Development of hatching ability in red-eyed treefrogs: escape from complications

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Introduction

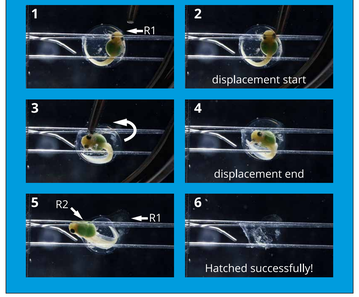
Hatching early allows embryos to escape threats to eggs, but increases risks to larvae. Red-eyed treefrogs, *Agalychnis callidryas*, hatch by rapidly releasing enzymes to digest a small hole in their membrane, then squeezing out aided by turgor pressure. Displacement from the initial hole can occur spontaneously and in predator attacks, complicating hatching by capsular collapse as fluid escapes.

Objective: Assess developmental changes in ability to recover from such complications

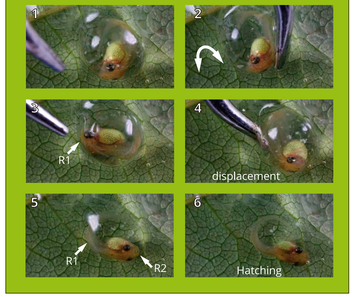
Methods

- Collected young *A. callidryas* clutches from pond, maintained in lab at Smithsonian Tropical Research Institute, Gamboa, Panama
- Induced individual 3– 5 day old embryos to begin hatching, using hypoxia and mechanosensory stimuli
- Manually displaced embryo in egg, interrupting exit through initial rupture
- Recorded macro-video until embryo exited its capsule; rescued individuals that failed to hatch after 30 minutes
- Analyzed only videos where manipulation was performed successfully

Flooding – we submerged younger eggs in hypoxic water (N = 25, 27 at ages 3 and 4 d)

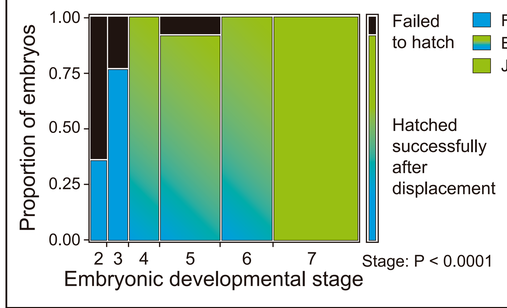


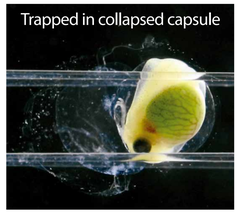
Jiggling – we used a blunt probe to move older eggs on clutches (N = 64, 51 at ages 4 and 5 d)



Results

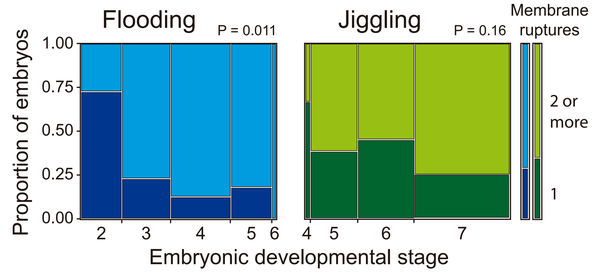
Escape-hatching success after complications





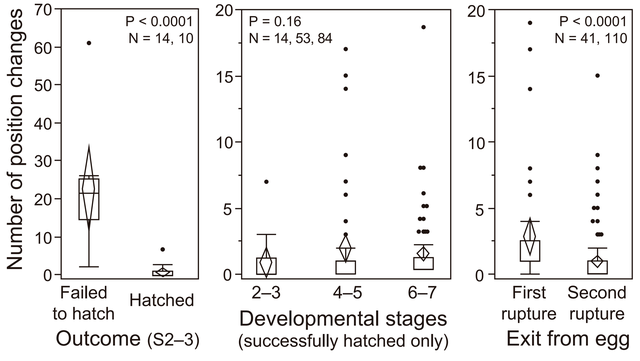
- Only the least developed embryos had high rates of hatching failure
- Ability to cope with hatching complications improved rapidly

Multiple membrane ruptures indicate reserve enzyme stores



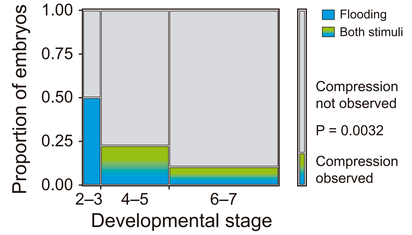
- Embryos hatched by re-finding initial rupture site or by making another
- Few stage 2 embryos made a second hole
- From stage 3, many embryos made multiple ruptures (2 or 3)

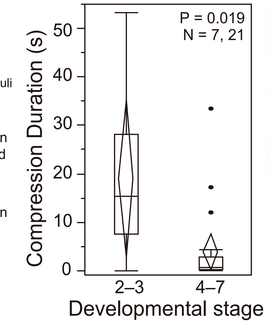
Position changes after displacement – search for initial rupture site

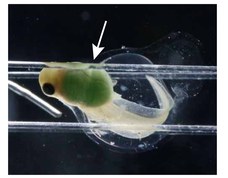


- S2-3 embryos that failed to hatch changed position many times, searching for exit
- Different stages expended similar search effort before successful hatching
- Across stages, embryos often made second ruptures after little or no searching

Body compression during exit

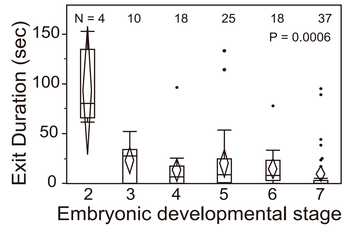






Compression indicates that hole is smaller than body

Exit duration



- We observed body compression as embryos passed through the membrane – more often in less developed embryos
- Less developed embryos showed body compression for longer periods
- More developed embryos passed through the membrane more rapidly

Conclusions

- Embryos escaped either by re-finding their first hole or by making another. Few Stage-2 embryos made a second hole but the likelihood of doing so increased rapidly, suggesting a build-up of a reserve supply of hatching enzyme.
- Many S2 embryos changed position many times, presumably in effort to re-find their first hole. More developed embryos also searched, but others immediately made another hole.
- Body compression during exit decreased with development; later stages made larger holes and passed through them more rapidly, likely due to both increased ennzyme reserves and greater muscular strength.

Along with high risk after hatching, less ability to escape from hatching complications may select against unnecessary hatching attempts at early developmental stages.

* Warkentin, K.M., J. Cuccaro Diaz, B.A. Güell, J. Jung, S.J. Kim & K.L. Cohen. In review. Developmental onset of escape-hatching responses in red-eyed treefrogs depends on cue type.

Acknowledgements

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