

A new fast easy inexpensive permanent noninvasive

# method for batch-marking amphibians across developmental stages

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## 1. INTRODUCTION

Marking amphibians via submersion in liquid **calcein** (a fluorochrome that binds to **calcified tissues**) provides a new option in field identification that overcomes limitations of current marking methods.

|                                  | Speed    | ease     | Noninvasive          | Inter-stage | Batch    | Field administer | Size independent | Stage independent | Permanent | Safe     |
|----------------------------------|----------|----------|----------------------|-------------|----------|------------------|------------------|-------------------|-----------|----------|
| Toe clip                         | Positive | Positive | Neutral or uncertain | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Subcutaneous (VIE, tattoo, etc.) | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| PIT                              | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Skin branding                    | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Banding                          | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Topical dyes                     | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Oxytetracycline                  | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Calcein (intra-stage)            | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |
| Calcein (inter-stage)            | Positive | Positive | Positive             | Positive    | Positive | Positive         | Positive         | Positive          | Positive  | Positive |

Legend: Positive (Green), Neutral or uncertain (Hatched), Negative (White)

## 2. EXPERIMENT

Tested impacts of calcein on growth and mortality and detection probabilities over time in two age classes of **Wood frogs (*Rana sylvatica*)**.

### INTRA-STAGE

Larvae were marked at < **Gosner stage 30** and monitored through **metamorphosis (stage 42)**.

### INTER-STAGE

Larvae were marked between **stages 30-42** and monitored for >**125 days** as juveniles.

