microCT imaging of threespine stickleback

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Abstract

Can we predict evolution? The threespine stickleback (Gasterosteus aculeatus) is a well-recognized system for understanding adaptation to divergent habitats. Populations of benthic and limnetic stickleback differ in a number of phenotypic traits that are associated with shifts in dietary specialization. However, analyses of the structures required for feeding – especially the jaws and complex internal branchial anatomy – requires considerable time and expertise, with destructive sampling and fine dissection skills needed for quantitative analysis. The advent of μCT and 3D-scanning technology affords non-destructive sampling and an increase the resolution of data available for study, but at the substantial cost of increasing complexity and processing time for each specimen. To address these concerns, we developed a rapid and semi-automated segmentation and analysis pipeline based around the Biomedisa Image Segmentation platform for investigating three-dimensional morphological adaptation within the threespine stickleback. The pipeline includes splitting a multi-specimen scan into regions of interest for each specimen, reconstruction of targeted anatomy, and morphometric analyses. We then applied this pipeline to a sampling effort encompassing hundreds of samples of divergent benthic and limnetic stickleback populations, showcasing the possibility of using high-throughput scanning data to provide tests of ecological and evolutionary hypotheses.

Introduction

- Embedded into Alaska Stickleback Restoration Project, Genomics axis where Katie Peichel, Ben Sulser and Sheila Christen are affiliated.

Materials & Methods

Sample preparation

microtomographic imaging

- Scanned on a Bruker SkyScan 2214
- Sample holder generated with OpenSCAD, available online at GitHub [1]
  - Scanning several fish toghether to efficiently use machine time
  - Full in Bruker workflow
  - Results in PNG stacks on disk
- All log files available here: https://github.com/habi/sticklebacks/tree/main/logfiles

Data analysis

Preparation and handling of tomographic datasets

- Jupyter notebooks [2]
  - Efficiently loading data from disk with `dask` [3]
  - Extract position of single fish (all scanned together), based on the MIP of the scan (see Figure 2).
  - Crop out each fish (with a buffer) and write to cropped dataset (see Figure 4).
    - Cropped datasets are saved to discrete folders for easy handling. In both original gray-scale plus as thresholded dataset, e.g. binarized into bone and "not bone". These are saved out as `zarr` [4] and `nrrd` files.

