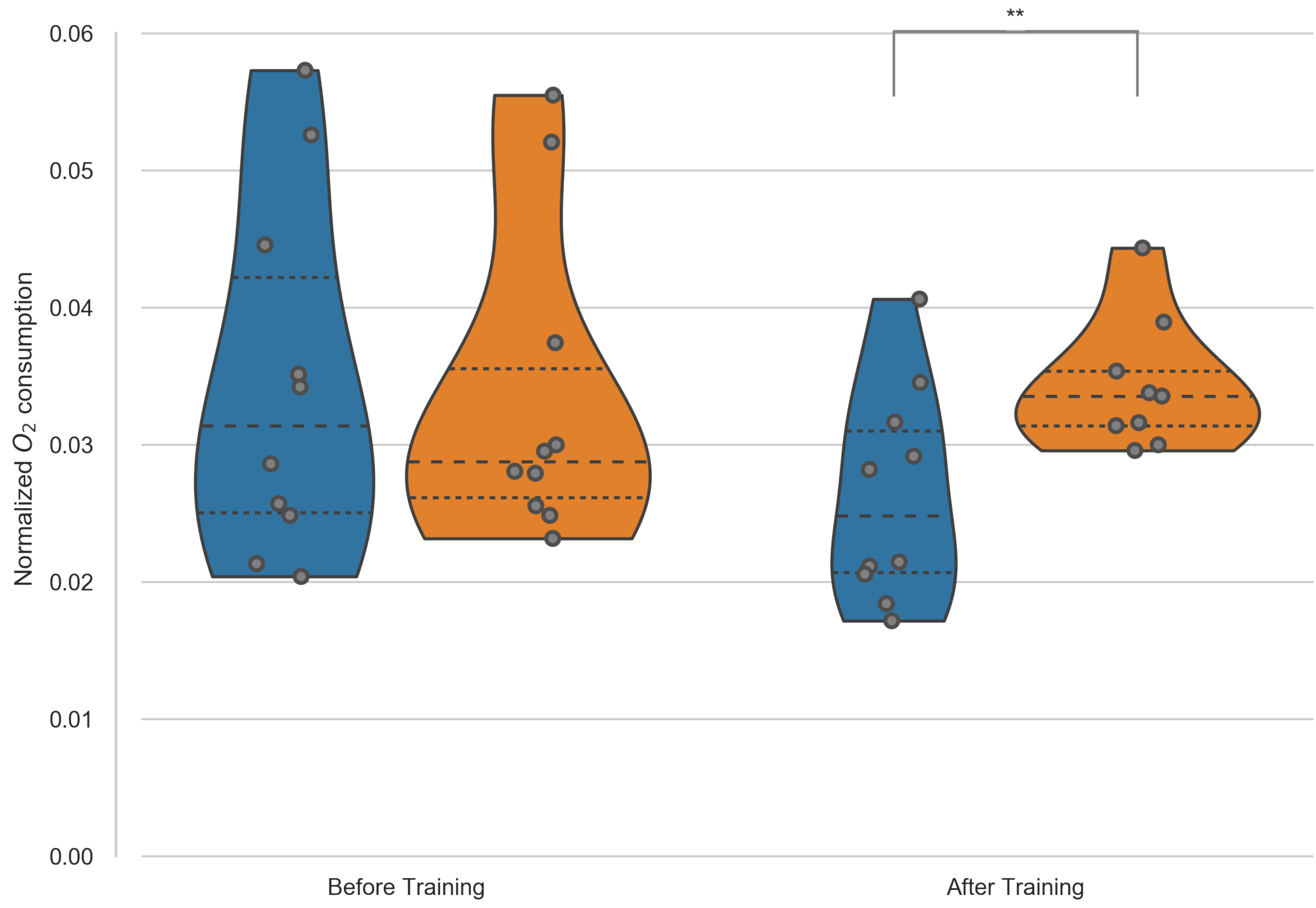

Figure 2: *Gill volume and filling factor of gills calculated from micro-CT data. Left: the total volume of the gills was calculated from micro-tomographic assessment, after selecting a VOI and binarizing the image into gills and background. Data from controls and swimmers, showing a significant increase after 5 weeks of training (p=0.048, n=10 for each group). Right: Ratio of gills per organ area correlating to gill complexity. The swimmers have significantly less gills per organ, e.g. more room between the filaments (p=0.0088, n=10). *: p<0.05, **: p<0.01, lines within the plots show the quartiles of the respective distributions.*

We propose that gill filaments may re-initiate their growth by a process we call *gill filament budding*. Whether mammalian lung can regrow after exercise too, remains to be investigated.

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