

SUPPLEMENTAL INFORMATION

A NEW, NONINVASIVE METHOD OF BATCH-MARKING AMPHIBIANS ACROSS DEVELOPMENTAL STAGES

A. Z. ANDIS

School of Forestry and Environmental Studies, Yale University, 370 Prospect Street, New Haven,

Connecticut, USA, e-mail: a.andis@yale.edu

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TABLE S1. Experiment design and treatment groups.

Treatment group	Bath 1 (3.5 min)	Bath 2 (3.5 min)	Rinse
A (control)	RD water	RD water	RD water
B (salt only)	1% NaCl	RD water	RD water
C (calcein only)	RD water	1% calcein	RD water
D (osmotic induction)	1% NaCl	1% calcein	RD water

TABLE S2. Inter-stage experiment: summary of hierarchical logistic regression for variables predicting mortality (65 d post-treatment) of Wood Frogs marked as larvae (n = 80).

variable	Model 1				Model 2			
	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>
Treat B	0.46	0.97	0.20	0.635	0.47	0.97	0.20	0.631
Treat C	1.21	1.20	0.53	0.314	1.22	1.20	0.53	0.311
Treat D	0.46	0.97	0.42	0.635	0.44	0.98	0.19	0.651
Initial Mass					0.90	2.21	0.16	0.682
DF	76				75			
AIC	58.86				60.69			

TABLE S3. Inter-stage experiment: summary of hierarchical regression for variables predicting growth of Wood Frog larvae from initial mass at time of treatment to 65 d post-treatment. Animals that died prior before 65 d were excluded from the analysis (n = 72).

variable	Model 1				Model 2				Model 3				Model 4			
	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>
Treat B	-0.01	0.03	-0.05	0.742	-0.01	0.03	-0.04	0.740	-0.01	0.03	-0.04	0.739	-0.01	0.03	-0.04	0.751
Treat C	0.02	0.03	0.08	0.596	0.01	0.03	0.07	0.575	0.01	0.03	0.07	0.594	0.01	0.03	0.07	0.585
Treat D	0.02	0.03	0.11	0.447	0.01	0.03	0.05	0.694	0.01	0.03	0.05	0.697	0.01	0.03	0.05	0.705
Mass					0.24	0.05	0.50	0.000	0.23	0.07	0.48	0.001	2.66	2.60	0.44	0.310
Time									0.00	0.00	-0.04	0.779	0.00	0.00	-0.12	0.431
Mass*Time													-0.01	0.01	-0.12	0.354
DF	68				67				66				65			
R^2	0.02				0.27				0.27				0.28			
<i>F</i> for change in R^2					22.65				0.08				0.87			
(<i>P</i> -value for <i>F</i>)					(0.00)				(0.78)				(0.35)			

TABLE S4.

Inter-stage experiment: summary of hierarchical regression for variables predicting final mass of Wood Frog larvae from initial mass at time of treatment to metamorphosis. Animals that metamorphosed within one week of treatment were excluded from the analysis ($n = 49$).

variable	Model 1				Model 2				Model 3				Model 4				
	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>	<i>b</i>	SE	β	<i>P</i>	
Treat B	0.01	0.05	0.03	0.85	-0.01	0.05	-0.03	0.87	-0.03	0.02	-0.12	0.17	-0.03	0.03			0.21
Treat C	-0.05	0.05	-0.19	0.31	-0.06	0.05	-0.22	0.21	-0.05	0.02	-0.17	0.06	-0.05	0.02			0.07
Treat D	0.01	0.05	0.04	0.81	0.00	0.05	0.00	1.00	-0.02	0.03	-0.07	0.38	-0.02	0.03			0.40
Initial Mass					0.34	0.12	0.38	0.01	0.80	0.07	0.91	0.00	-1.33	3.98			0.74
Time to meta									0.02	0.00	0.95	0.00	0.01	0.01			0.03
Mass*Time													0.01	0.01			0.59
DF	45				44				43				42				
R^2	0.05				0.19				0.80				0.80				
<i>F</i> for change in R^2					29.76				126.56				0.29				
(<i>P</i> -value for <i>F</i>)					(0.00)				(0.00)				(0.59)				

TABLE S6. Inter-stage experiment: Comparison of detecting calcein label via noninvasive whole-animal observation of live individuals to invasive observation of tibiofibular and phalange cross-section.