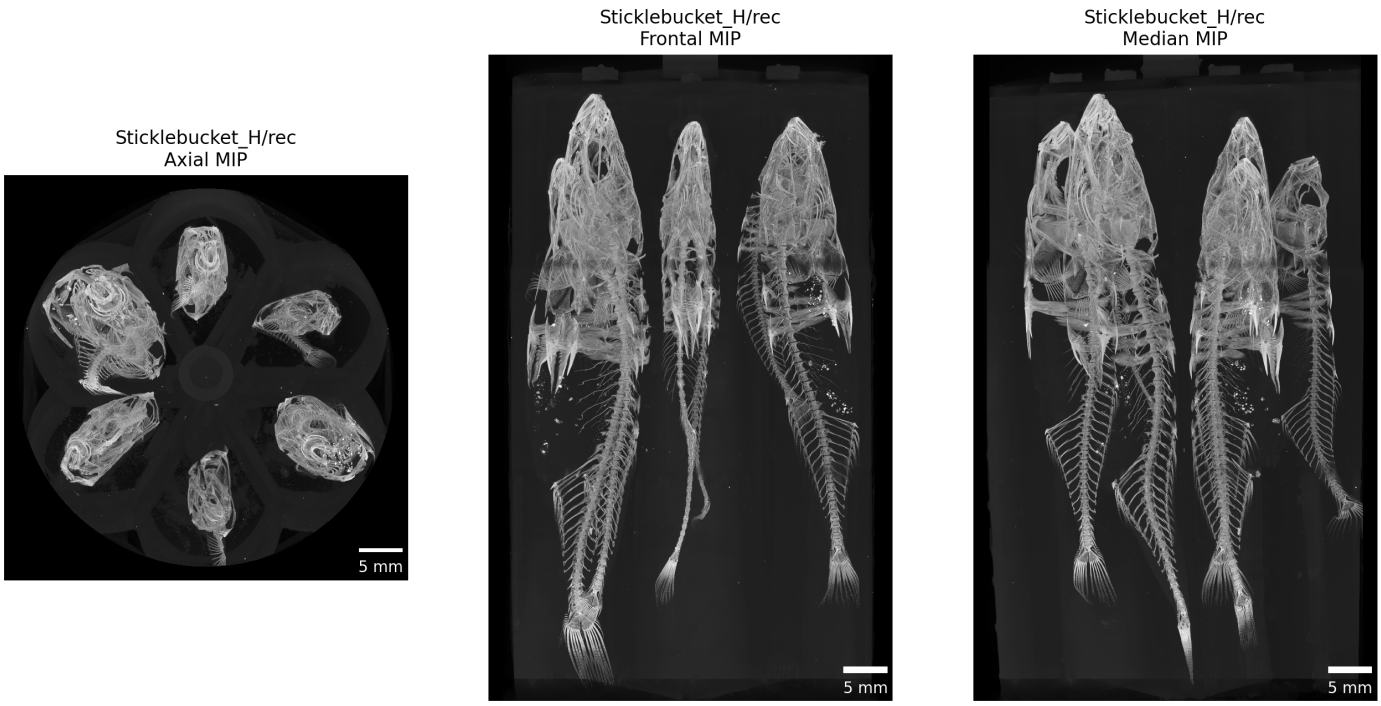

Figure 1: Maximum intensity projections of one acquired dataset along all three cardinal axes.

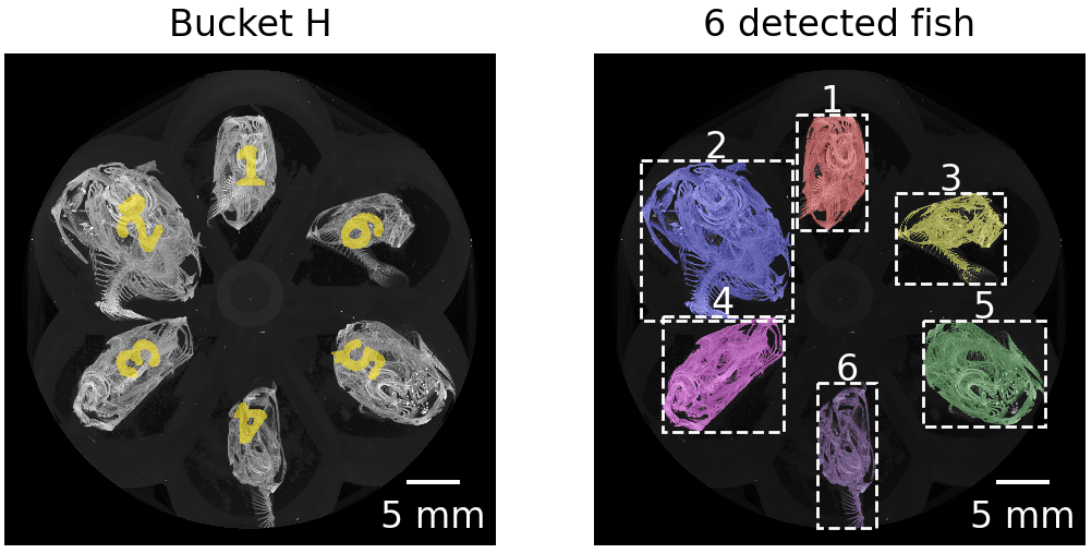

Figure 2: Automatically detected regions based on maximum intensity projection along the rotation axis of the tomographic scan. The regions are numbered consecutively from the top left to the bottom right. These numbers are mapped to the correct fish ID in the next step.

