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Geographic Variation in Northern Green Frog Larvae, *Lithobates Clamitans Melanotus*, in Northwestern New Jersey

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Abstract

A total of 124 larvae of the northern green frog, *Lithobates clamitans melanotus* (Rafinesque 1820), were collected at five localities in three adjacent counties of northwestern New Jersey from 2001 to 2007. Data were recorded for 19 varying character states that included 18 morphometric features (body dimensions and characteristics of the oral disc) and developmental stage. Developmental stage differed significantly among the localities. Tables of univariate descriptive statistics are provided for the 18 morphological features from all sites. Regression analyses of body length over developmental stage and tail length over developmental stage determined that larvae from one locality (Allamuchy State Park) differed markedly from the larvae from the other four localities. Four localities expressed the larval tooth row formula (LTRF) 2(2)/ 3, while Chubb Park was 2(2)/ 3(1). Phenotypic plasticity likely accounts for some of the variation of all characters.

Introduction

Northern green frogs are commonly found throughout New Jersey in a variety of permanent, freshwater habitats, and are named *Rana clamitans melanota* by Schwartz and Golden (2002). Using mtDNA data, Hillis and Wilcox (2005) retained the use of the genus *Rana*, placing them in their Aquarana group. Based on molecular data, Frost et al. (2006) placed the species *clamitans* in the genus *Lithobates*, requiring the use of this genus with the subspecific name changing to *melanotus*, but retaining the common name as green frog. In contrast, Austin and Zamudio (2008) presented mtDNA data that suggested the recognition of the subspecies was not supported. However, their finding was rejected by an eminent anuran systematist group (Center for North American Herpetology, http://cnah.org/detail.asp?id=1163). As a result, Collins and Taggart (2009) designated the green frog as *Lithobates clamitans melanotus* (Rafinesque 1820), as does Frost (2011).

Since natural selection operates on anuran larvae (tadpoles) as well as adults, studies focused on the larvae are warranted. This study entails the examination of 124 field-collected larvae from five sites in three adjacent counties of northwestern New Jersey (Morris, Warren, Sussex), known as The Highlands. The purpose of the study was to: (1) document the occurrence of the taxon with habitat notes, (2) document geographic variation of 18 morphometric characters from selected sites using descriptive statistics, and (3) analyze patterns of variation among populations.

Methods

Field collections: Field collections of 124 tadpoles were made by one of us (JKK) at five different sites between 2001 and 2007. Three of the sites were collected twice in two different years. The following are the collection localities and dates of collection. (1) Chubb Park, State Route 24, Chester, Morris County (40° 46' 57.5" N, 74° 42' 36.1' W; elevation 253 m AMSL), a 0.71-ha rectangular, man-made pond of an approximately 1-m uniform depth used for winter ice skating in 34-ha park of grassland and woodlands. A total of 21 specimens were collected on 3 July 2001 and 26 June 2002. (2) Intersection of Colby Farm Road and Knollwood Terrace, Chester, Morris County

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(40° 46' 48.5" N, 74° 40' 58.9' W; elevation 249 m), a 0.05-ha artificial retention basin in a housing subdivision, with marginal aquatic vegetation surrounding a ≥1.25-m deep circular basin. A total of 13 specimens were collected on 3 July 2001 and 26 June 2002. (3) Allamuchy State Park, Deer Park Road, Hackettstown, Warren County (40° 53' 15.0" N, 74° 49' 23.5' W; elevation 273 m), a 0.05-ha natural pond of approximately 1 m depth near a house on the access road to interior of approximately 3500 ha of diverse habitat. Twenty-seven specimens were collected on 21 June 2007. (4) Schooley's Mountain, West Springtown Road, Long Valley, Morris County (40° 47' 6.0" N, 74° 48' 29.4' W; elevation 329 m), a 1.50-ha spring fed pond, approximately 3.5 m deep at center on residential property. Thirty-three specimens were collected on 11 September 2006. (5) New Jersey School of Conservation, Montclair State University, Branchville, Sussex County (41° 13' 1.7" N, 74° 44' 50.2' W; elevation 268 m), a 0.2-ha flooded beaver pond area with slowly moving water and fallen trees. A total of 30 specimens were collected on 8 July 2002 and 14 July 2003.

