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

UNIVERSITÄT BERN

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, the gills of the zebrafish were manually delineated in CT-Analyser (Bruker, Version 1.17.7.2+). These volumes of interest (VOI) were then exported as a set of PNG images for each fish head and 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

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.

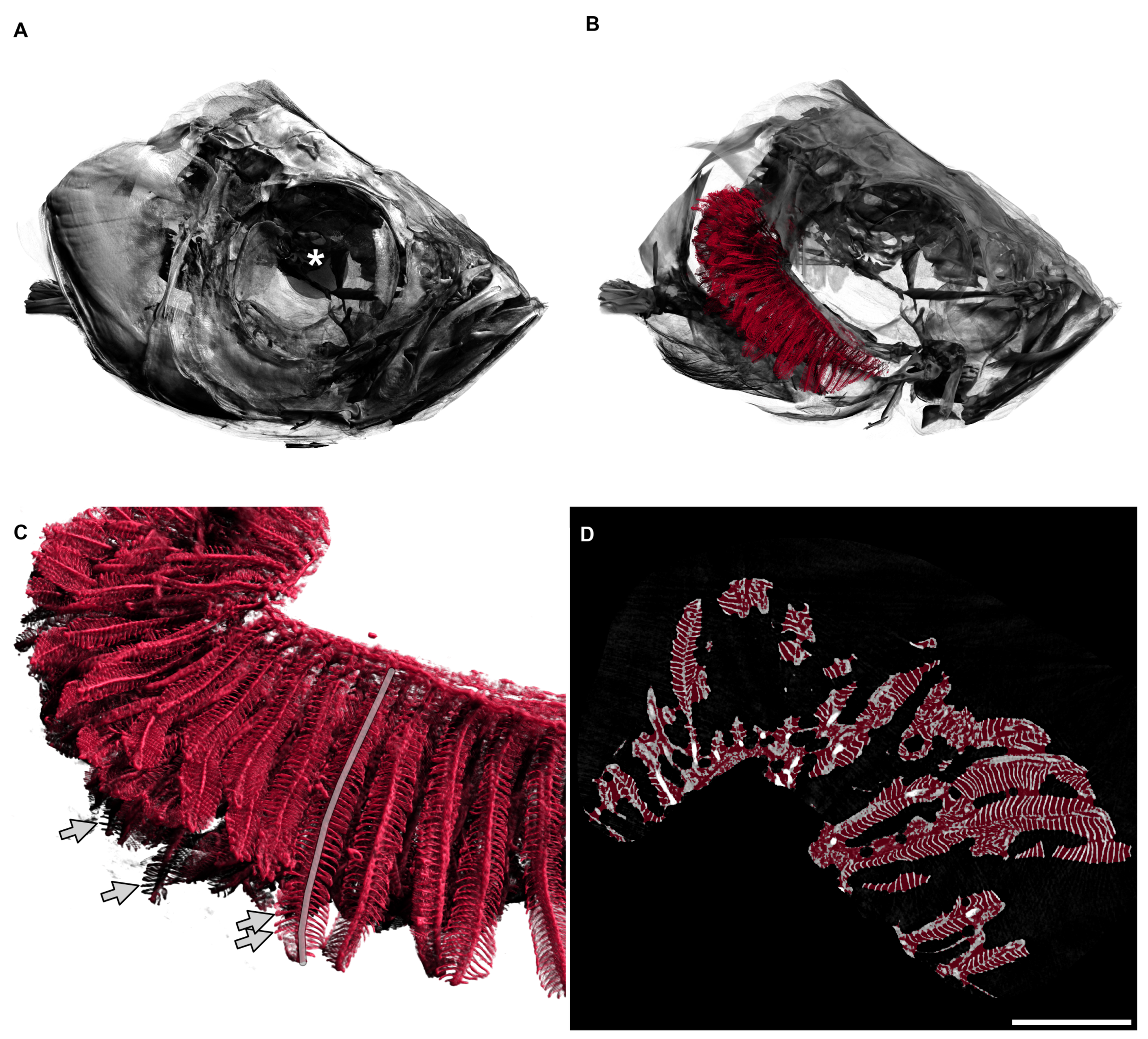

Figure 1: *3D visualization of a tomographic scan of a fish head from the control group. A: Fish head. The diameter of its eye (center marked with a white asterisk) is approximately 0.83 mm. B: The delineated gills in red are shown inside the head of the fish. The primary filaments are mainly pointing 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 Figure 3 has been calculated by dividing the red volume by the white volume. Scale bar 0.5 mm.*

Results (continued)

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.

