



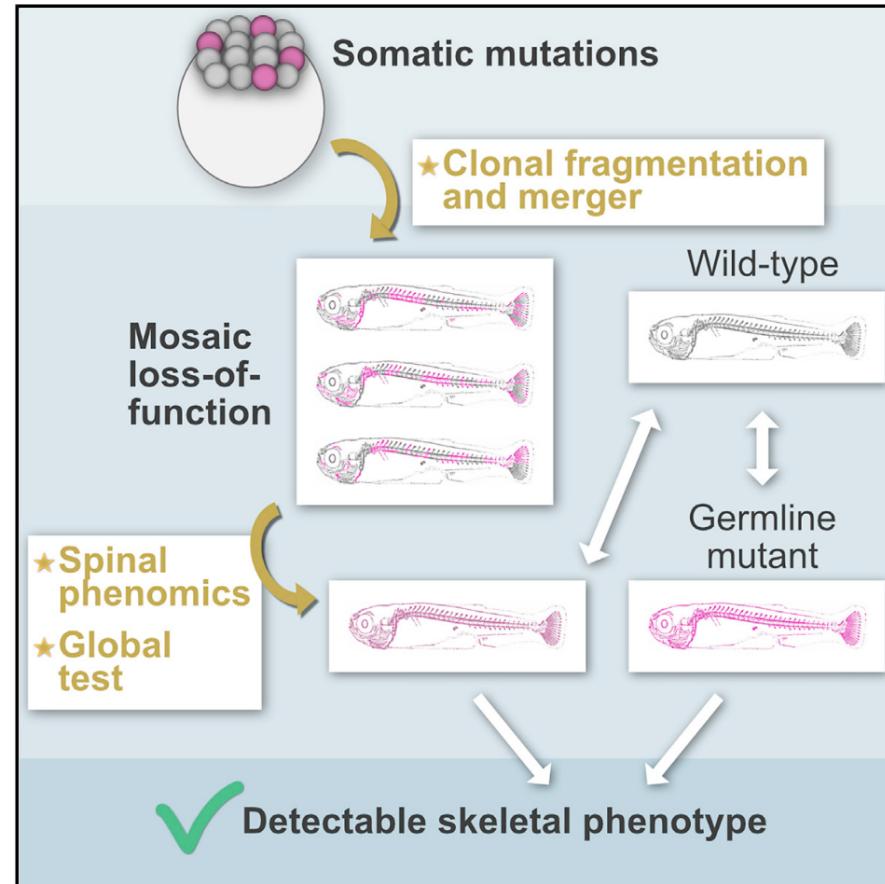
Putting it all together

Using 3D Slicer & SlicerMorph to quantify phenotypic variation

Kelly Diamond

Quantifying genotype to phenotype

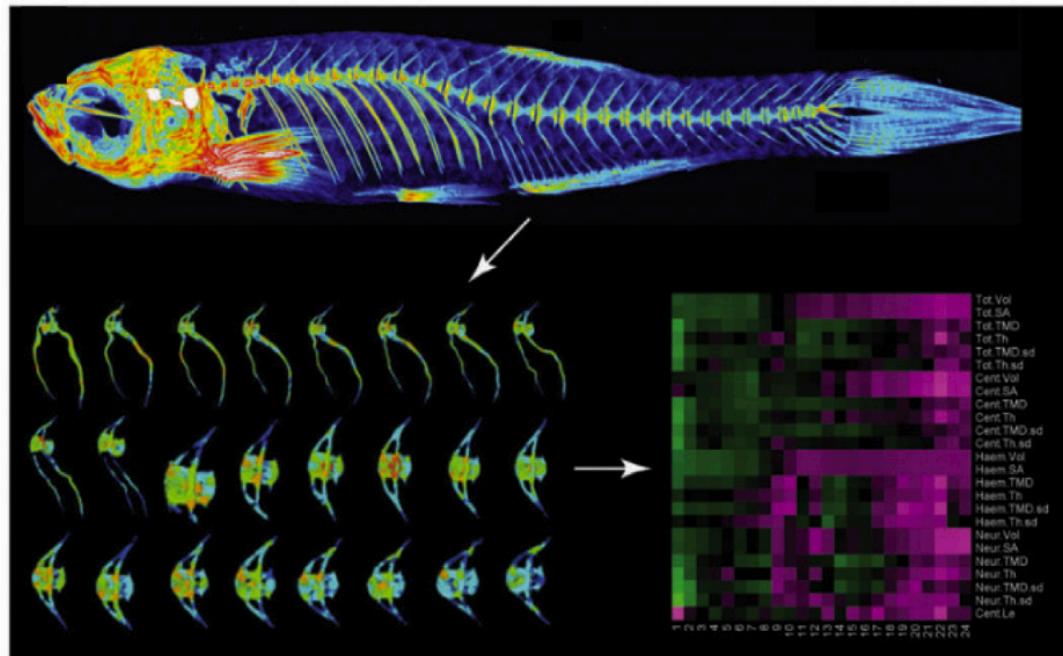
One way to quantify relationships between genotype and phenotype is to compare the phenotype of known genetic mutants to their wildtype siblings



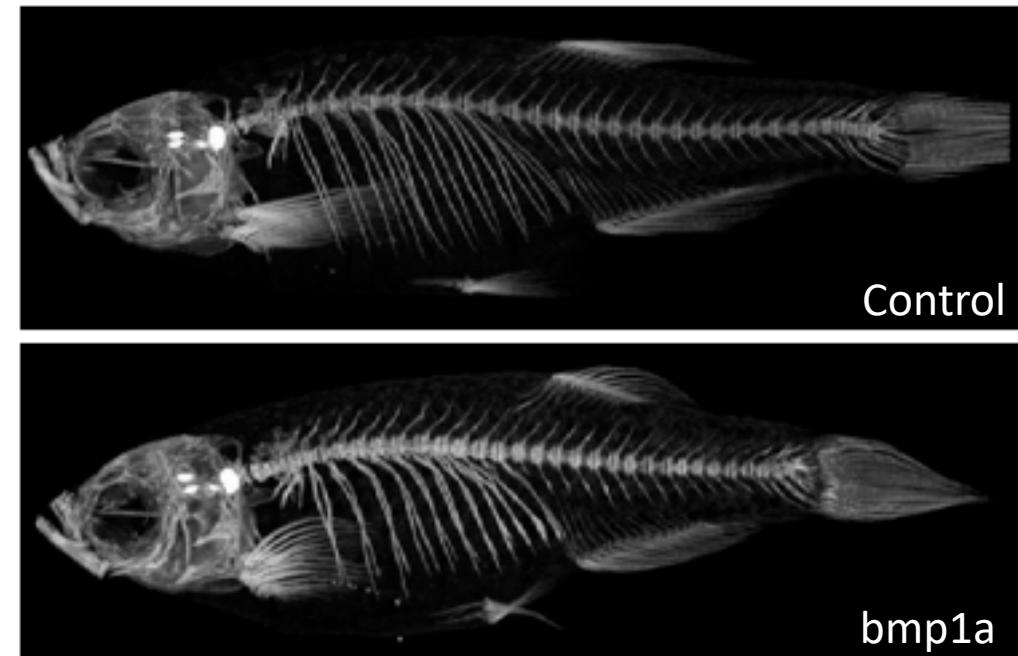
[Watson et al., 2020 Cell Systems](#)

