Multiple Cases of Hypomelanism in Wood Frog Larvae (*Rana sylvatica*) Associated with Developmental Retardation and Mortality

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Abstract - Hypomelanism in natural populations is rare and has been anecdotally associated with developmental retardation and mortality when present in anuran larvae. We report multiple, recent cases of hypomelanism across 5 populations in 2 counties in Connecticut. We also experimentally confirm that this phenotype is associated with extremely delayed metamorphosis and increased mortality.

Introduction

Observations of atypical color morphologies that deviate far from putatively optimum phenotypes are of interest as they may be the first indicators of deleterious biotic responses to environmental change (Henle et al. 2017). Color anomalies in amphibian larvae arise from variation in integumentary pigment (commonly melanin) amount, distribution, and density during development (Henle et al. 2017, McDiarmid and Altig 1999). Both hyper- and hypomelanistic morphologies have been observed in amphibians (Bechtel 1995). In the most extreme cases of hypomelanism (albinism), melanin can be completely absent from the integument and retina (Dyrkacz 1981). In other cases, pigment is present but imperfectly distributed, usually highly reduced throughout the skin, but remains in non-integumentary tissues such as the retina and iris (Dyrkacz 1981).

Observations of hypomelanistic individuals in the wild are rare because the lack of pigmentation reduces crypsis and exposes affected individuals to higher predation (Bond 2007, Childs 1953) and also may reduce thermoregulatory abilities (Andrén and Nilson 1981), thereby reducing relative fitness. Given their rarity, observations of aberrant phenotypes in the wild are important to note and may be useful in diagnosing other issues of environmental physiology (Boero 2013, Sokos et al. 2018).

Specifically, aberrant phenotypes may also be associated with underlying pathologies that contribute to reduced fitness (Eagleson et al. 2010, Henle et al. 2017, Smith-Gill et al. 1972). Amphibians in particular are strongly influenced by their environment. Variable or extreme environmental conditions, especially in the natal environment, can adversely affect physiology and morphology (Hillman et al. 2008). In anurans specifically, it has been suggested that hypomelanism is

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associated with developmental retardation (Childs 1953, Krings and Reich 2016, Mitchell 2005) and early mortality (Smith 2014, Smith-Gill et al. 1972). Here we report multiple, recent cases of hypomelanism in *Rana sylvatica* (LeConte) (Wood Frog) larvae from multiple populations in Connecticut, as well as evidence of an association between this phenotype and developmental delay and early mortality.

Field-site Description and Methods

Our lab has been collecting long-term data since 1999 on Wood Frogs in 60 breeding pond populations at Yale Myers Forest in Tolland County, CT. Since 2015, we have collected data on 30 populations in the towns of Madison and Guilford, New Haven County, CT. The ponds at Yale Myers Forest are situated in a rural forest with little development, while those in New Haven County are situated in fragmented, suburban habitat. Our annual data include observations of oviposition dates, egg-mass counts, and dipnet surveys for larvae throughout the season. During larval surveys, we euthanize individuals in the field and bring them to the lab where we use a dissecting microscope to assess size and developmental stage. Since 1999, we have individually assessed tens of thousands of Wood Frog larvae.

In 2018 and 2019, we discovered 13 hypomelanistic larvae from 4 populations in New Haven County and 1 population at Yale Myers Forest during dipnet surveys for larvae. The skin of the hypomelanistic larvae was light gray to golden (Figs. 1, 2). The pupils were black and the irises dark with flecks of light pigment, similar to that of a standard morph. This morphology matches that of a hypomelanistic (i.e. leucistic) Wood Frog larva described by Mitchell (2005).