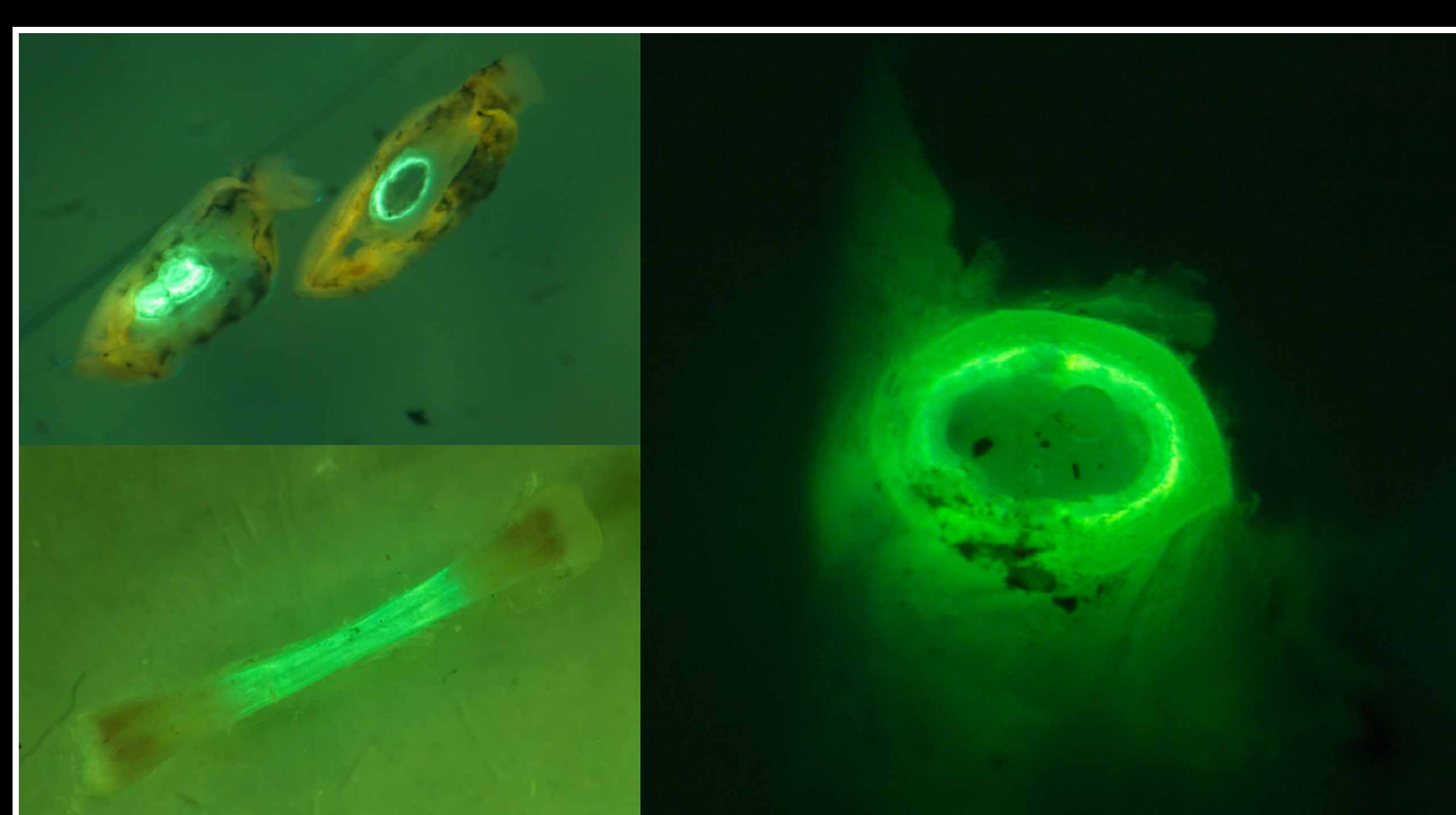
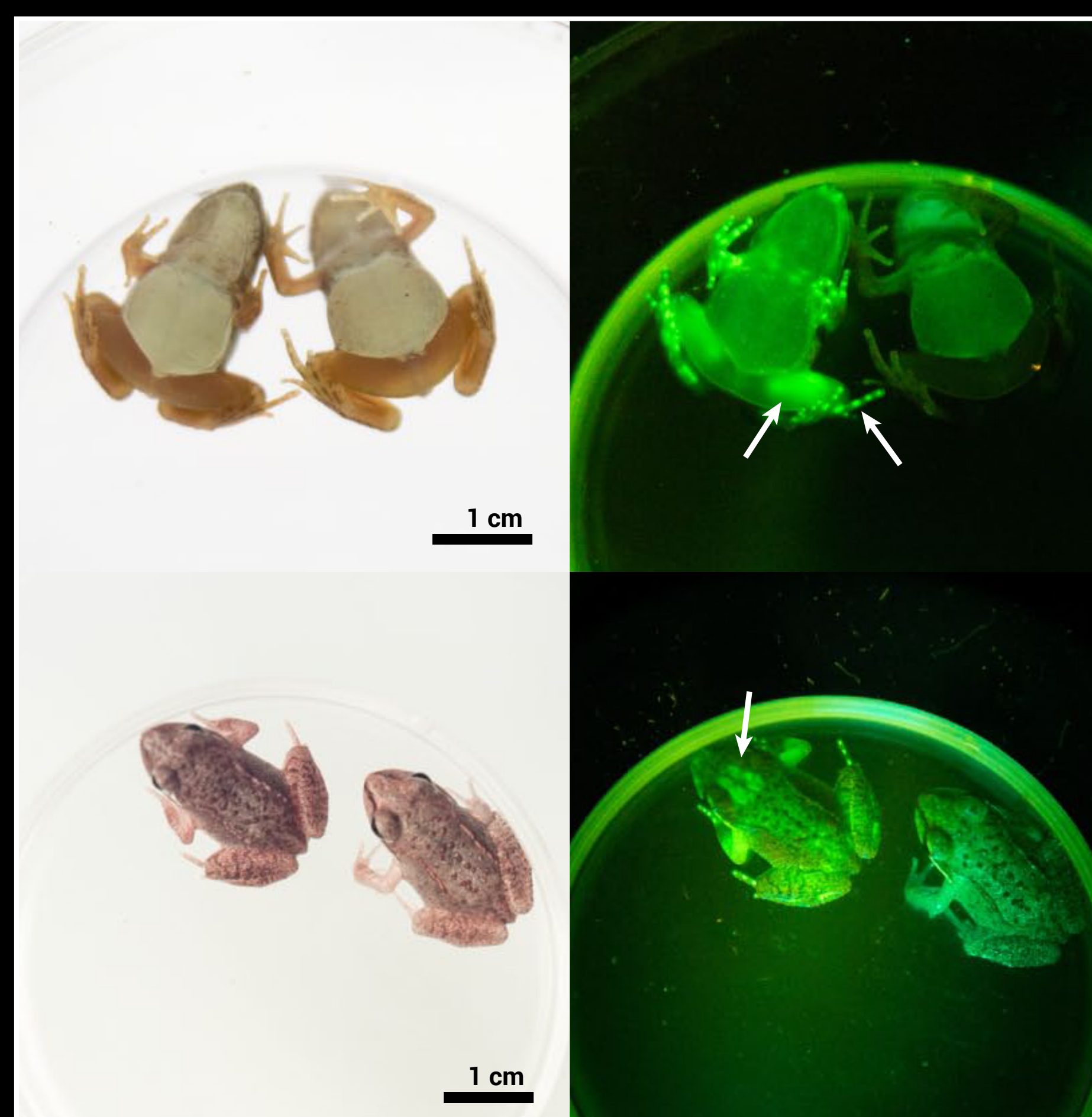