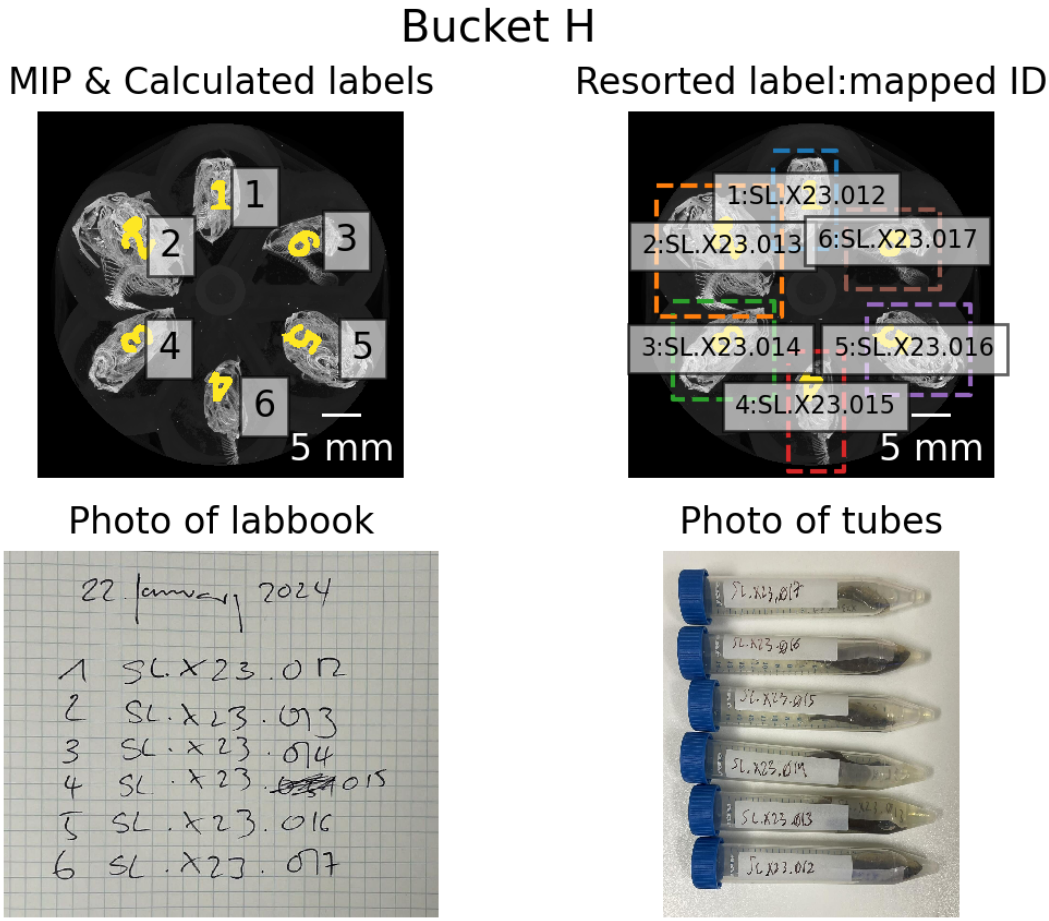

Figure 3: Mapping lab book notes, photos and detected regions to fish ID.