	Total marked	Live, noninvasive observation	Tibiofibular cross-section	Phalange cross-section
Individuals detected	39	30	28	18
Percent of total	100%	77.0%	71.8%	46.2%
Percent of individuals detected noninvasively		100%	93.3%	60.0%

TABLE S7. Intra-stage experiment: Percent true positive, non-lethal detections of calcein label in larval Wood Frogs by days post-treatment and immediately upon metamorphosis (Gosner stage 42 [GS 42]).

	n	Days post-marking				
		1	8	15	24	GS 42
Treatment C	8	100%	62.5%	62.5%	50.0%	62.5%
Treatment D	5	100%	100%	100%	80.0%	40.0%

TABLE S8. Field catalog numbers of specimens included in this study and accessioned to the Yale Peabody Museum of Natural History.

AZA 0885	AZA 1287	AZA 1331	AZA 1361	AZA 1381
AZA 0886	AZA 1288	AZA 1332	AZA 1362	AZA 1382
AZA 0887	AZA 1289	AZA 1333	AZA 1363	AZA 1383
AZA 0890	AZA 1290	AZA 1334	AZA 1364	AZA 1384
AZA 0895	AZA 1291	AZA 1335	AZA 1365	AZA 1385
AZA 0896	AZA 1297	AZA 1336	AZA 1366	AZA 1386
AZA 0897	AZA 1317	AZA 1337	AZA 1367	AZA 1387
AZA 0898	AZA 1318	AZA 1338	AZA 1368	AZA 1388
AZA 0899	AZA 1319	AZA 1339	AZA 1369	AZA 1389
AZA 0900	AZA 1320	AZA 1340	AZA 1370	AZA 1390
AZA 0904	AZA 1321	AZA 1341	AZA 1371	AZA 1391
AZA 0905	AZA 1322	AZA 1342	AZA 1372	AZA 1392
AZA 0906	AZA 1323	AZA 1343	AZA 1373	AZA 1393
AZA 0907	AZA 1324	AZA 1344	AZA 1374	AZA 1394
AZA 0913	AZA 1325	AZA 1345	AZA 1375	AZA 1395
AZA 0914	AZA 1326	AZA 1346	AZA 1376	AZA 1396
AZA 1283	AZA 1327	AZA 1347	AZA 1377	AZA 1397
AZA 1284	AZA 1328	AZA 1348	AZA 1378	AZA 1398
AZA 1285	AZA 1329	AZA 1359	AZA 1379	AZA 1399
AZA 1286	AZA 1330	AZA 1360	AZA 1380	AZA 1400
				AZA 1401