We discovered 13 hypomelanistic larvae in total: 12 from populations in 4 ponds (BHHL, BART, COPT, and ZOE) in suburban environments in New Haven County

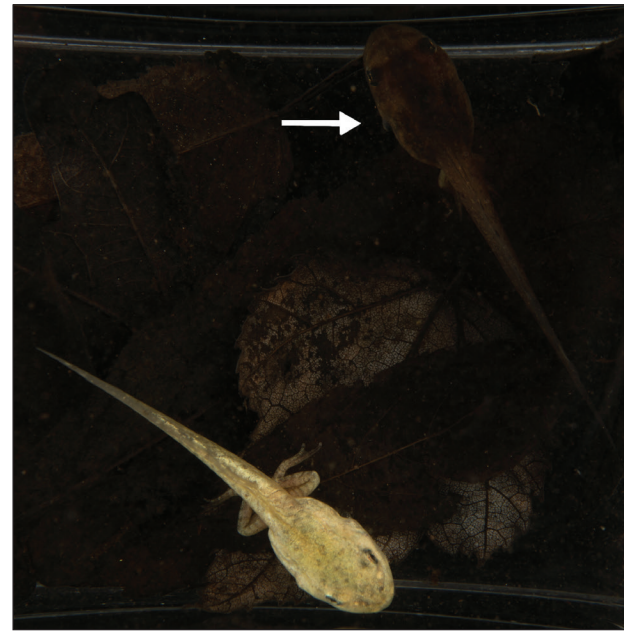

Figure 1. Hypomelanistic and wild-type (arrow) Wood Frog larvae from BART collected on 22 June 2018 and photographed on native substrate to illustrate the contrast in crypsis between phenotypes.

and a single larva from a rural population in a pond (WP) in Tolland County (Yale Myers Forest) (Table 1). On 14 June 2018 during our regular dipnet surveys, we found a single hypomelanistic larvae in BHHL that we euthanized in the field. It was at Gosner stage 31 (Gosner 1960), while other conspecifics at the site varied from Gosner stage 34 to 42. On 22 June 2018, we found 2 additional larvae in BART with the same phenotype. These 2 larvae were 3–6 Gosner stages less developed than conspecifics. These larvae were collected live and reared in the lab.

On 27 June 2018, we found 8 additional hypomelanistic larvae in COPT. These 8 larvae were 5–9 Gosner stages behind conspecifics (Fig. 2). We collected these 8 larvae from COPT along with 16 wild-type conspecific from the same COPT cohort and returned these to the lab where we reared them in temperature (21.5 °C)- and light (12:12 h photoperiod)-controlled incubators in individual 1-L glass containers. We fed larvae a 3:1 ratio of powdered rabbit chow and fish flakes ad libidum

