The Characterization of Remodeling and Non-Remodeling Craniofacial Defects in Pre-Metamorphic *Xenopus laevis* tadpoles

ALLEN DISCOVERY CENTER at Tufts University

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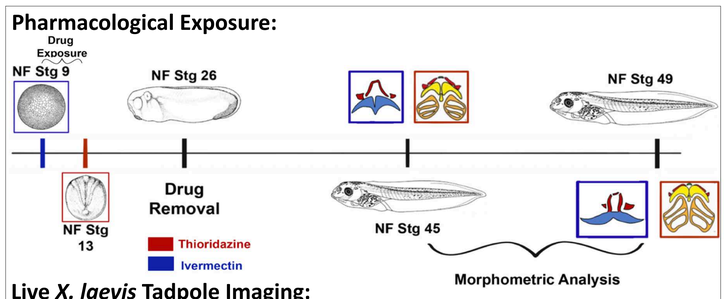
Background

- Craniofacial (CF) development is a highly complex and conserved process amongst vertebrate species, with the complexity resulting in an elevated likelihood of developing permanent CF defects.
- The *Xenopus laevis* (African clawed frog) model is used to study the biophysical cues of tissue remodeling in the CF defect correction pathways.
- We previously showed that CF defects defined by abnormal, but not missing, cartilage structures show some degree of remodeling prior to the onset of metamorphosis, defined by the Nieuwkoop and Faber (NF) stage 49.
- Recently, we identified Ivermectin (IVM) as a pharmacological treatment inducing CF defects that fail to remodel, despite containing all existing cartilage structures.
- In other species, the eyes have been associated with early CF development and cell migration processes.
- We hypothesized that eye tissue acts as a key player in the detection of CF defects.
- To test this hypothesis, we exposed *X. laevis* embryos to Thioridazine, resulting in eye defects, and Ivermectin, resulting in primarily ventral cartilage disfigurations.

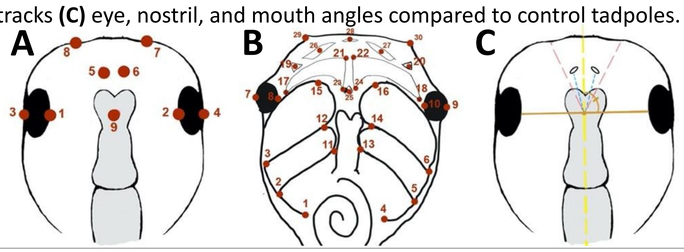
Hypothesis

Failing to induce eye tissue malformations will decrease the craniofacial remodeling of ventral cartilage disfigurations in *Xenopus laevis* tadpoles.

Methods



Landmark-based Geometric Morphometric Analysis: Parameters are shown for **(A)** dorsal and **(B)** ventral landmarks. Morphometric analysis tracks **(C)** eye, nostril, and mouth angles compared to control tadpoles.



Aims

1. **Elucidate the underlying mechanisms that initiate and direct craniofacial remodeling in pre-metamorphic *Xenopus laevis* tadpoles.**
2. **Characterize Ivermectin-induced, non-remodeling craniofacial defects compared to known remodeling phenotypes during pre-metamorphic stages using geometric morphometric mapping.**