MSB Lab developed an approach for decoding spatially variable phenotypes of the axial skeleton

Mutation in *bmp1a* results in brittle bones and shortened axial skeleton



[Hur et al., 2017 eLife](#)

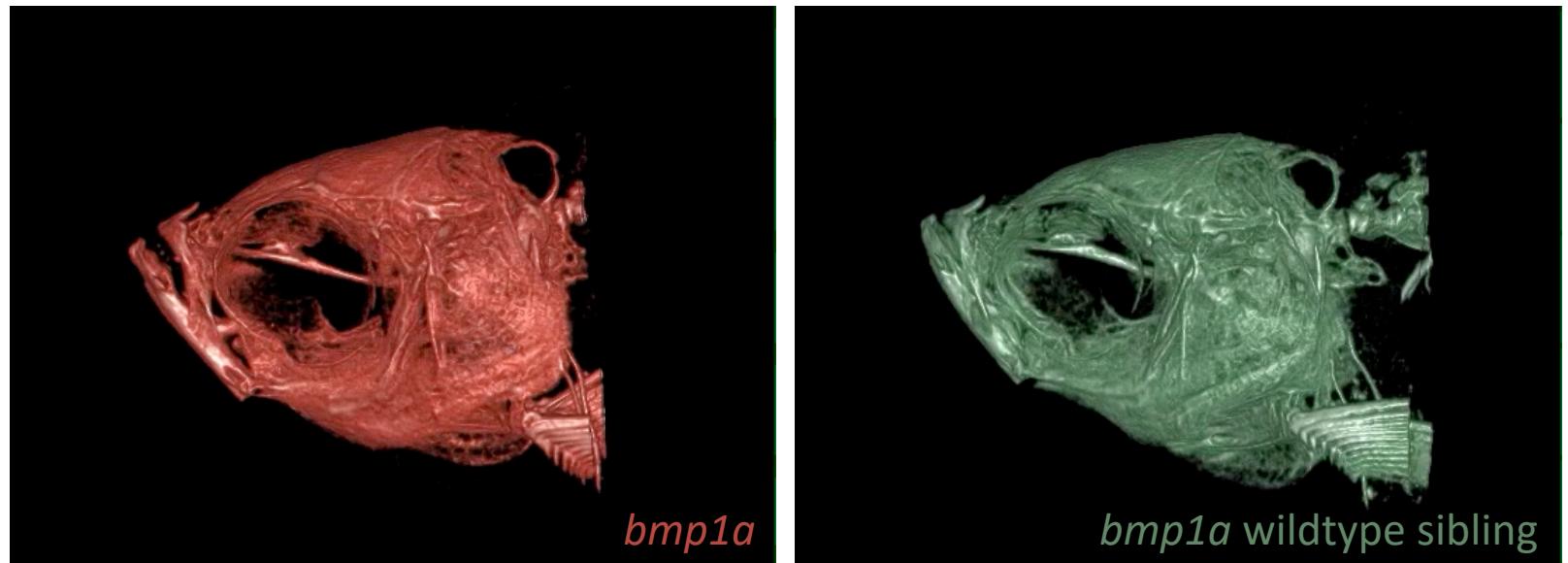


[Watson et al., 2020 Cell Systems](#)

But what about the cranial skeleton?

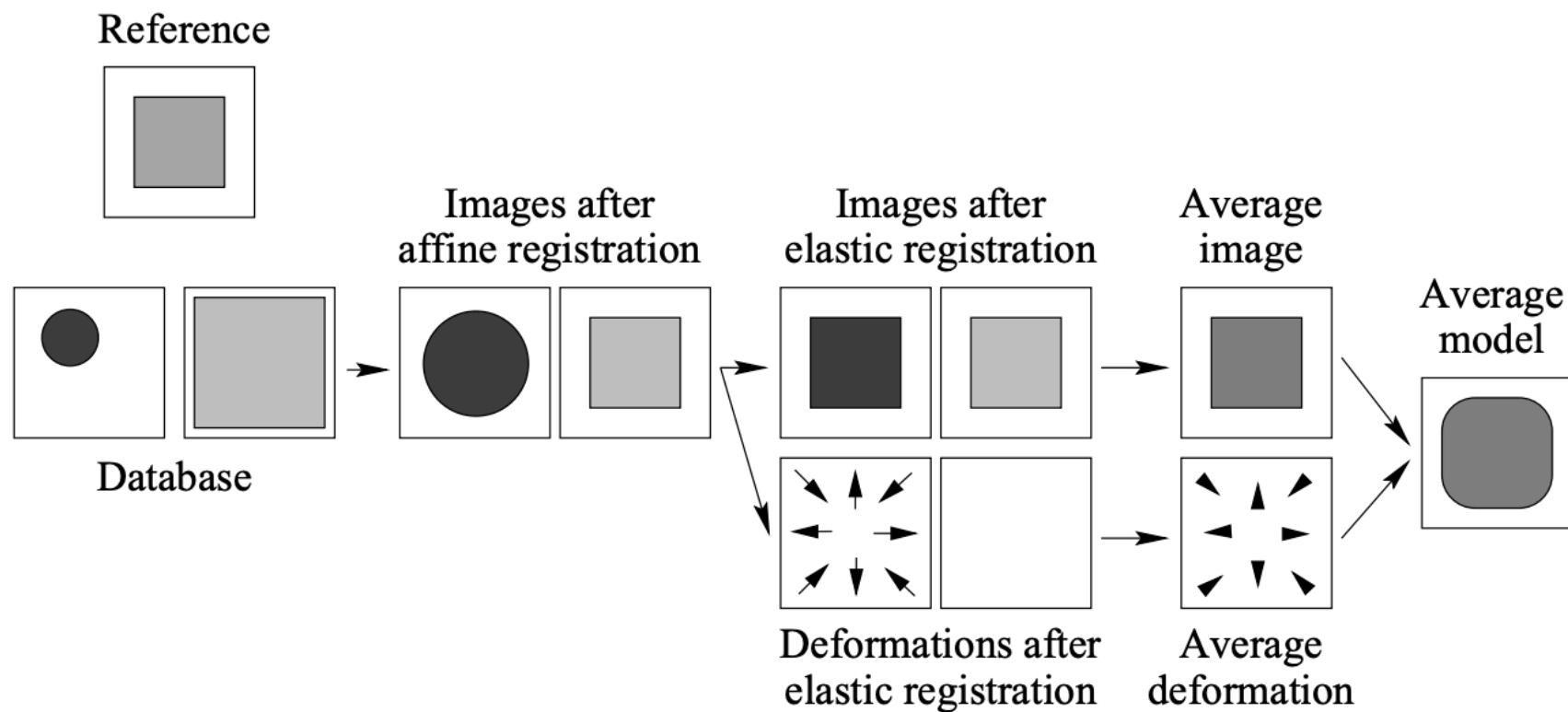
Goal: Quantify phenotype of cranial skeleton