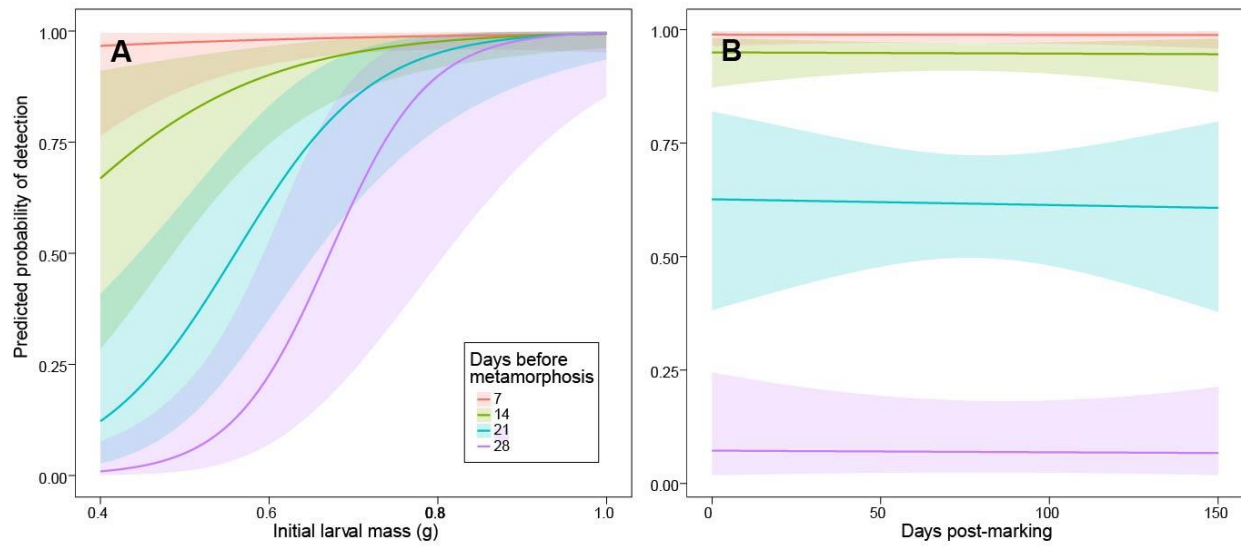


Figure S1. Inter-stage experiment: predicted probability of detecting a calcein label 146 d after marking in a Wood Frog at various age classes and mass (A). For a Wood Frog of average mass within each age class, detection rates are not predicted to diminish over time (B).