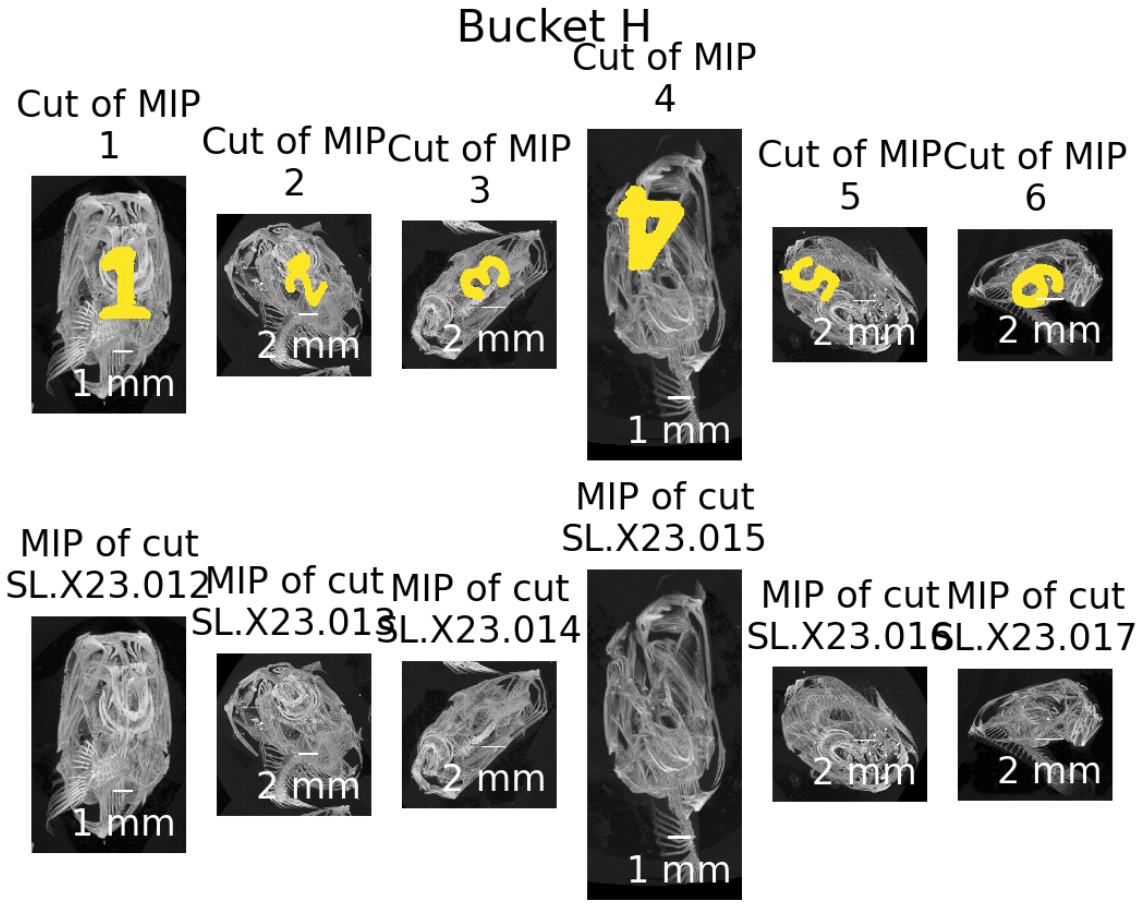

Figure 4: Doublechecking crop extent and fish ID.

Extraction of features of interest

- Biomedisa [5]

Results

- 3D shape variation on internal pharyngobranchial bone. Only possible to get this information in 3D.

Discussion

- Both *repeatable* and *reproducible* research
- Automated cropping out of single fish from combined scan very efficiently uses machine time. Several fish can be scanned together, splitting is performed after the fact, reproducibly and without manual input.
- Combination of methdes cust down on time **a lot**.
- Biomedisa makes more “extraction” possible. Other biological questions can be answered, too.

Conclusion

Author Contributions

Contributor Roles Taxonomy (CRediT), as defined in [6]:

- Conceptualization: Ben Sulser
- Data curation: David Haberthür, Ben Sulser
- Formal analysis: David Haberthür, Ben Sulser
- Funding acquisition: Ben Sulser, Catherine L. Peichel
- Investigation: David Haberthür, Ben Sulser
- Methodology: David Haberthür, Ben Sulser
- Project administration: David Haberthür, Ben Sulser, Catherine L. Peichel
- Resources: Ben Sulser
- Software: David Haberthür, Ben Sulser
- Supervision: Ben Sulser, Catherine L. Peichel
- Validation: David Haberthür, Ben Sulser
- Visualization: David Haberthür, Ben Sulser
- Writing – original draft: David Haberthür, Ben Sulser
- Writing – review & editing: David Haberthür, Ben Sulser, Catherine L. Peichel

Competing Interest

Author	Competing Interests	Last Reviewed
David Haberthür	None	2026-01-14
Ben Sulser	Nothing to Declare	
Sheila Christen		
Catherine L. Peichel	none	19.01.2026
Ruslan Hlushchuk		

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