Starting data:
microCT scans of
bmp1a and their
wildtype siblings



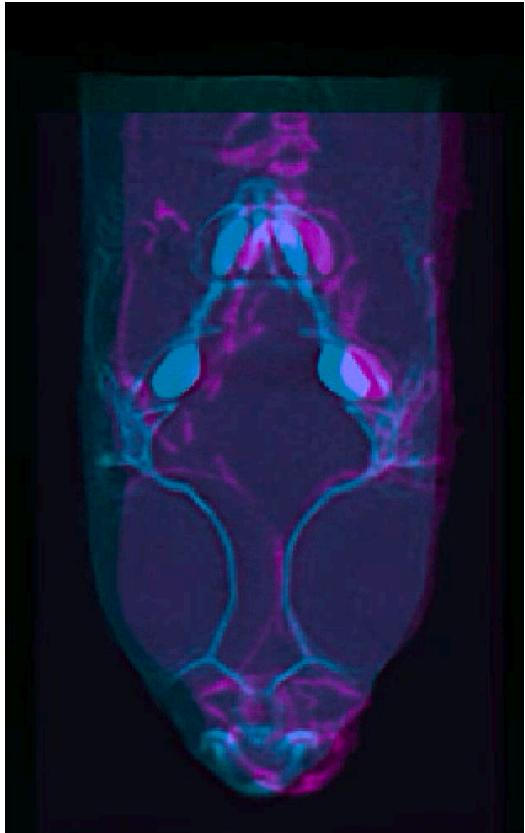
Atlas building

The atlas construction framework iteratively finds a virtual space which resides in the centroid of the population (i.e. deformations needed to transform all subjects into this virtual space sum to zero everywhere in this virtual space)



Visualizing Image Registration

Rigid Registration



Rotation and translation only
Degrees of Freedom (DOF) in
movement

Affine Registration



Rotation, translation,
independent scaling and
shearing on each axis 12 DOF

Deformable Registration

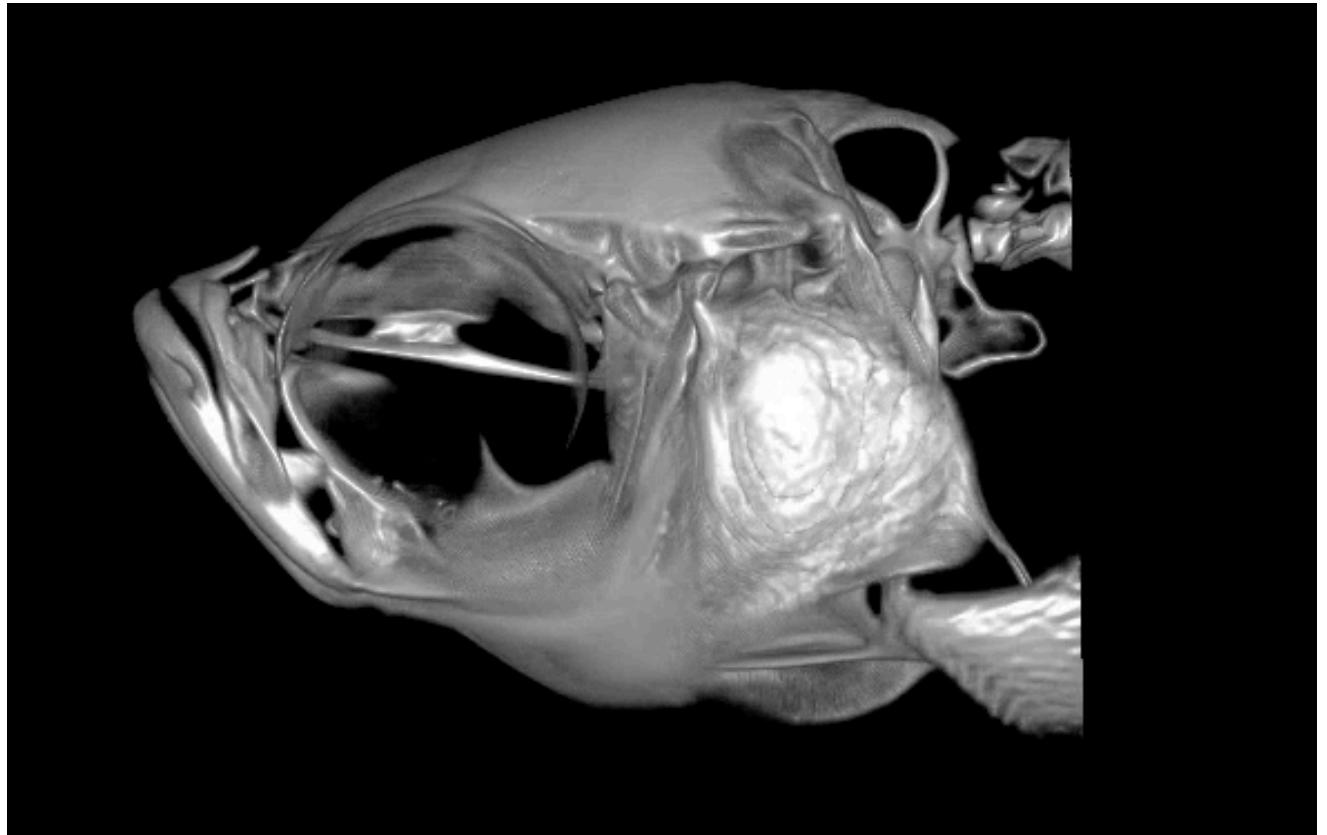


Every point can move

Atlas building

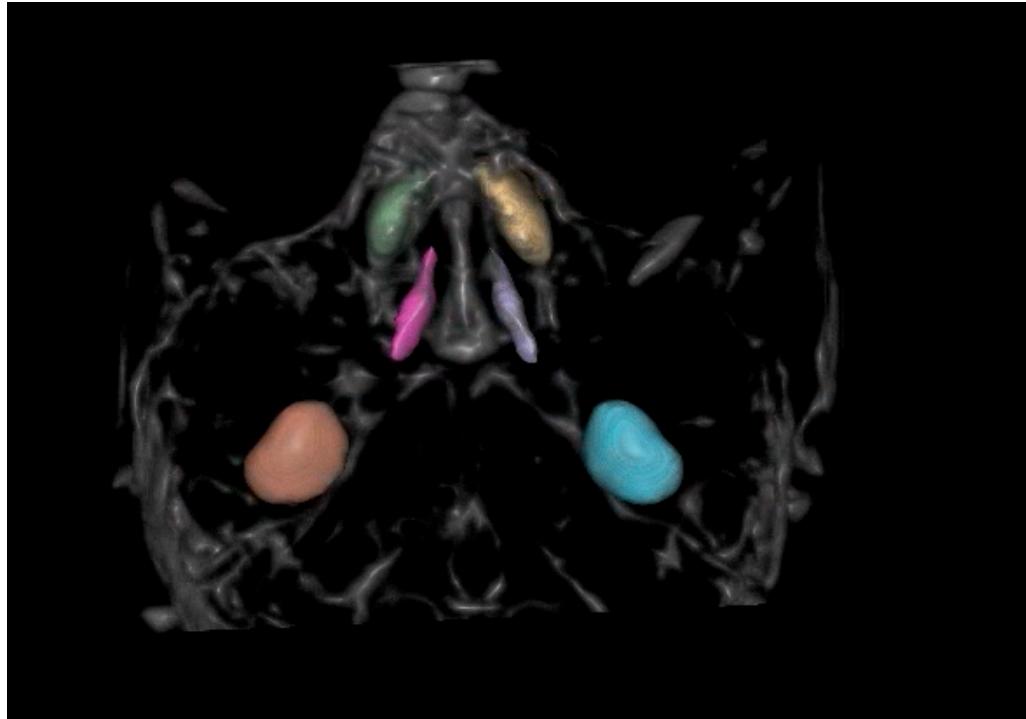
Built atlas using a process of
deformable image registration
([ANTs](#))