TABLE 1. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Chubb Park, Morris County, New Jersey, 2001-2002. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 ($n = 3$), stage 28 ($n = 2$), stage 30 ($n = 2$), stage 36 ($n = 3$), stage 37 ($n = 1$), stage 38 ($n = 2$), stage 39 ($n = 2$), stage 40 ($n = 4$), and stage 41 ($n = 2$).

Variable	*n*	Mean	Median	SD	Range
Body length	21	29.3	33.0	9.1	13.0-39.0
Tail length	21	42.7	48.0	13.9	18.0-59.0
Total length	21	72.0	80.0	23.0	31.0-97.0
Tail height	21	14.6	16.0	3.7	7.0-19.5
Tail muscle height	21	7.5	8.1	2.3	3.5-10.0
Dorsal fin height	21	4.7	4.8	1.2	2.5-7.0
Ventral fin height	21	3.5	3.5	0.9	2.0-5.2
Interocular distance	21	10.0	11.0	3.8	3.0-14.5
Internareal distance	21	3.4	3.5	0.9	1.5-4.6
A-1 length	21	3.6	3.7	1.5	0.7-6.0
Left A-2 length	12	0.9	0.9	0.4	0.3-1.4
Right A-2 length	14	1.0	1.0	0.4	0.5-1.7
A-2 gap	12	2.5	2.5	0.6	1.5-3.6
A-2 gap ratio	12	0.5	0.5	0.2	0.2-0.9
P-1 length	21	3.5	3.6	1.2	1.1-4.8
P-1 gap	6	0.2	0.2	0.1	0.1-0.3
P-2 length	21	3.4	3.5	1.1	0.9-4.6
P-3 length	14	2.4	2.4	1.1	0.2-3.7

Tadpoles were obtained by hand net and preserved in 10% formalin, and are in the custody of the senior author. Larvae were identified by keys (Altig 1970, Altig and Johnston 1986), the online guide of Altig et al. (http://www.pwrc.usgs.gov/ tadpole/), and the presence of adults in some cases. Larvae were staged according to Gosner (1960).

Morphological measurements: Measurements of body features were made with Cenco calipers, whereas those of the oral disc were made with dissecting microscope and ocular micrometer calibrated to the nearest 0.1 mm. Descriptive features follow Altig (1970) and McDiarmid and Altig (1999), and included body length, tail length, total length, tail height, tail muscle height, dorsal fin height, ventral fin height, interocular distance, internareal distance, A-1 length, left A-2 length, right A-2 length, A-2 gap, A-2 gap ratio, P-1 length, P-1 gap, P-2 length, and P-3 length. Thus,

TABLE 2. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Colby Farm Road, Morris County, New Jersey, 2001-2002. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 (n = 1), stage 27 (n = 1), stage 28 (n = 1), stage 29 (n = 1), stage 36 (n = 1), stage 37 (n = 2), stage 38 (n = 1), stage 41 (n = 1), stage 42 (n = 1), stage 43 (n = 1), and stage 44 (n = 2).

