DATA DESCRIPTION, RAW DATA, AND CODE KEYS

VISIBLE IMPLANT ELASTOMER (VIE) SUCCESS IN EARLY LARVAL STAGES OF A TROPICAL AMPHIBIAN SPECIES

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Data Collection Methods

Study organism – Dendrobates tinctorius is a neotropical poison frog that transports their recently hatched larvae to ephemeral pools of water. In addition to the risk of desiccation, tadpoles face predation by their cannibalistic conspecifics, as well from heterospecifics (e.g. Odonate naiads) that occur in these pools (Rojas, 2014; 2015). The larval period lasts approximately 2 months in the wild (Rojas and Pašukonis, 2019) though the laboratory population has had a longer range (2.5-3 months).We used tadpoles from a breeding laboratory population of *Dendrobates tinctorius* kept at the University of Jyväskylä, Finland. Adult pairs were each housed in a 55L terrarium that contained layered gravel, leaf-litter, moss substrate and was equipped with a shelter, ramps, and live plants. Terraria were maintained at 26C (±2C) and were automatically misted with reverse osmosis water four times a day, maintaining a humidity around 95% and lit with a 12:12 photoperiod. Frogs were fed live *Drosophila* fruit flies coated in vitamin supplements three times per week. Tadpoles were raised singly in 10 x 6.5 x 5 cm cups which are filled with spring water, and fed an *ad libitum* diet of fish food (JBL NovoVert flakes) three times a week. Adult and tadpole health and water levels were checked daily, and experimental tadpoles were weighed and photographed weekly. Experiments began in October 2019 and continued through April 2020. This experiment was permitted by the National Animal Experiment Board (ESAVI/9114/04.10.07/2014).

Tags— Visible implant elastomers are a 2-part silicone-based polymer that is injected as a liquid that hardens to a pliable consistency once warmed (VIE; Northwest Marine Technology Inc.). The result is a small color band on the surface of the animal that can be detected by the naked eye. There is a range of 10 possible colors for application, 6 of which

are fluorescent. Visualization of fluorescent tags can be enhanced using a UV light. VIE tags have been successfully used in diverse taxa across developmental stages (e.g. echinoderms: Martinez et al. 2013; fish: Croft et al., 2003; salamanders: Campbell Grant, 2008).

Anesthesia—Prior to tagging, tadpoles were anesthetized in a 14mL solution of a 1µl:1mL ratio of 2-PHE to spring water. 2-PHE is an oily liquid at room temperature and does not need to be buffered for anesthetic purposes (Coyle et al., 2004). The solution was reused multiple times for multiple tadpoles within a single day of tagging (max. 10 tadpoles tagged each session); its effect did not deteriorate after multiple uses. Each day of tagging a new solution was made. Tadpoles were placed in an anesthetic solution until there was no muscular contraction in response to agitation; this process took approximately 3 minutes. We assumed the anesthetic's potency did not degrade because the latency of its effects remained consistent after being applied to multiple tadpoles. The effect of anesthesia on tadpoles lasted approximately 6 minutes; within 10 minutes individuals had regained full muscular function. The effects of anesthesia were similar across developmental stages (Gosner 24-26). We had no deaths in response to our anesthesia procedure which was applied to a total of 40 individuals across both our pilot study and experimental manipulations.

Tadpole tagging—We applied VIE tags to early larval stages of *D. tinctorius* and monitored tadpoles across development (Fig 1) to ensure the presence of the tags over time, and to test the effects of larval tagging and tag retention. Previous studies reporting tadpole tagging have been done primarily with late-term tadpoles (Gosner stage 30+) whose snout-vent lengths (SVL) were double or triple the SVL of tadpoles in our experiment (Andis, 2018; Bainbridge et al., 2015; McHarry et al., 2018). Other studies also worked with amphibians who produce large egg clutches (*Litoria aurea*, 37000 eggs/clutch (Pyke & White, 2001); American bullfrog, 12000 eggs/clutch (Howard, 1978); *Alytes obstetricans*, 50 eggs/clutch (Reading & Clarke, 1988)), which allowed for large tag sample sizes (n = 53-90, depending on study). *Dendrobates tinctorius* lay clutches that range from 2 to 5 eggs with a high level of mortality (Rojas & Pašukonis, 2019). Due to the reproductive limitations of the system, our sample total (n = 27 tagged, n = 11 control) is less than previously published data.

Elastomer was mixed and loaded into syringes prior to each tagging session, according to the Northwest Marine Technology VIE tag protocol. Elastomer was stored in a freezer (-20C) during extended periods of disuse and in a refrigerator between individual tagging sessions;

we found that mixed elastomer was no longer applicable after a storage period of longer than three months. Average tagging procedure was executed in under 90 seconds. Throughout our pilot study we found that tag retention was most effective when placed dorsally; thus, this experiment only contained dorsally marked tadpoles. Each tadpole was marked only once.

Immediately after being anesthetized, tadpoles were prepared for tagging. This was done by removing tadpoles from the anesthetic solution and placing them on a laminated surface where they were dried with a paper towel to improve grip. Once excess moisture was removed from the body (without completely drying out the tadpole), a 3 mL insulin syringe with a 30 G/12 7 mm needle was placed subcutaneously and dye (approx. 1 µl) was injected. For this experiment, we used a fluorescent green elastomer, though any color tag would have been suitable for application. After tag injection, tadpoles were placed under UV light to ensure proper placement of the tag. Proper placement was qualified as the tag being injected deep enough to not fall out (directly under epidermis) but shallow enough to be visible with the help of a UV light. Tadpoles were then cleaned with spring water and the status of the tag was checked again. Tadpoles post-tagging were placed in a pool of spring water and observed for 10 minutes to ensure proper return of muscular function. After the observation period, tadpoles were returned to the pool of water in which they were living

Data Key

Raw data can be found on Elastomer_Final.xlsx data file.

Treatment: Two level factor where “Tag” is a tagged tadpole and “Control” is a non-tagged tadpole.

ID: Each experimental tadpole was given a unique identification code. Once assigned to a tadpole, the code did not change for the entire experiment.

Week: Indicates the time period of the experiment. Tadpoles were tagged at “Week 0” and experiment followed their development (tag state, size, survival) each week.

Tag_Date: The calendar date of data recording. Each week all experimental tadpoles (tagged and control) were looked at.

Weight: Tadpole weekly weight at time of observation.

Tag_Y_N: Binomial response variable where tag was observed (Y) or not (N). For control tadpoles these rows are marked NA.

Photo_Y_N: Although this variable was not used for analysis, it was used to keep track of tadpoles in the laboratory population.

Dead: Binomial response variable where tadpoles were either alive (0) or dead (1).

DOD: Date of death of tadpoles.

Observer: Initials of person conducting observation of experimental tadpoles for a given week. Although there were 3 total observers, CF did over 60% of total observations. For a given week, a single person did all of the observations.

Code key

vie_models_final.R: Bayesian R code of observation and retention models for elastomer tags across development.

vie_plots.R: Accompanying code to vie_models which visualizes the models with the lowest DIC value. Generated graphs from this code are used for the figures presented in the final version of the manuscript.

vie_growth_survival.R: R code for modeling and visualizing growth and survival of tadpoles across time. Surival rates are compared using Cox mixed effects models.