Only wildtype fish were used to
build our atlas

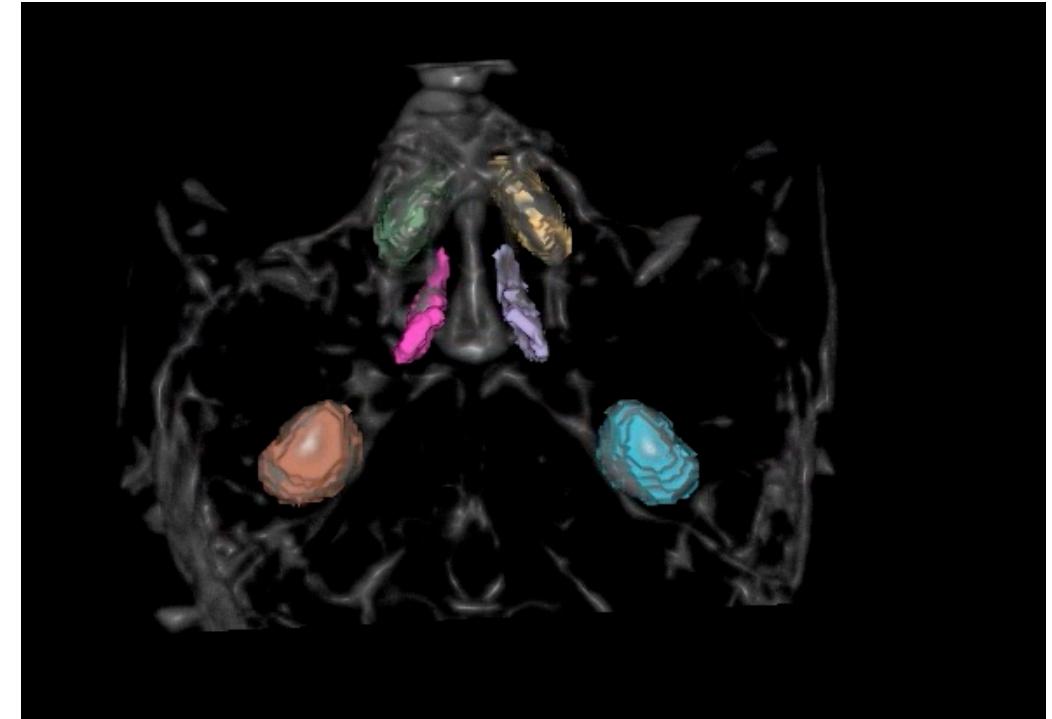


Testing the atlas

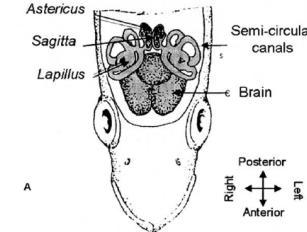
Manually segmented out the otoliths from each microCT scan using Segment Editor