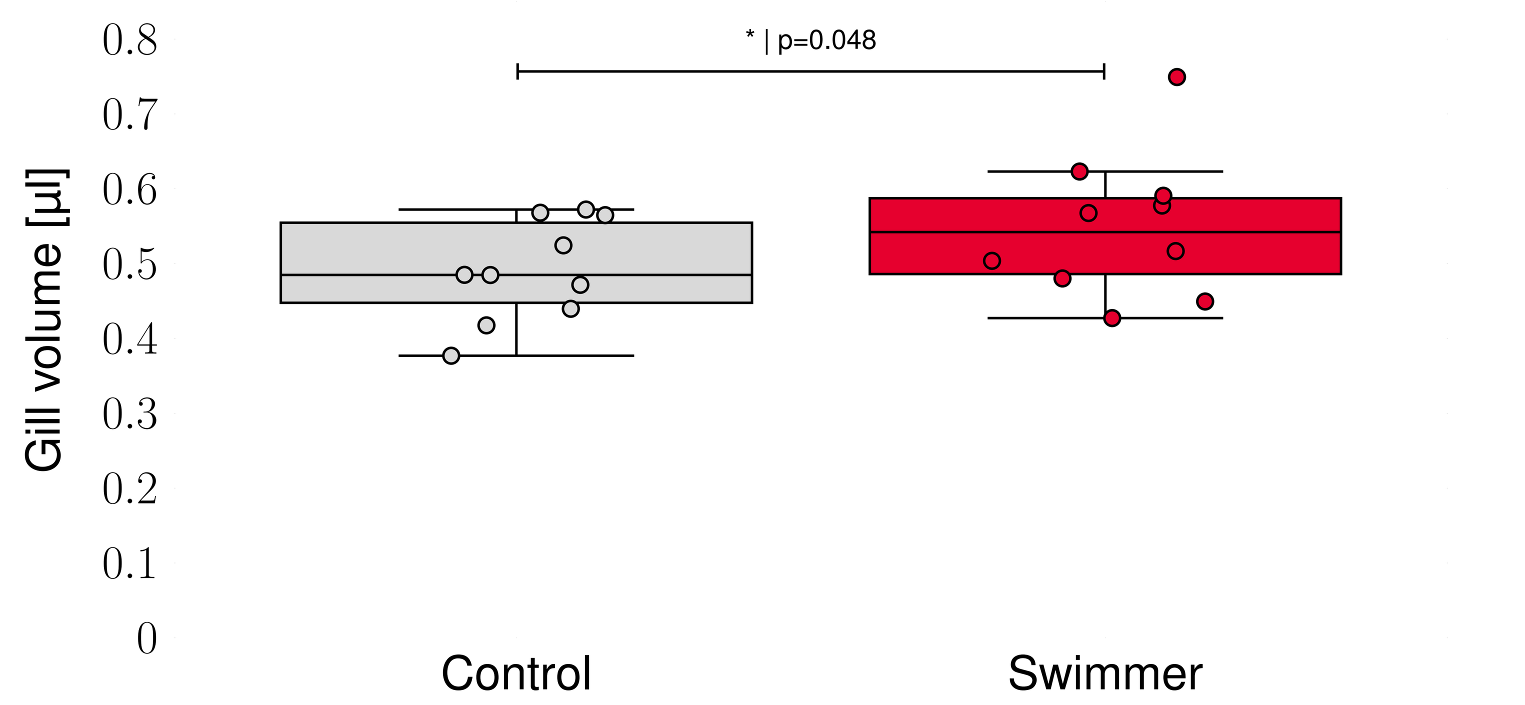

Figure 2: *Gill volume calculated from µCT data. The total volume of the gills was calculated from micro-tomographic assessment, after selecting a VOI and binarizing the image into gills and background. Data from controls and swimmers, showing a significant increase after 5 weeks of training (p=0.048, n=10 for each group).*