Variable	*n*	Mean	Median	SD	Range
Body length	13	24.2	26.0	6.4	13.0-36.0
Tail length	13	33.8	36.0	10.2	20.0-47.0
Total length	13	57.5	60.0	14.3	33.0-74.0
Tail height	13	11.9	11.0	4.6	5.0-19.5
Tail muscle height	13	5.8	5.1	1.9	2.5-8.8
Dorsal fin height	13	4.0	3.5	1.6	1.5-6.5
Ventral fin height	13	2.8	3.0	1.4	1.0-5.4
Interocular distance	13	6.8	6.8	1.8	3.5-9.4
Internareal distance	13	3.1	3.4	0.6	1.8-3.7
A-1 length	10	2.8	3.1	0.9	1.5-3.7
Left A-2 length	3	0.5	0.6	0.2	0.3-0.6
Right A-2 length	4	0.6	0.5	0.4	0.2-1.2
A-2 gap	3	2.0	1.9	0.2	1.8-2.2
A-2 gap ratio	3	0.4	0.3	0.2	0.2- 0.6
P-1 length	10	2.6	2.7	0.7	1.4-3.4
P-2 length	10	2.3	2.6	0.9	1.1-3.5
P-3 length	7	1.6	1.6	0.4	1.0-2.0

16 direct measurements, 2 derived variables (total length and A-2 gap ratio), and developmental stage were recorded for each tadpole from the five selected sites. Some specimens had missing or damaged body or oral disc features.

Data analyses: We calculated descriptive statistics of central tendency and variability for each of the 18 morphometric variables. However, developmental stage differed significantly among the five locations (Kruskal-Wallis Rank Sums Test, chi-square approximation = 42.6, df = 4, $P < 0.0001$). Thus, we did not compare these variables directly among the five locations. Instead, following Strauss and Altig (1992) we first converted measurements to natural logarithms, and then for each location we used regression models to describe the change in selected morphometric variables in relation to developmental stage. We then compared those regressions among locations. Tests of significance were performed using JMP version 8.0.2, and regression models were calculated with TableCurve 2D version 5.01.

TABLE 3. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Allamuchy State Park, Warren County, New Jersey, 2007. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 31 ($n = 1$), stage 32 ($n = 5$), stage 33 ($n = 9$), stage 34 ($n = 5$), stage 35 ($n = 5$), and stage 36 ($n = 2$).

Variable	*n*	Mean	Median	SD	Range
Body length	27	9.6	10.0	0.9	7.5-10.5
Tail length	27	10.6	11.0	1.6	8.0-13.5
Total length	27	20.2	21.0	2.2	15.5-24.0
Tail height	27	3.7	3.5	0.5	2.8-4.5
Tail muscle height	27	1.8	2.0	0.3	1.2-2.2
Dorsal fin height	27	1.3	1.2	0.3	0.5-2.0
Ventral fin height	27	1.4	1.4	0.3	1.0-2.0
Interocular distance	27	2.4	2.4	0.2	2.0-2.8
Internareal distance	27	1.5	1.5	0.2	1.0-1.8
A-1 length	27	1.6	1.6	0.2	0.9-2.0
Left A-2 length	27	0.5	0.5	0.2	0.2-0.8
Right A-2 length	27	0.6	0.6	0.2	0.3-0.8
A-2 gap	27	0.4	0.3	0.1	0.1-0.7
A-2 gap ratio	26	1.7	1.7	0.7	0.6-3.5
P-1 length	27	1.4	1.5	0.2	1.1-1.7
P-2 length	27	1.3	1.3	0.2	1.1-1.7
P-3 length	26	1.1	1.0	0.2	0.8-1.5

Results

Measures of central tendency and variability in the 18 morphometric characters of the samples collected from Chubb Park are presented in Table 1; 6 of 21 larvae had the unique LTRF of 2(2)/3(1), indicating a P-1 median gap. P-1 gap was not expressed on any tadpoles from the other four localities; these larvae showed a LTRF of 2(2)/3. Measures of central tendency and variability in the remaining 17 morphometric characters of specimens from the four other collection localities are presented in Tables 2 through 5.

The following regression model most consistently provided the best fit for the relationship of body length with developmental stage: length = a + b/stage^2. The samples from Allamuchy State Park differed markedly from the other four locations (Figure 1). The same regression model also most

TABLE 4. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Schooley's Mountain, Morris County, New Jersey, 2006. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 (n = 1), stage 27 (n = 2), stage 28 (n = 2), stage 29 (n = 4), stage 30 (n = 4), stage 31 (n = 15), stage 32 (n = 1), stage 33 (n = 2), and stage 36 (n = 2).