Used ANTsR to segment the otoliths using the atlas as a template



Example specimen is mutant fish bmp1a_06

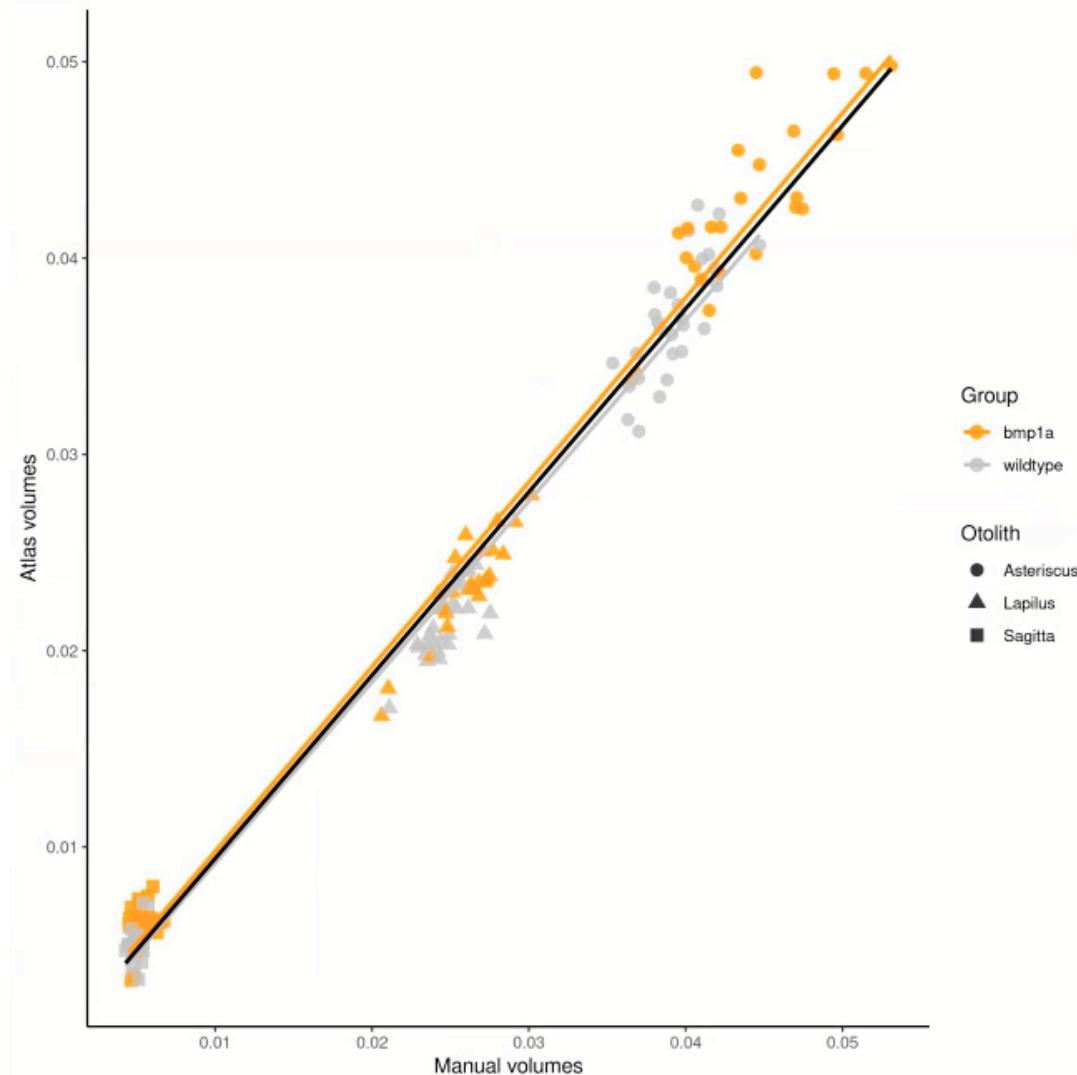


Payan et al., 2004

Testing the atlas

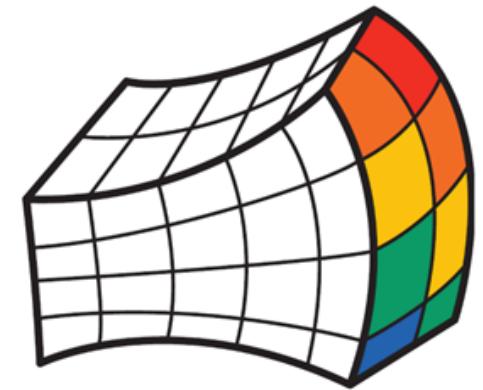
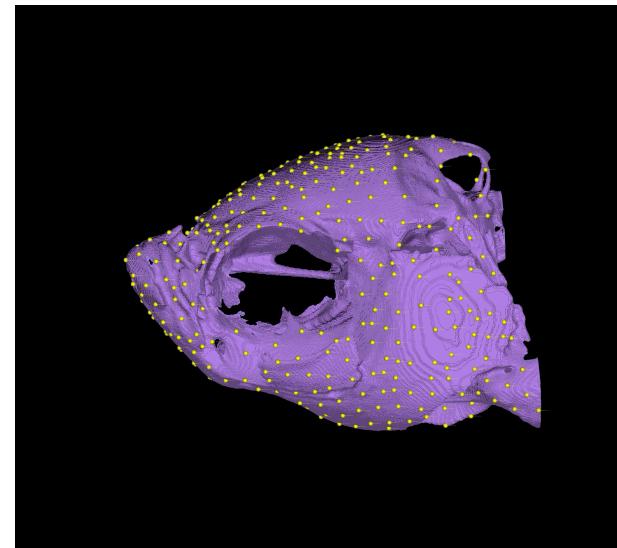
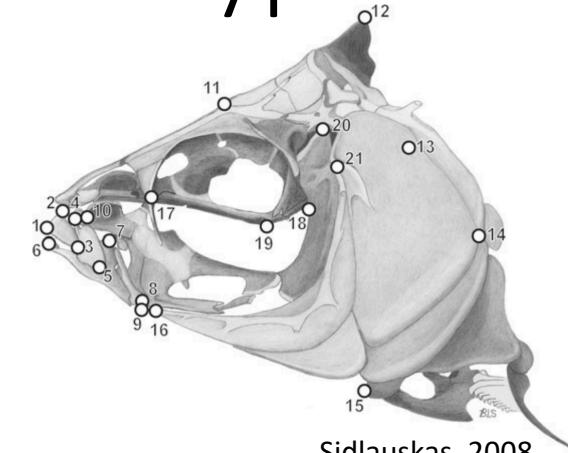
**Overall current atlas does a
decent job of segmenting
otoliths**

Model:
 $\text{Atlas volume} \sim \text{Manual volume}$
 $r = 0.991$

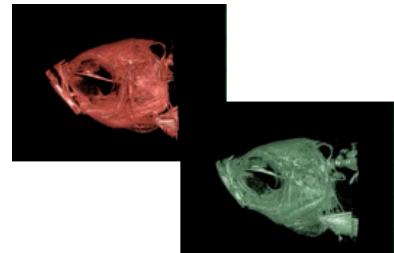


Using the atlas to quantify phenotype

- Traditional ways to quantify cranial morphology = landmarks
- Limitations: What if phenotype differs in areas where there are not good landmarks to use?
- **Proposal: Using pseudo-landmarks to quantify skull phenotype**

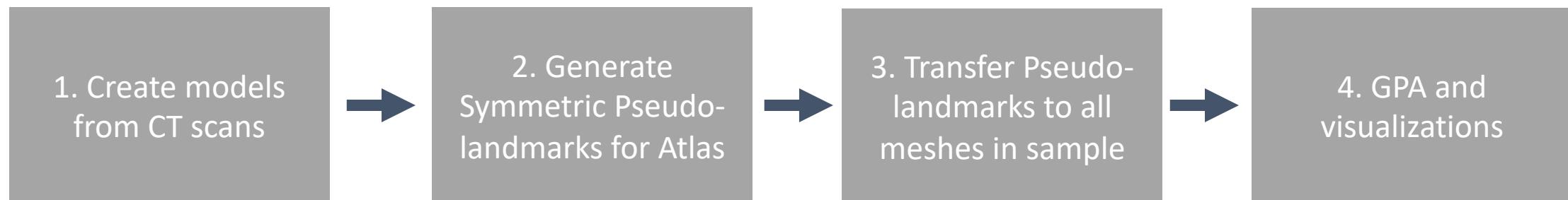


SLICERMORPH



Import CT scans

Slicer analysis pipeline



Segment Editor

Segmentations

Surface Toolbox

Markups

PseudoLMGenerator

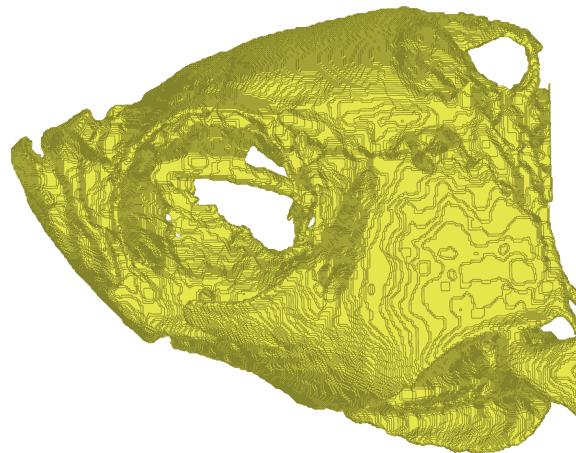
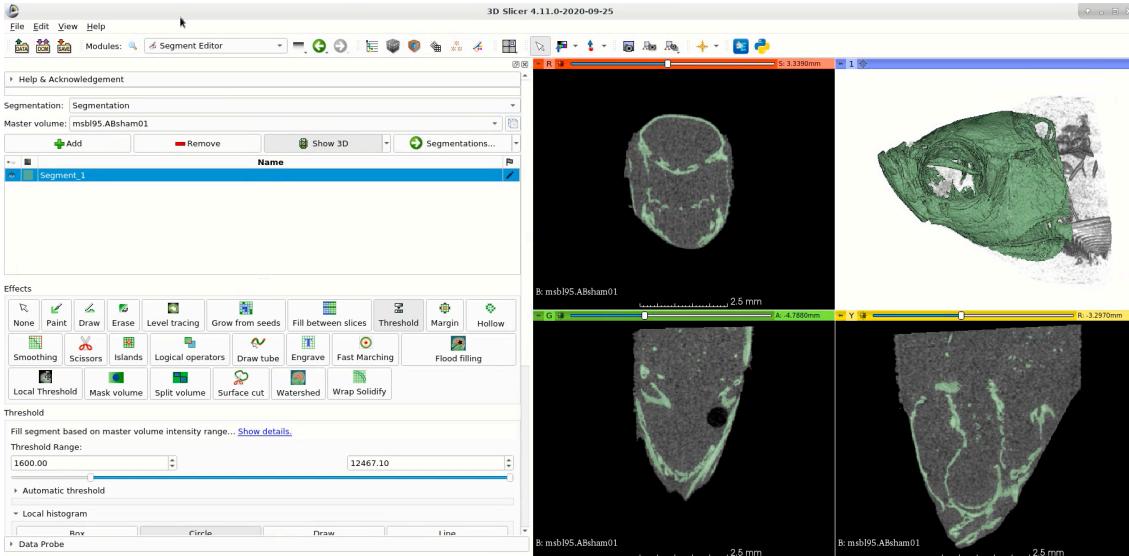
ALPACA