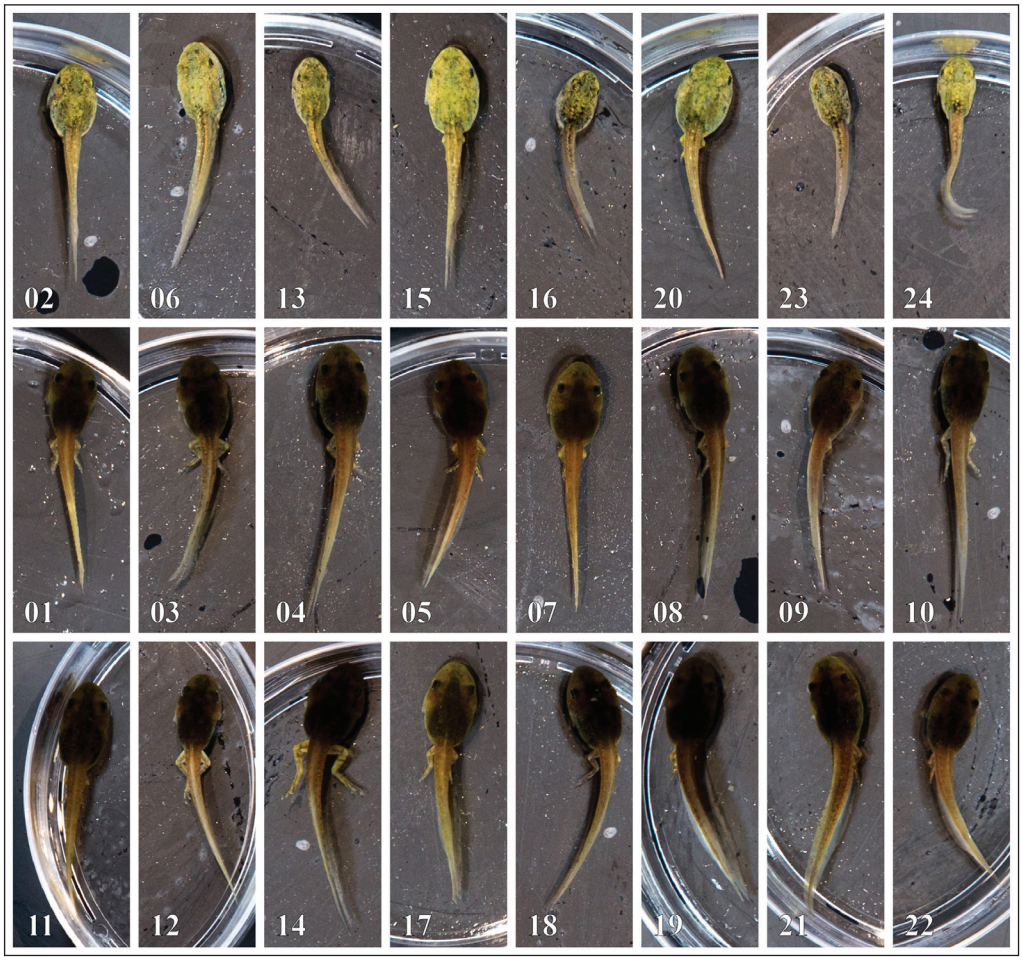

Figure 2. Eight hypomelanistic (top row) and 16 wild-type (bottom 2 rows) Wood Frog larvae collected from COPT on 27 June 2018 that were reared in a common garden experiment. Larvae were photographed on 28 June 2018 under the same lighting conditions. Color was standardized to a colorimetric key and size was standardized to a common scale.

(Ligon and Skelly 2009). Our intent was to compare development of wild-type and hypomelanistic larvae.

The following year, on 29 May 2019, we found a hypomelanistic larvae in ZOE in Madison. We found an additional hypomelanistic on 29 June 2019 in WP in Tolland County. We brought both of these larvae to the lab on the day they were found and reared them in temperature- and light-controlled incubators following our standard protocols, as in 2018, but without wild-type conspecifics for comparison.

We measured larval age as the number of days since the average oviposition date for the given population. Wood Frogs exhibit explosive, synchronous breeding, and all clutches are deposited within a few days (Petranka and Thomas 1995).

Results

Despite sharing the same pond environment and identical care and conditions in the lab, the 8 hypomelanistic larvae from COPT exhibited extremely delayed development and high mortality compared to wild-type conspecifics in the 2018 common garden experiment (Table 2, Fig. 3). Fifteen of the 16 wild-type larvae in the common garden survived to metamorphosis within an average of 16.5 days (min–max = 12–20 d). In comparison, only 2 of the 8 hypomelanistic larvae survived to metamorphosis 33 and 46 days after collection, respectively. Of the remaining hypomelanistic larvae, 2 died prior to metamorphosis at Gosner stages 36 and 41, after 75 and 69 days, respectively (Fig. 2). We euthanized 2 hypomelanistic larvae due to integumentary problems at Gosner stage 36 and 35, at 115 days into the experiment. We terminated the experiment on November 16, 2018 after 142 days with 2 remaining hypomelanistic larvae at Gosner stage 35 and 39.

Neither of the 2 larvae collected in 2019 survived to metamorphosis. The larva from ZOE died prior to metamorphosis at Gosner stage 36 on 17 February 2020 (~324 days after ovipositon). The larva from WP died on 14 November 2019 (224 days after ovipositon) after attaining Gosner stage 40. In comparison, wild larvae from ZOE completed metamorphosis ca. 26 June 2019 (~88 days after ovipositon),

Table 1. Collection year, pond, location, and number of hypomelanistic larvae (*n*) reported in this study, approximate oviposition date, and date of collection from the natal pond.

Year	Pond	County	Coordinates	*n*	Oviposition date	Collection date
2018	BHHL	New Haven	41°19'31.37"N, 72°39'36.90"W	1	2 March	14 June
2018	BART	New Haven	41°19'54.48"N, 72°38'34.44"W	2	2 March	22 June
2018	COPT	New Haven	41°22'47.14"N, 72°35'42.22"W	8	28 March	27 June
2019	ZOE	New Haven	41°21'42.91"N, 72°38'7.58"W	1	1 April	29 May
2019	WP	Tolland	41°56'32.78"N, 72°7'46.01"W	1	5 April	29 June

and wild larvae from WP had completed metamorphosis ca. 2 July 2019 (~89 days after oviposition).