Variable	*n*	Mean	Median	SD	Range
Body length	33	19.0	19.0	2.4	14.0-24.0
Tail length	33	26.6	27.0	4.1	18.0-35.0
Total length	33	45.6	47.0	6.3	32.0-59.0
Tail height	33	9.7	10.0	1.3	7.0-12.2
Tail muscle height	33	4.1	4.2	0.5	3.2-5.0
Dorsal fin height	33	3.4	3.3	0.5	2.5-4.5
Ventral fin height	33	2.8	2.8	0.4	2.0-3.5
Interocular distance	33	5.3	5.5	0.9	3.8-7.2
Internareal distance	33	3.1	3.2	0.4	2.2-3.9
A-1 length	33	2.3	2.4	0.3	1.6-3.1
Left A-2 length	31	0.3	0.3	0.2	0.1-0.6
Right A-2 length	30	0.4	0.4	0.2	0.1- 0.7
A-2 gap	29	1.2	1.2	0.3	0.9-2.1
A-2 gap ratio	29	0.4	0.4	0.2	0.1-0.7
P-1 length	33	2.1	2.1	0.4	1.1-3.0
P-2 length	33	2.1	2.1	0.4	1.4-3.0
P-3 length	32	1.2	1.2	0.4	0.6-2.1

consistently provided the best fit for the relationship of tail length with developmental stage, and again the samples from Allamuchy State Park were markedly different from the samples obtained at the other four locations (Figure 2). Mean total length of the Allamuchy State Park larvae (20.2 mm) was less than half the mean lengths from the four other localities (45.6-72.0 mm; Tables 1-5).

Discussion

Altig and Johnston (1989, Table 1) showed the LTRF 2/3 to be most common (51%) and highly conserved of 320 anuran species studied of a total of 627. All our 124 larvae were slight derivatives of this prime formula, and are consistent with the species being a lentic-benthic pond form with reduced oral apparatus complexity.

TABLE 5. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from the School of Conservation, Sussex County, New Jersey, 2002-2003. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 28 ($n = 2$), stage 29 ($n = 1$), stage 32 ($n = 1$), stage 35 ($n = 1$), stage 36 ($n = 4$), stage 37 ($n = 2$), stage 38 ($n = 1$), stage 39 ($n = 6$), stage 40 ($n = 4$), stage 41 ($n = 7$), stage 42 ($n = 1$), and stage 46 ($n = 1$).

Variable	*n*	Mean	Median	SD	Range
Body length	30	26.9	27.5	3.3	17.0-33.0
Tail length	30	41.7	43.0	8.9	21.0-59.0
Total length	30	68.6	70.0	11.6	39.0-88.0
Tail height	30	13.9	14.5	2.4	7.5-19.0
Tail muscle height	30	7.6	7.7	1.3	4.0-9.5
Dorsal fin height	30	4.2	4.5	0.7	2.5-5.5
Ventral fin height	30	2.8	2.9	0.8	1.0-4.5
Interocular distance	28	8.5	8.8	0.7	6.5-9.6
Internareal distance	30	3.8	4.0	0.4	3.0-4.4
A-1 length	27	2.5	2.8	0.7	0.5-3.3
Left A-2 length	10	0.3	0.4	0.1	0.1-0.6
Right A-2 length	8	0.5	0.5	0.3	0.2-1.1
A-2 gap	4	2.1	2.0	0.5	1.8-2.8
A-2 gap ratio	4	0.3	0.3	0.1	0.2-0.3
P-1 length	27	2.7	2.7	0.5	1.3-3.8
P-2 length	27	2.2	2.3	0.5	1.0-2.8
P-3 length	21	1.1	1.1	0.5	0.2-2.0

While developmental stage was determined to be statistically different between localities, variability in the oral apparatus cannot be explained solely by stage difference as the assumption that stage and oral disc development are tightly correlated is not warranted McDiarmid and Altig (1999, p.45).