GPA

Output GPA for further analysis
and visualizations in R and
other 3D Slicer modules



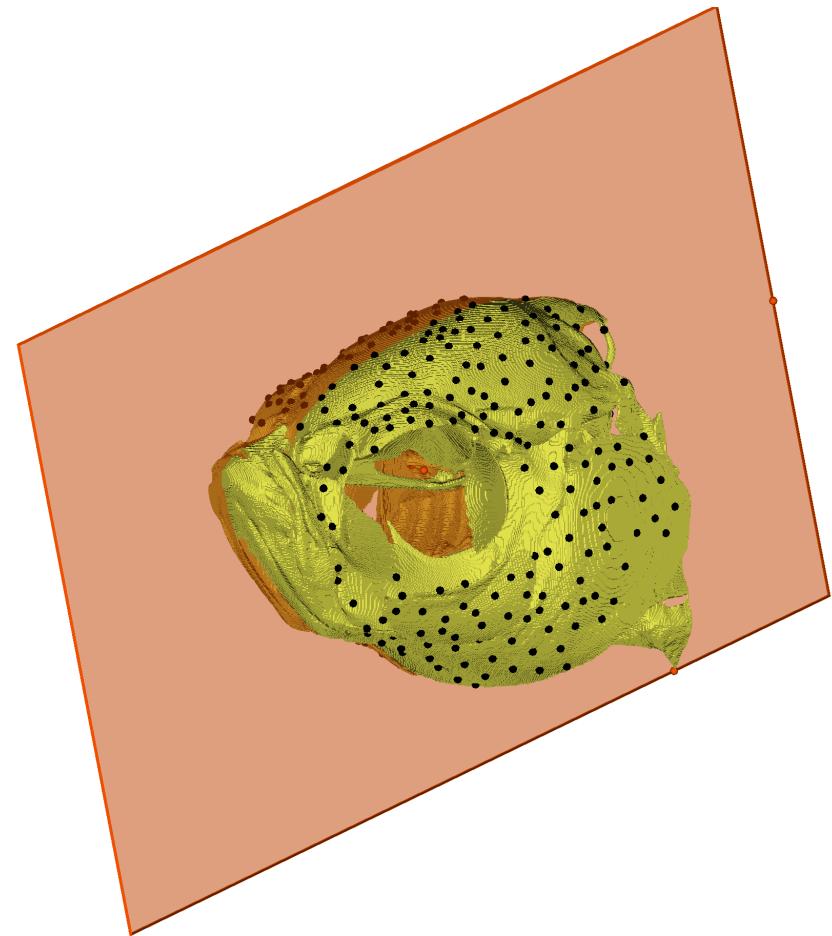
1. Create mesh models from ct volumes



- Used the **Segment Editor** module to create a segment of my ct scans that included only the bone using the tools:
 - Threshold (include only bone)
 - Islands (remove small islands)
 - Scissors (remove postcranial elements)
- Used the **Segmentations** module to create a model from my segmentation
 - Used the **Surface Toolbox** for model smoothing and decimation

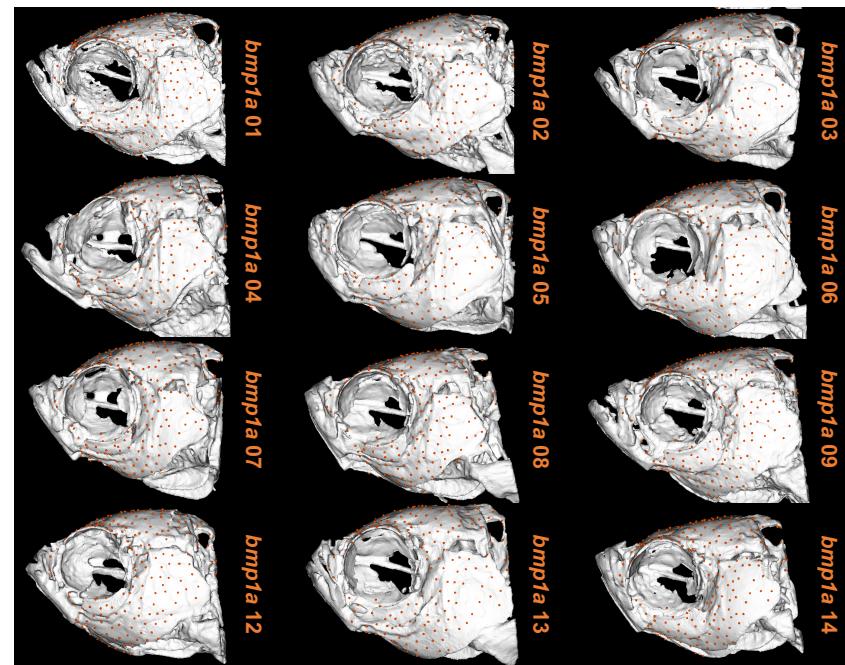
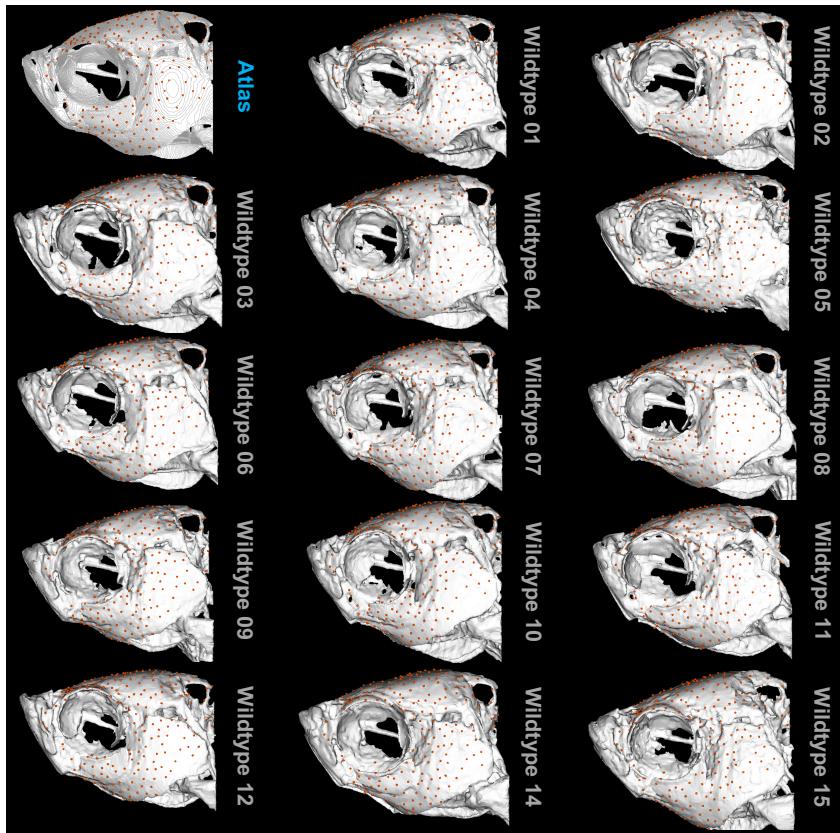
2. Generate symmetrical pseudo-landmarks