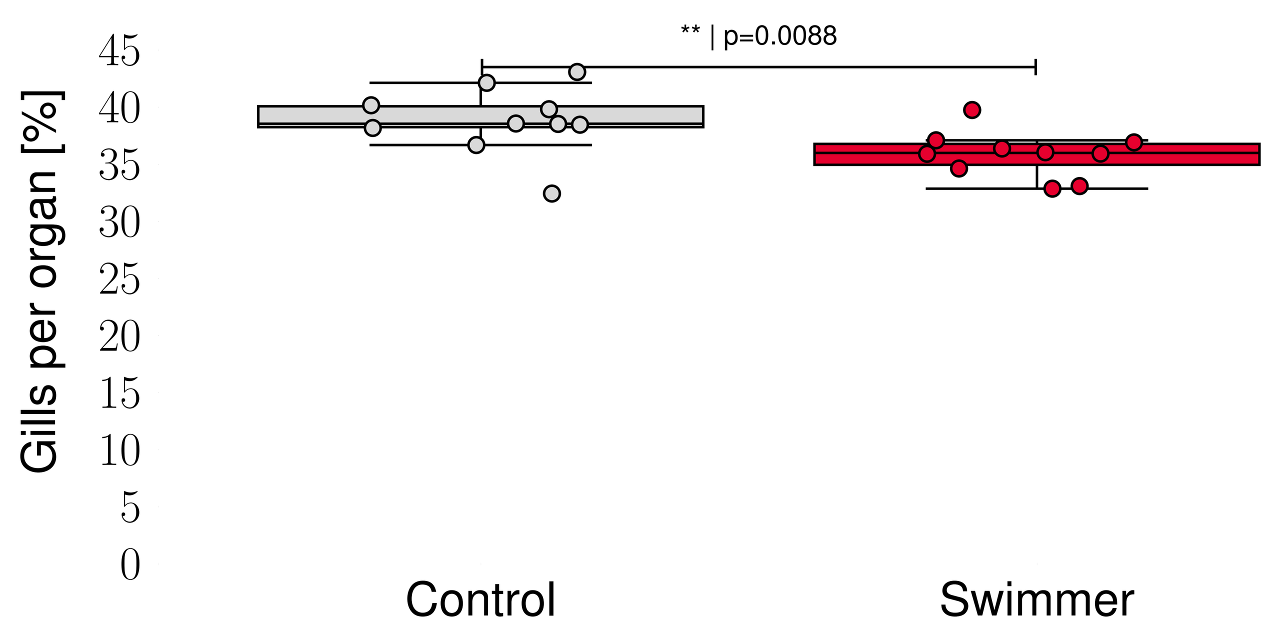

Figure 3: *Filling factor of gills (i. e. gill complexity) calculated from µCT data. The ratio of gills per organ area correlattes to the gill complexity. The swimmers have significantly less gills per organ, e. g. more room between the filaments (p=0.0088, n=10).*

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.

Acknowledgments

WE thank Stefan Tschanz and Yury Belayev for valuable inputs about imaging and quantification; Regula Buergy, Eveline Yao and Sara Soltermann for essential help in the fish facility. The present work was supported by the Swiss National Foundation grant 31003A_176038 and Swiss Cancer Research grant KFS-4281-08-2017.

References

[1] Nathan D Lawson and Brant M Weinstein. "In Vivo Imaging of Embryonic Vascular Development Using Transgenic Zebrafish". In: *Developmental Biology* 248.2 (Aug. 2002), pp. 307–318. ISSN: 00121606. DOI: 10.1006/dbio.2002.0711.

[2] Arjan P. Palstra et al. "Establishing Zebrafish as a Novel Exercise Model: Swimming Economy, Swimming-Enhanced Growth and Muscle Growth Marker Gene Expression". In: *PLoS ONE* 5.12 (Dec. 2010). Ed. by Jose A. L. Calbet, e14483. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0014483.

[3] David Haberthür. *habi/Zebra-Fish-Gills: Zenodo*. Tech. rep. July 2019. DOI: 10.5281/ZENODO.3342451.

[4] Matthias Messerli et al. "Adaptation mechanism of the adult zebrafish respiratory organ to endurance training". In: *PLOS ONE* 15.2 (Feb. 2020). Ed. by Eric A Shelden, e0228333. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0228333.