It is notable the two localities in Chester, Chubb Park and Colby Farm Road, are about 2.5 km apart. Yet, the former had 6 of 21 specimens with a P-1 gap, while none of the 13 at the latter had any with that gap. The specific reason for this is unclear.

Variation in the data may be attributed to some combination/permutation of the following factors: ontogenetic variation, nonadaptive variation, and phenotypic plasticity. The percentage each factor may contribute singularly or synergistically has not been determined in studies, but phenotypic plasticity has been investigated the most, particularly with tadpoles as subjects.

Phenotypic plasticity is an adaptive phenomenon wherein one genotype can produce multiple phenotypes as a function of abiotic and biotic factors. This plasticity can result in behavioral, physiological, morphological, and life history alterations (Miner et al. 2005).

The nature of the habitat alone, field-collected versus laboratory-reared, was shown by Hillis (1982) to induce morphological variation in conspecifics. Not surprisingly, food availability affected both age and size at metamorphosis (Hensley 1993). Impending pond desiccation increased

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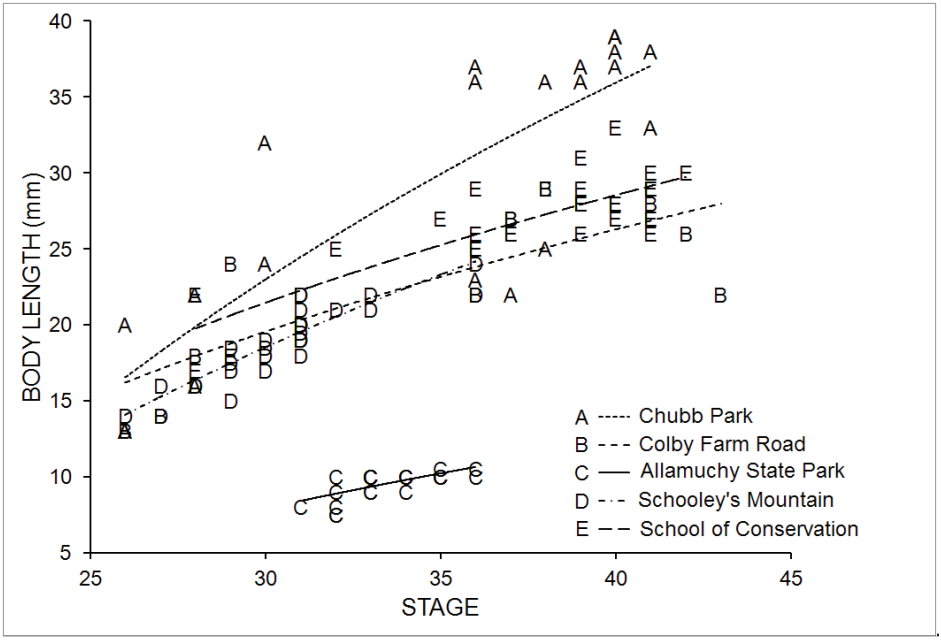
FIGURE 1. The relationship of body length and developmental stage for *Lithobates clamitans melanotus* larvae collected at five locations in northwestern New Jersey, 2001-2007. Regression model for Chubb Park: ln(length) = 4.1 – 949.3/stage^2, $r^2 = 0.73$; for Colby Farm Road: ln(length) = 3.7 – 668.7/stage^2, $r^2 = 0.71$; for Allamuchy State Park: ln(length) = 3.1 – 951.9/stage^2, $r^2 = 0.46$; for Schooley's Mountain: ln(length) = 3.8 – 789.3/stage^2, $r^2 = 0.82$; and for School of Conservation: ln(length) = 3.7 – 611.9/stage^2, $r^2 = 0.74$.