- First, I created a plane in the **Markups** module that separated the skull into left and right halves
- Next, I created a set of symmetric pseudo-landmarks using the **PseudoLMGenerator** module
- Finally, I used the **Markups** module to remove points from part of the atlas that I didn't want to include
 - After cleaning up the points there are 372 points on the atlas that I used for analysis



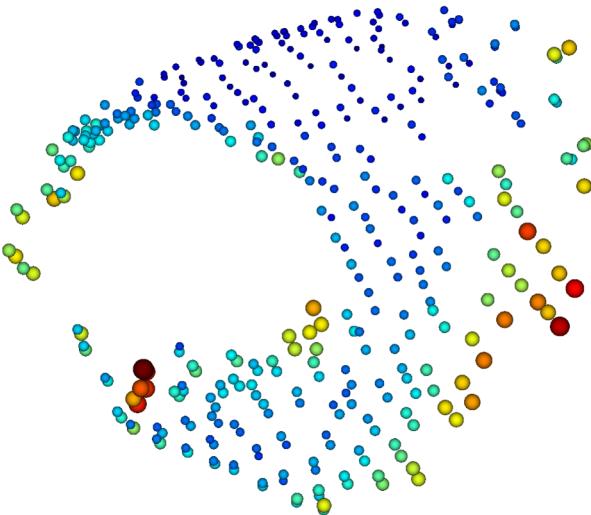
3. Transfer pseudolandmarks to samples

Used the ALPACA module to transfer the 372 pseudo-landmark points from the atlas to every fish in our sample

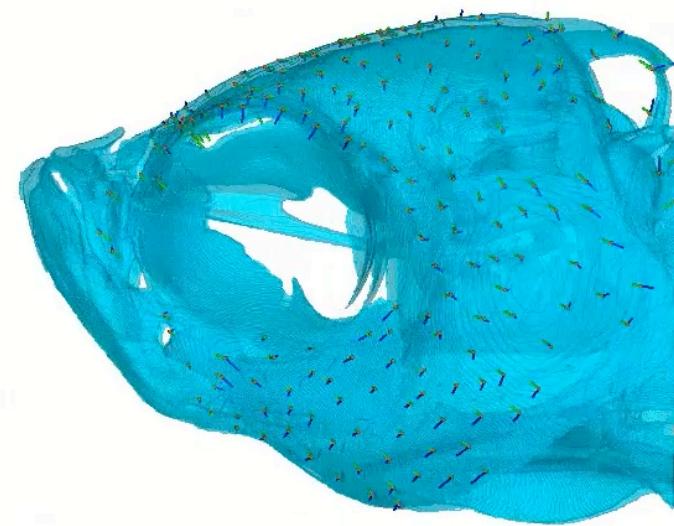


4. Analyzing pseudo-landmark points

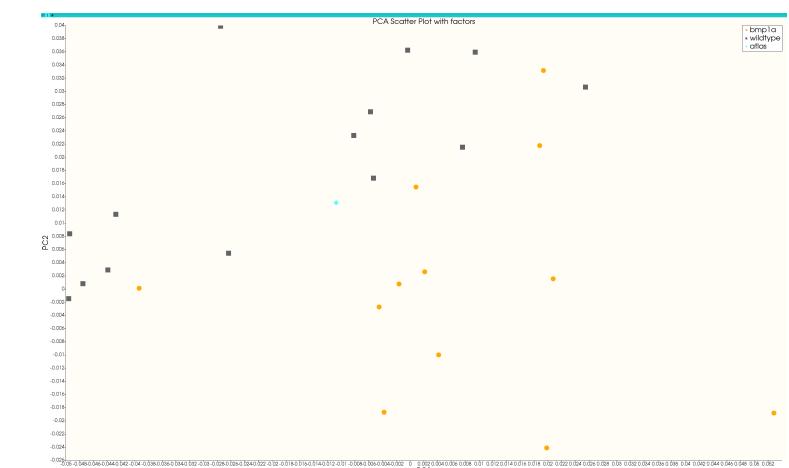
Ran a General Procrustes Analysis on pseudo-landmarks and visualized results using the **GPA** module in slicer



Visualizing variation in pLMs
(brighter colors = more variation)



Lollypop vector plot for pLMs



PC plot from GPA analysis

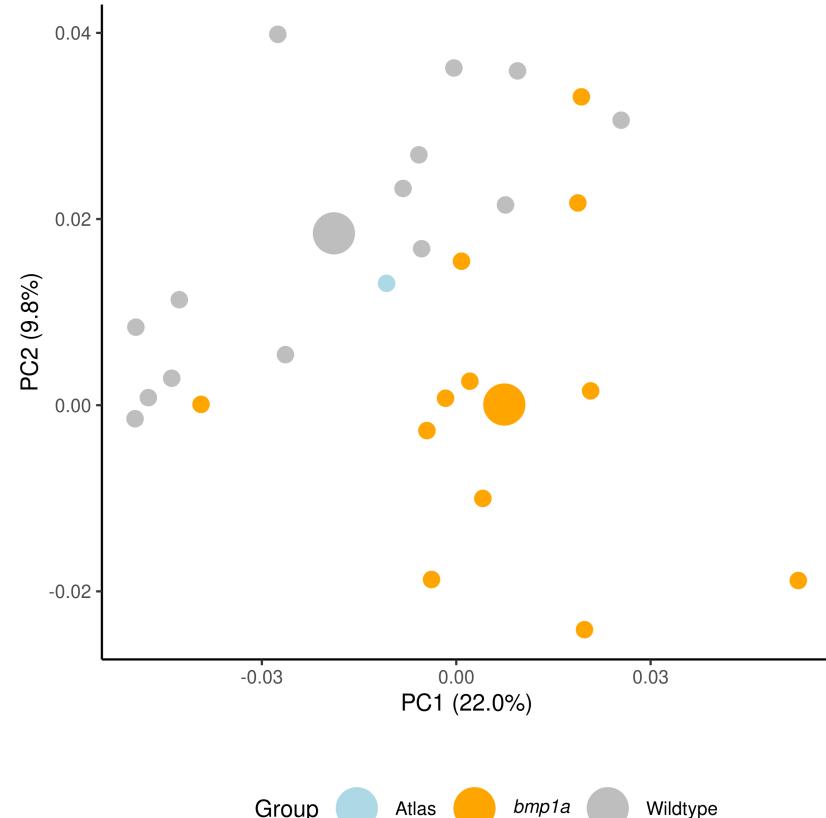
[Rolle et al., 2020 Biorxiv](#)