Results

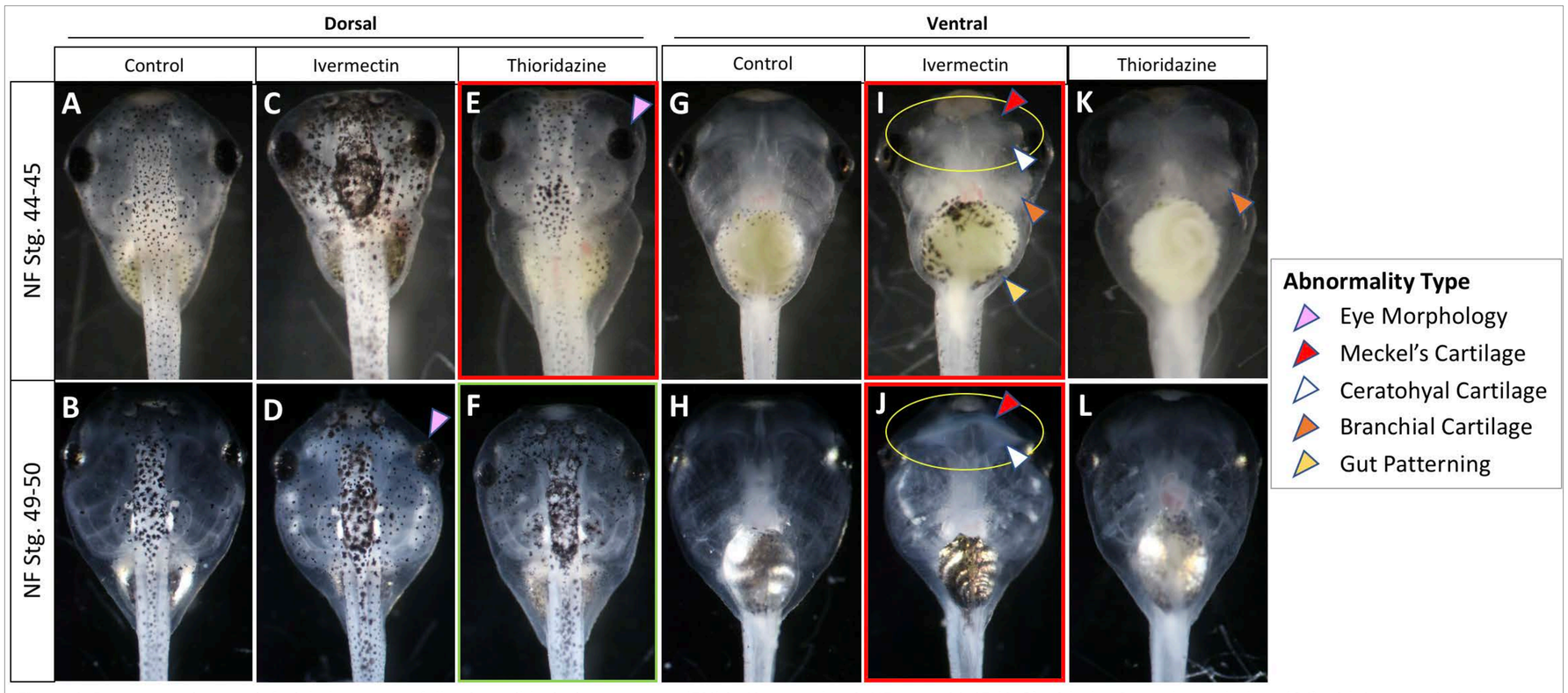


Figure 1. Representative craniofacial phenotypes following mild tricaine exposure (5%) in *Xenopus laevis* NF stage 44-45 **(A,G)** with the Control morphology (**C,I)** with Ivermectin-induced defects **(E,K)** with Thioridazine-induced defects at dorsal and ventral views, and NF stage 49-50 tadpoles **(B,H)** with the Control morphology (**D,J)** with Ivermectin-induced defects (**F,L)** with Thioridazine-induced defects at dorsal and ventral views. Arrowheads indicate common abnormalities present in treatment groups, such as eye shape. *n* = 25-30 tadpoles/group per stage.

Results, Continued

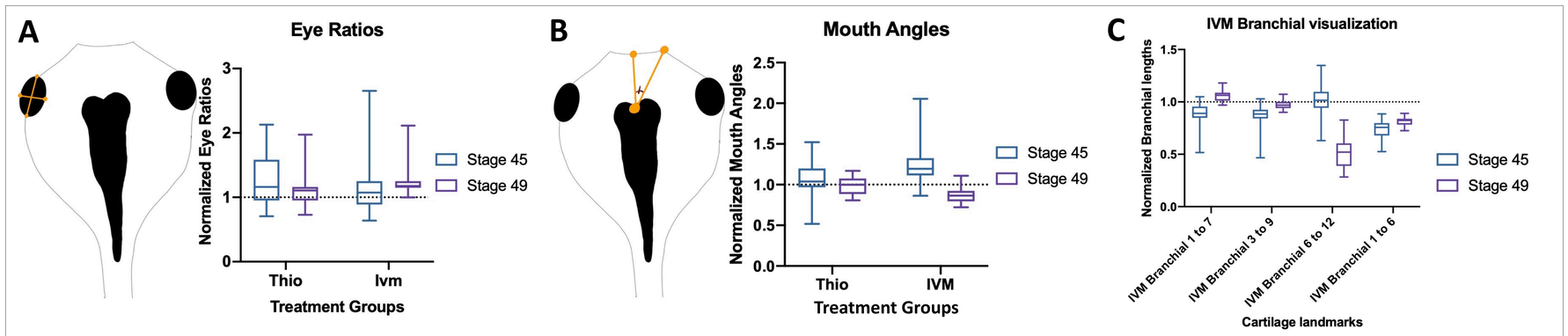


Figure 2. Geometric morphometric analysis of corrected abnormal craniofacial morphology. Morphological metrics at NF stage 44-45 and 49-50 for **(A)** eye ratios in Thioridazine- and Ivermectin-treated tadpole groups with the representative landmark schematic **(B)** mouth angles in Thioridazine- and Ivermectin-treated tadpole groups with the representative landmark schematic and **(C)** Branchial cartilage lengths for Ivermectin-treated tadpole groups, setting the control tadpole groups as the normalized baseline. *N* = 1-2 biological replicates.