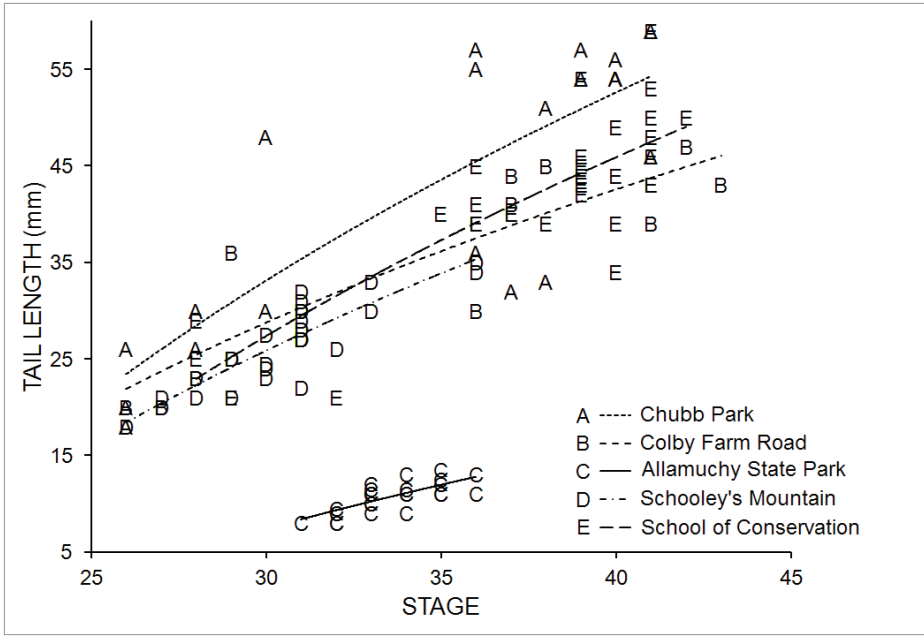
speed of development with earlier metamorphosis and smaller size (Laurila and Kujasalo 1999), an obvious survival facilitator. Predator presence is another factor. Miner et al. (2005), Kraft et al. (2006), and Van Buskirk and Relyea (2008) all demonstrated such presence resulted in smaller bodies with larger tail fins, presumably aiding predator escape by better swimming and being able to survive a tail bite compared to a fatal body bite.

Predator presence also may increase the toxic effects of pesticide use (Relyea 2003). Carbaryl is a water soluble pesticide used globally. He tested green frogs as one of six amphibian species exposed to the pesticide and simultaneous predator stress. The lethality of exposure increased many times with predator presence.

Sometimes the changing subtle interaction of factors may lead to study discordance, as in those of green frog tadpoles. Schalk et al. (2002), using a leech as a high risk larval predator, showed delayed metamorphosis and a larger size at metamorphosis. Ireland et al. (2007), using a leech as a green frog egg predator, determined metamorphosis occurred at an earlier stage with a smaller size at hatching. Thus, the same predator at a different life history stage of the same species produced markedly different plasticity outcomes.

Suffice to say, further investigations of the abiotic and biotic factor interplay influencing larval morphology are warranted, and will be challenging evolutionary biology, but will better illuminate the mechanisms of Natural Selection.

FIGURE 2. The relationship of tail length and developmental stage for *Lithobates clamitans melanotus* larvae collected at five locations in northwestern New Jersey, 2001-2007. Regression model for Chubb Park: ln(length) = 4.6 – 983.7/stage^2, $r^2 = 0.73$; for Colby Farm Road: ln(length) = 4.3 – 843.1/stage^2, $r^2 = 0.80$; for Allamuchy State Park: ln(length) = 3.8 – 1630.9/stage^2, $r^2 = 0.57$; for Schooley's Mountain: ln(length) = 4.3 – 926.8/stage^2, $r^2 = 0.77$; and for School of Conservation: ln(length) = 4.5 – 1052.0/stage^2, $r^2 = 0.69$.



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