Discussion

Our lab has been surveying Wood Frog populations in New Haven County and Yale Myers Forest annually for 5 and 20 years, respectively, yet these are the first observations of hypomelanism we have recorded, which suggests that the proximate cause of the anomalies derives from a recent change.

A heritable, genetic cause is the most likely explanation for the majority of hypomelanism in the wild (reviewed in Henle et al. 2017); however, this is not likely in the cases we report. First, the cases arose simultaneously in the same cohort in 2018 and in populations separated by over 100 km in 2019. Thus, gene flow can be ruled out, and a genetic origin for the phenotype would have had to manifest at least 4 times.

Chemicals, irradiation, nutritional deficiencies, parasites, and disease have been attributed as causes of depigmentation in amphibians (Henle et al. 2017). Others

Table 2. Wild-type and hypomelanistic specimens collected from COPT pond in New Haven County, CT, in 2018 and included in the common garden experiment. Color morph: W = wild-type, H = hypomelanistic; Initial SVL = initial snout-to-vent length immediately after capture upon stocking into the experiment; Final SVL = snout-to-vent length at the time of metamorphosis or death; Sex: F = female, M = male, U = undetermined; Age = duration from average oviposition date to metamorphosis or death(*).

Specimen	Morph	Initial SVL (mm)	Final SVL (mm)	Sex	Age (days)	Field catalog #
1	W	13.44	14.68	F	111	SKE 4409-1
2	H	13.92	NA	U	207*	NA
3	W	15.12	16.25	F	105	SKE 4354-3
4	W	16.46	14.88	F	107	SKE 4393-4
5	W	14.59	14.63	M	108	SKE 4394-5
6	H	14.11	19.60	U	234*	AZA 3319
7	W	15.23	14.51	F	111	SKE 4322-7
8	W	14.86	14.88	F	106	SKE 4357-8
9	W	13.78	14.88	U	111	SKE 4412-09
10	W	15.43	16.17	M	107	SKE 4392-10
11	W	15.68	15.30	F	111	SKE 4410-11
12	W	13.54	14.89	M	104	SKE 4353-12
13	H	11.33	NA	U	207*	NA
14	W	15.13	14.66	U	104	SKE4352-14
15	H	15.37	17.69	M	161*	SKE 4724-15
16	H	11.21	13.06	U	167*	SKE4733-16
17	W	15.25	13.75	M	108	SKE 4395-17
18	W	15.47	16.33	M	107	SKE 4391-18
19	W	15.68	16.50	M	112	SKE 4413-19
20	H	14.55	16.51	M	138	SKE 4723-20
21	W	16.33	15.40	M	108	SKE 4403-21
22	W	14.38	14.64	M	111	SKE 4411-22
23	H	11.20	16.10	U	234*	AZA 3318
24	H	10.47	13.50	F	125	SKE 4620-24

have implicated *Ranavirus* infection as a provisional cause of discoloration in Wood Frogs (Krynak and Dennis 2014). *Ranavirus* infections have been reported in our study populations in the past, although no obvious outbreaks were observed to co-occur in the populations with hypomelanism in 2018 nor 2019.

It is also notable that 12 of the 13 hypomelanistic larvae (in 4 of 5 ponds) were from populations in a suburban area, as others have found a correlation between urbanization and deformities. For instance, Vershinin (2005) found morphological anomalies, including pigmentation defects, among *Rana arvalis* Nilsson (Moor Frog) populations increased from forest to urban zones.