Results, Continued

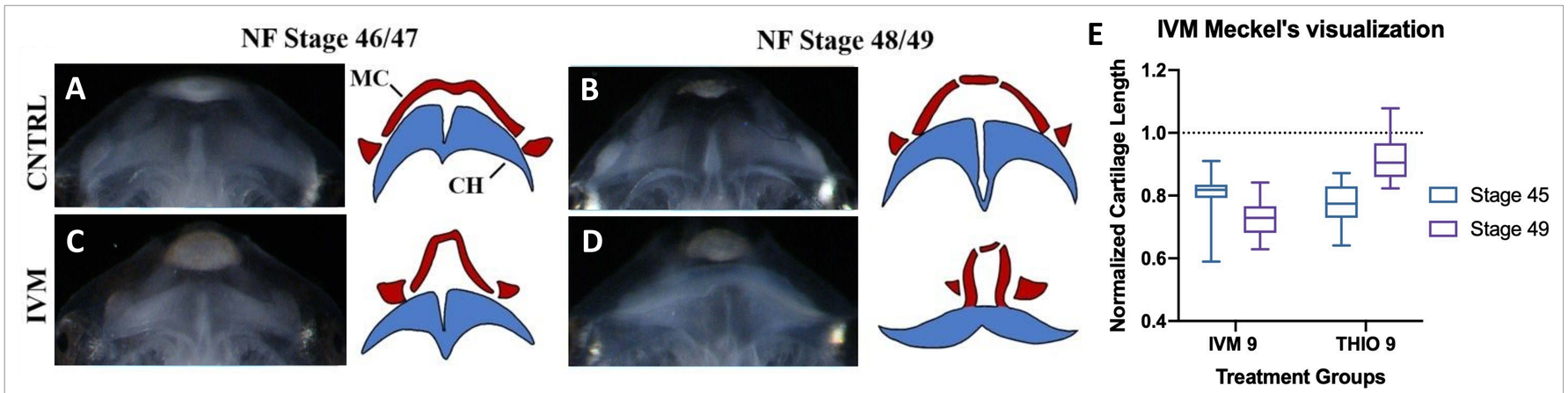


Figure 3. Representative images of anterior ventral cartilage phenotypes in Control tadpoles at **(A)** NF stage 46-47 **(B)** NF stage 48-49, and Ivermectin-treated tadpoles at **(C)** NF stage 46-47 **(D)** NF stage 48-49 with corresponding craniofacial schematics. Geometric morphometric analysis at NF stage 44-45 and 49-50 for **(E)** Meckel's cartilage orientation for Thioridazine- and Ivermectin-treated tadpole groups, setting the control group as the normalized baseline.

Conclusions

- **Thioridazine-treated tadpoles remodeled the observed cartilage disfigurations and eye abnormalities.**
- **Ivermectin treatment (2%) resulted in no significant remodeling of craniofacial structures.**
- **Eye abnormalities were absent in Ivermectin-treated tadpoles at NF stage 45. Ivermectin treatment resulted in significant eye and anterior ventral cartilage disfigurations at NF stage 49.**
- **Our findings suggest a link between the organizational role of eyes in later stages of CF development.**

Acknowledgment(s)

I would like to express my gratitude to Professor Kelly McLaughlin for welcoming me into her laboratory with guidance and mentorship. Special thank you to Ms. Emilie Jones for her assistance, teaching, and direction throughout this project. Thank you to everyone at the Allen Discovery Center for your willingness to share your research projects with me and to the National Science Foundation Louis Stokes Alliance for Minority Participation (LSAMP) Program for the opportunity to conduct this research.

This poster was supported by the Grant or Award Number, 1619629, funded by the National Science Foundation's Northeast Louis Stokes Alliance for Minority Participation (LSAMP) Program. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Science Foundation or the Directorate for Education and Human Resources.