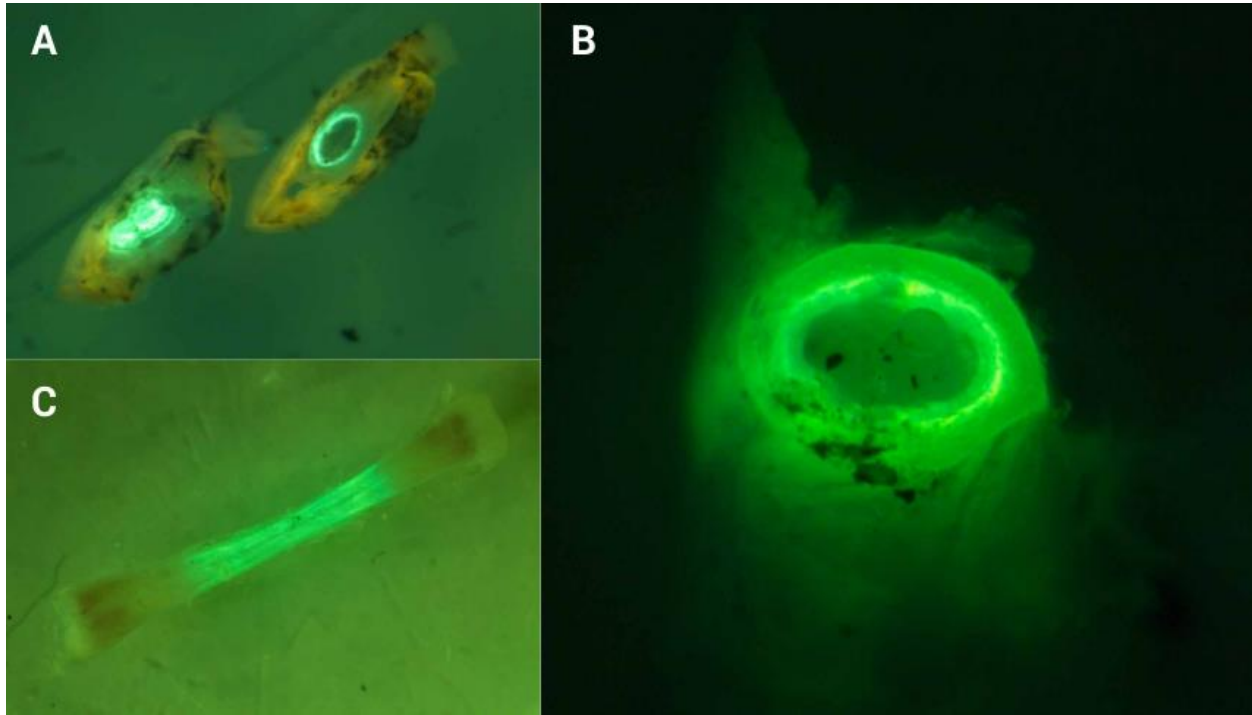


Figure S2. Photos of phalange cross-section (A), tibiofibular cross-section (B), and tibiofibula (C) from a Wood Frog marked with calcein illuminated with a NIGHTSEA BlueStar handheld 440-460nm flashlight through a cancellation filter. Illumination was augmented by white LED light in images (A) and (C). Photos were taken by A. Z. Andis with a Canon 6D and Wild Heerbrugg M5A Stereomicroscope at x12 (C) and x50 (A and B) magnification. Photos were corrected for exposure and white balance.

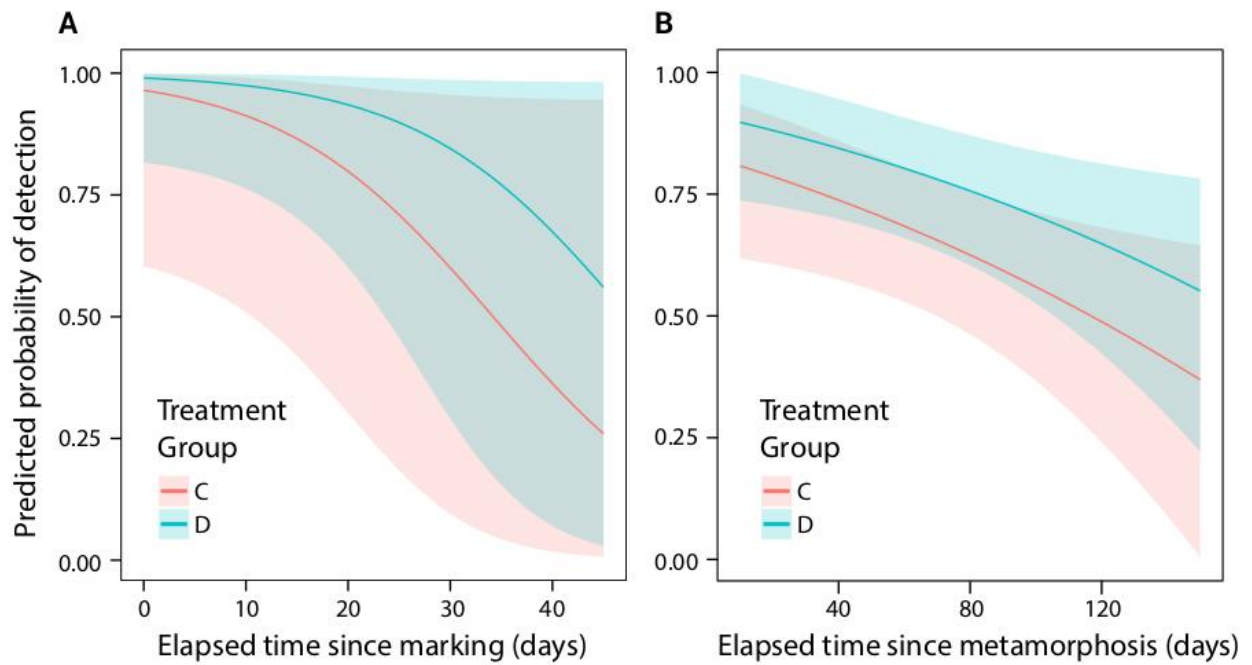


Figure S3. Inter-stage (A) and Intra-stage (B) predicted probability of detecting a calcein label administered with (Treatment D) and without (Treatment C) osmotic induction over time in Wood Frogs marked as larvae of average age (11.1 d prior to metamorphosis) and average mass (0.72 g).