Although the limited number of our observations do not allow us to conclude a mechanism, we do find evidence of an association between the phenotype and fitness, as proffered by multiple prior observations. For instance, Mitchell (2005) and Smith (2014) report retarded growth in hypomelanistic Wood Frog larvae in relation to wild-type conspecifics. However, each prior report involved only 1 hypomelanistic individual and precluded rigorous comparisons of development. Here, we show that when reared in common settings, hypomelanistic larvae exhibit extremely protracted development relative to wild-type larvae and also high

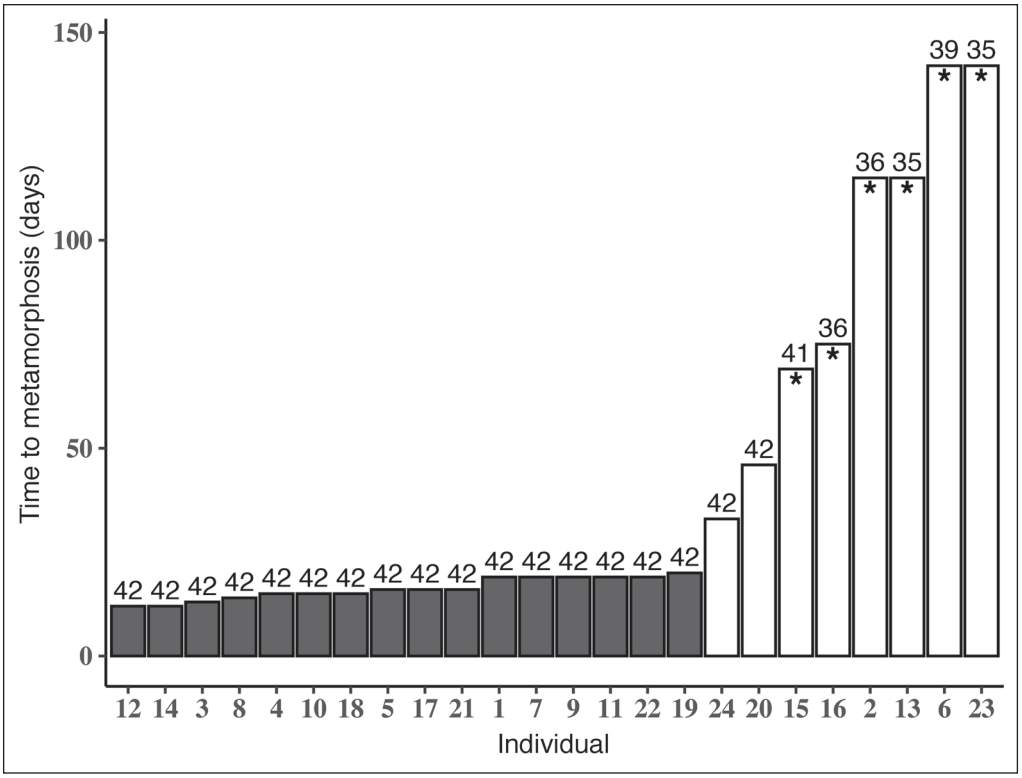

Figure 3. Time to metamorphosis (days) since time of capture (27 June 2018) for larvae reared in common garden conditions. Filled bars indicate wild-type larvae and open bars indicate hypomelanistic morphs. Asterisks in the open bars indicates individuals that died (ID = 15–16) or were euthanized (ID = 2, 13, 6, 23) prior to metamorphosis. Labels indicate Gosner stage when removed from the experiment.

mortality. We found that most of the hypomelanistic morphs in our experiment failed to complete metamorphosis. This result is consistent with Smith-Gill et al.'s (1972) finding that all of 7 hypomelanistic *Rana pipiens* (Schreber) (Northern Leopard Frog) from a Michigan population failed to attain metamorphosis after protracted development.

The ultimate effect of larval coloration on fitness is not well studied (Thibaudeau and Altig 2012). The fact that hypomelanistic adults (including Wood Frogs) have been observed indicates that the fitness consequences are not absolute (Thompson and Rea 2013). Rather, pathologies associated with anomalous coloration may reduce survival probabilities over time. Hypomelanistic phenotypes may be correlated with developmental abnormalities through underlying pathologies. Pigmentation patterns derive from cellular differentiation in early development, so anomalies in early development may manifest in both pigmentation and other systems (Fleck et al. 2016, Thibaudeau and Altig 2012). Amphibians are strongly influenced by their environments, and abnormal environmental conditions, especially in the natal environment, can adversely affect physiology and morphology (Hillman et al. 2008). Given the continued increase in urbanization and habitat alteration, tracking observations of atypical coloration (and other morphological anomalies) may be a useful indicator of the physiological impacts affecting amphibian populations.

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