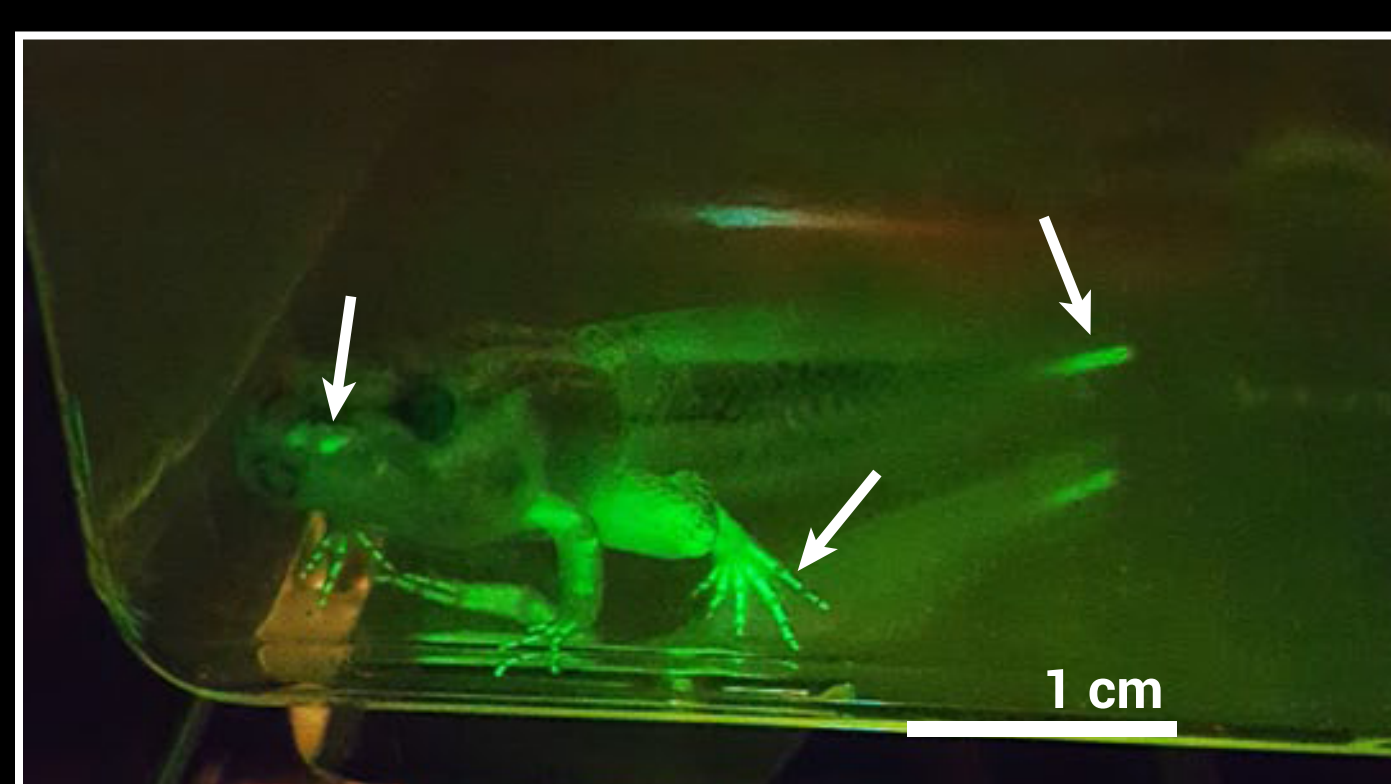
### MARKING