5. Further data analysis in R

Using the output from the GPA analysis I can do more visualizations and analysis in R

Including:

- Making better plots with *ggplot*
- Testing if PCs statistically vary between *bmp1a* and wildtype fish with *geomorph*
- Symmetry analysis with *morpho*



Larger shapes indicate average for each group and method

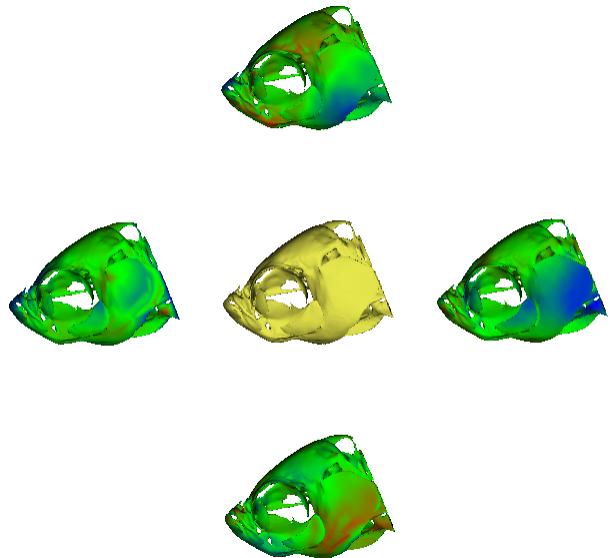
Diamond et al., BioRxiv

Symmetry visualizations

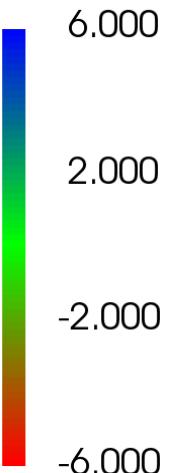
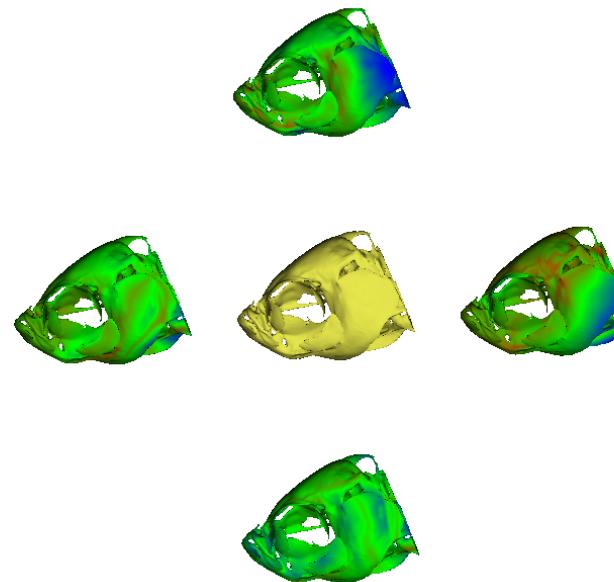
Using morpho in R I created average images of the symmetric and asymmetric components of shape variation. Then used the **Model to Model Distance** module to visualize where the different components of symmetry occur in our morphospace.

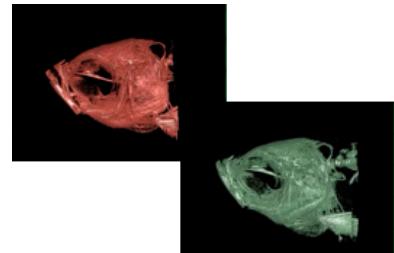
Then Loaded all meshes into the same view and used the **Transforms** module to create the visuals below.

Symmetric



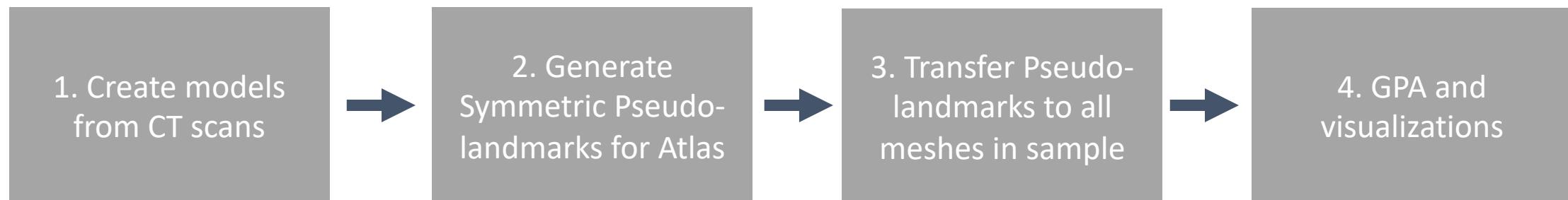
Asymmetric





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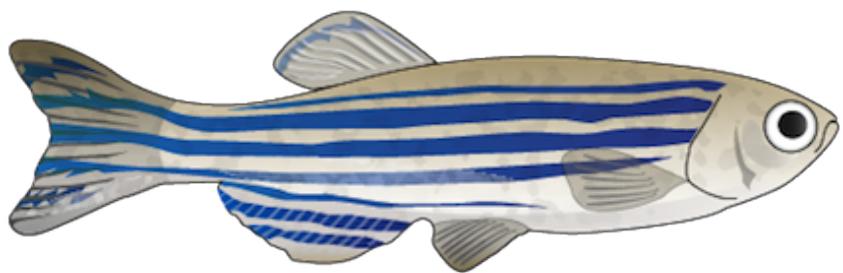
GPA

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Learn more here:

- Rolfe et al. SlicerMorph: An open and extensible platform to retrieve, visualize and analyze 3D morphology. BioRxiv
<https://doi.org/10.1101/2020.11.09.374926>
- Rolfe et al. 2021. Comparing semi-landmarking approaches for analyzing three-dimensional cranial morphology. Amer. J. Phys. Anthro.
<https://doi.org/10.1002/ajpa.24214>
- Porto et al. ALPACA: a fast and accurate approach for automated landmarking of three-dimensional biological structures. BioRxiv
<https://doi.org/10.1101/2020.09.18.303891>
- Diamond et al. Computational anatomy and geometric shape analysis enables analysis of complex craniofacial phenotypes in zebrafish mutants. BioRxiv
<https://doi.org/10.1101/2021.02.12.431035>



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