- 1% calcein solution for 3.5 minutes
- Marked with and without osmotic induction (pre-bath of 1% saline solution for 3.5 minutes)

### DETECTION

- External, in vivo, with NIGHTSEA BlueStar handheld 440-460nm flashlight and cancellation glasses (Electron Microscopy Sciences; Hatfield, Pennsylvania, USA)
- Bone cross-sections (phalanges and tibi-ofibula) post-mortem with stereo dissecting scope.

## 3. RESULTS



### GENERAL

- No impact on mortality or growth
- Invisible to the naked-eye
- Persistence of mark is contingent on mass and developmental stage
- No false positives

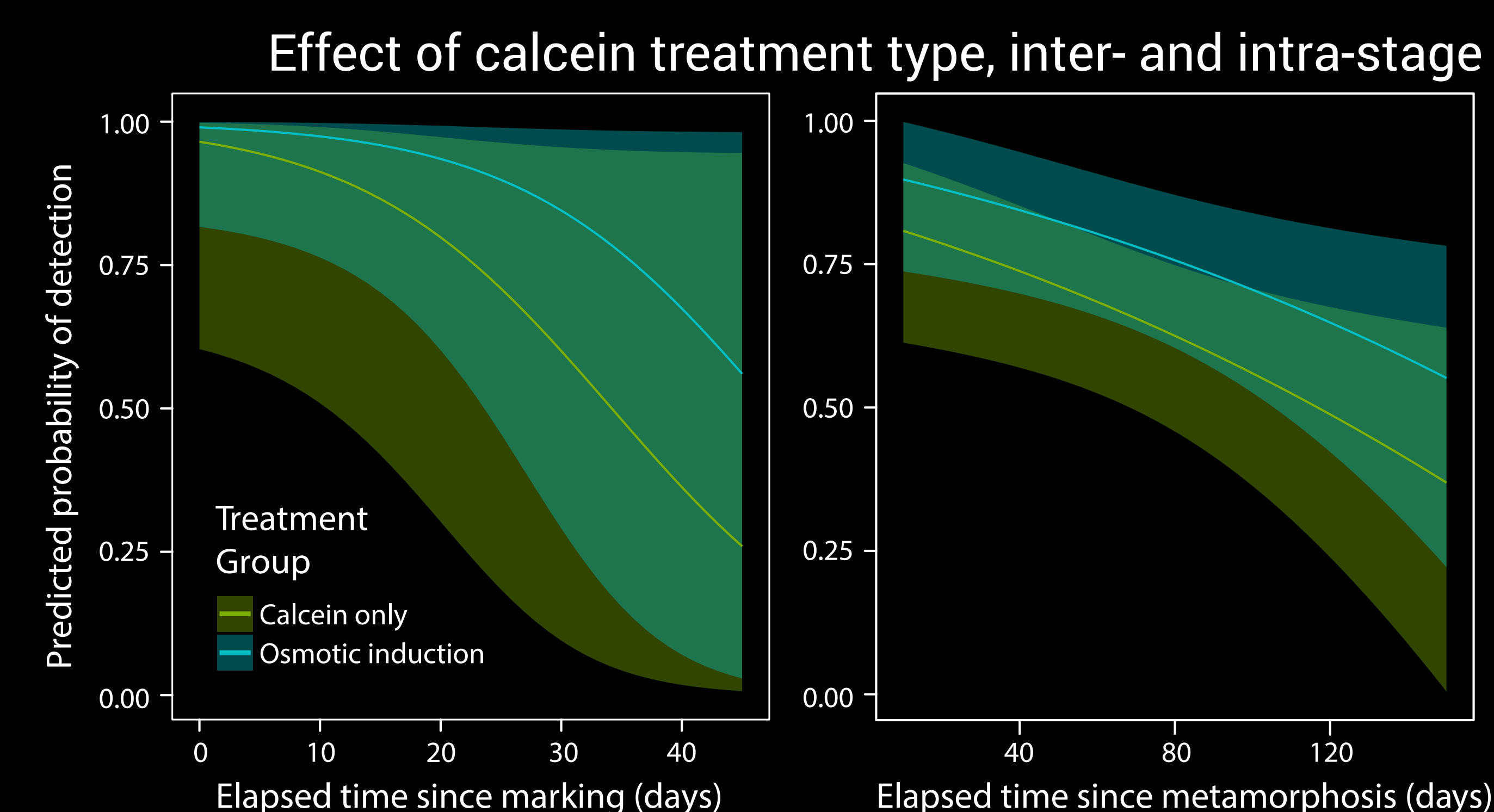
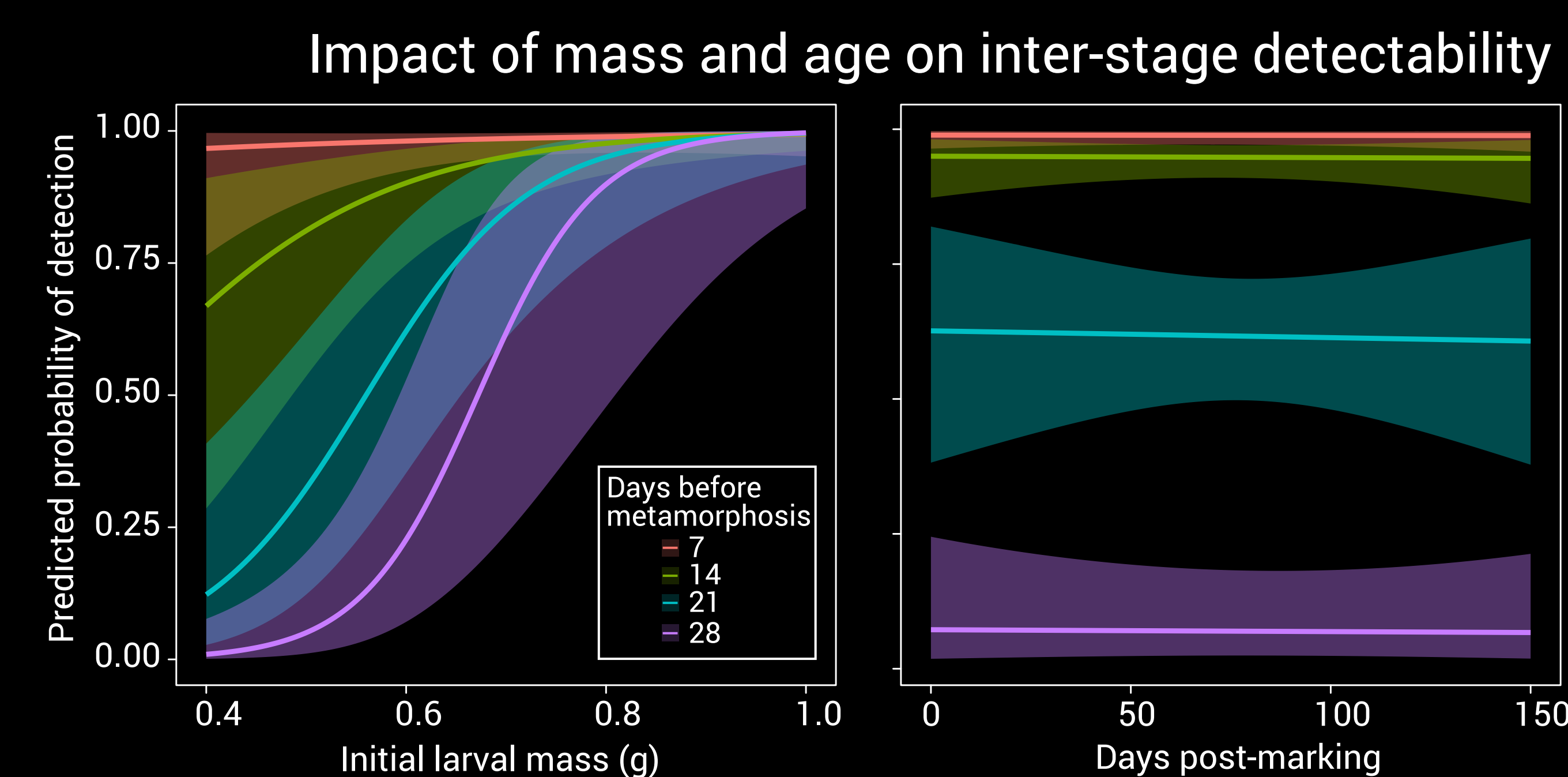
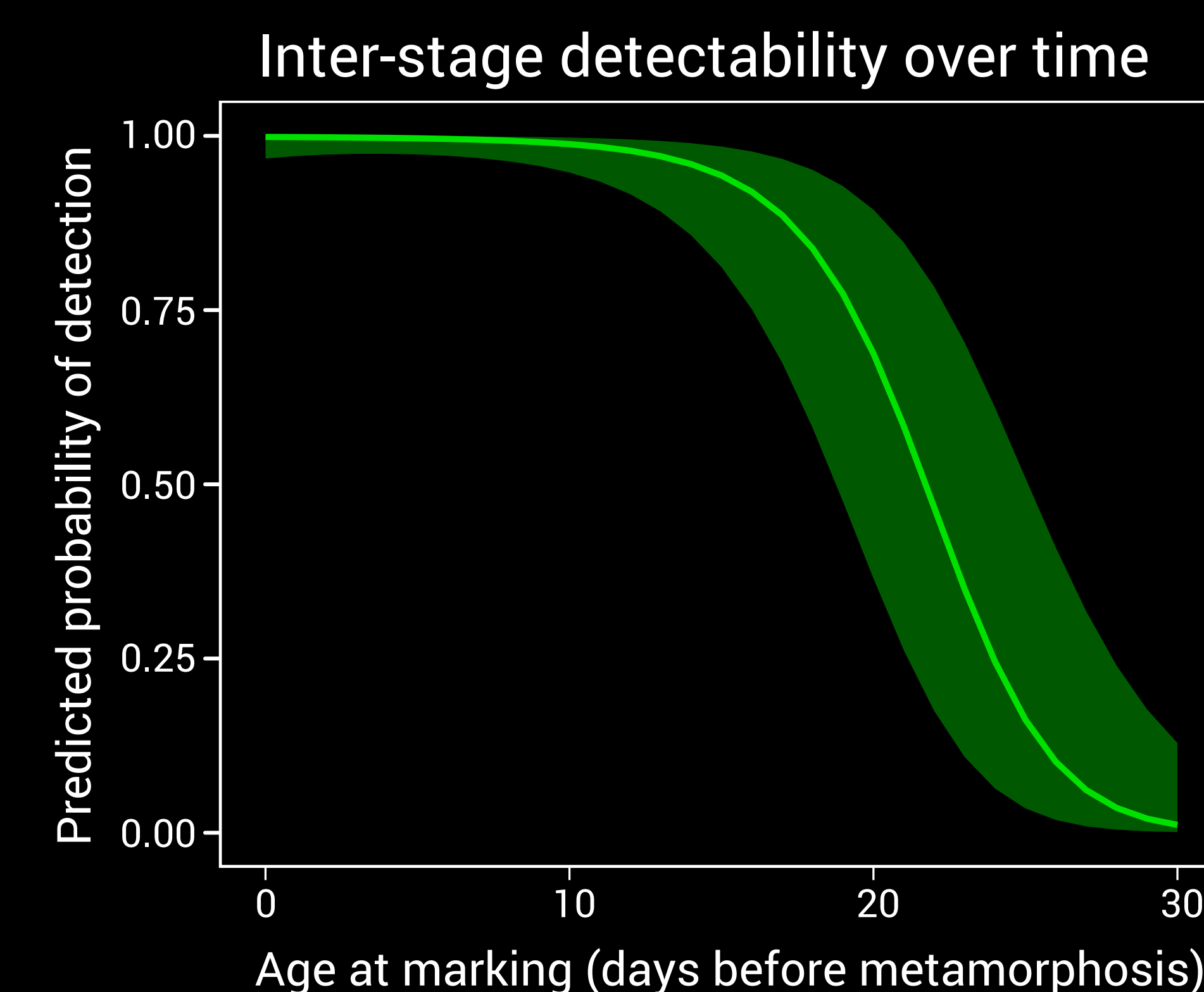
### SHORT-TERM

- Short-term, calcein is easily detectable throughout integument immediately and in the skeleton and tail after 3-4 days

- 99% detectability up to 20 days post-treatment for larvae marked within 28 days of metamorphosis
- 90% if marked within 34 days

### LONG-TERM

- Long-term, calcein is detectable in bones through translucent skin
- 99% detectability up to 146 days post-treatment for larvae marked within 10 days of metamorphosis
- 90% if marked within 16 days
- The mark is detectable in skeletal cross-sections, but more reliable in external observation



## 4. CAVEATS

- Ontogenic timing is critical
- UV exposure may attenuate the label
- Acidic or hypoxic environment may decrease marking efficacy
- Skeletal growth post-marking may dilute the label
- Detectability depends on minimal ambient light and intensity of the excitation beam

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