

COLOR PATTERN, ELEVATION AND BODY SIZE IN THE HIGH-ANDEAN FROG *HYLA LABIALIS*

por

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Resumen

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El patrón de coloración dorsal varía en la rana altoandina *Hyla labialis* y esta variación puede ser resumida en cinco categorías discretas (morfos cromáticos) a lo largo del desarrollo postmetamórfico. Los morfos pueden ser ordenados en una escala que va del café claro al verde, pasando por tres categorías intermedias. Existen diferencias entre poblaciones, relacionadas con la altitud, en la frecuencia relativa de los morfos, que parece relacionarse con las diferencias en estructura y coloración de los respectivos microhábitats. Al interior de las poblaciones, los morfos relacionados con el café son más grandes al final de la metamorfosis y como adultos. En el laboratorio, los morfos no difieren en la tasa de crecimiento cuando se les permite termoregular, por asoleamiento, bajo fuentes radiantes de calor. En conjunto, los resultados sugieren que el polimorfismo cromático de esta especie podría haber evolucionado bajo el efecto indirecto de selección natural actuando sobre el tamaño corporal, y bajo el efecto indirecto de selección apostática (aquella que favorece las variantes discretas de los morfos eliminando las formas intermedias) sobre el valor crítico de varios morfos adaptados en cada población.

Palabras clave: Anuros, Patrón de coloración, Variación geográfica, Historia de vida, Polimorfismo.

Abstract

Dorsal coloration varies in the high Andean frog *Hyla labialis* and this variation can be summarized in five discrete categories (color morphs) across postmetamorphic development. Color morphs can be arranged in a scale from light brown to green through three intermediate categories. There are interpopulational altitude-related differences, in the relative occurrence of color morphs, that seem partly related to microhabitat structure and coloration. Within populations, brown-related morphs are larger in both size at metamorphosis and adult body size. In the laboratory, color morphs do not differ in growth rate when allowed to thermoregulate by basking under a radiant heat source.

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Altogether, the results suggest that polymorphism in color pattern of this species may have evolved under the indirect effect of natural selection acting on correlated body size, and under the direct effect of apostatic selection on the value for crypsis of locally adapted morphs.

Key words: Anurans, Color pattern, Geographic variation, Life history, Polymorphism.

Introduction

Dorsal coloration of many frog species varies at the intraspecific level and this variation is often expressed as discrete categories or color morphs **Lynch** (1966), **Busse** (1981), **Duellman** (1983), **Hoyos** (1991). As for other animals, the existence of color morphs in frogs has been thought to play a role as antipredator strategy, for the variability in color pattern may difficult the development of search images in the frogs' predators **Endler** (1986), **Guilford** (1992), **Malcolm** (1992). Likewise, the occurrence of several morphs in a single population may lead to apparent rarity of the prey species to the predators **Endler** (1986). Natural selection favoring variation for its own sake has been called apostatic selection **Paulson** (1972). Some correlational and experimental studies have been done to test the adaptive role of color pattern as antipredator strategy in frogs, e.g. **Nevo** (1973), **Tordoff** (1980).

Less attention has received the possibility that dorsal coloration pattern be important for behavioral thermoregulation, specially in those species that bask to elevate body temperatures **Carey** (1978), **Freed** (1980), **Sinsch** (1989). Dark dorsal color patterns or short-term darkening of color pattern should lead to higher light absorption and increased rate of body temperature elevation.

Other authors have explored the possibility that color morphs of frogs evolved at least partly as a consequence of selection pressure on other phenotypical traits that are genetically linked to color pattern. For example, growth rate immediately after metamorphosis was higher in green than in brown morphs of *Pseudacris triseriata* but was not correlated with other larval or adult life history traits **Hoppe & Pettus** (1984). Nonetheless, in the closely related *P. ornata* no differences were detected between color morphs either in size and age at metamorphosis nor in juvenile growth rate and adult size **Travis & Trexler** (1984), **Blouin** (1989).

In Colombia, populations of the frog *Hyla labialis* occur along a wide geographical and altitudinal range, i.e. from about 1950 to 3600m. above sea level. In the course of other studies it has become evident that this species exhibits strong geographical, altitude-related variation in morphology, as well as in physiology and behavior.

Individuals from populations at higher elevations are significantly larger in adult and metamorphic size and grow more slowly until sexual maturity **Amézquita** (1995); they also invest more energy in larger eggs than in larger clutch sizes **Lüddecke & Amézquita** (unpubl. manuscript), select slightly lower temperatures **Lüddecke** (1995), and call at lower dominant frequencies and at lower call and note repetition rates **Amézquita** (unpubl. data). After noting the existence of several color patterns among the sampled individuals, I wondered whether frequency of color patterns also varied between populations and, if so, whether these differences were somehow related to other life history traits.

Thus, this study aims at answering the following questions: 1. Are there discrete color patterns recognizable among both juveniles and adults? 2. How are color patterns of juveniles related to adult color patterns? 3. Are there interpopulational differences in color patterns? 4. Is color pattern related either to size at metamorphosis or to adult body size? 5. Is color pattern related to postmetamorphic growth?

Materials and methods

Field study

The study populations of *Hyla labialis* are about 23-53km. distant from each other around the Colombian capital Santafé de Bogotá. The low-mountain population (LOWM) occurs at 2000m. (4°27'N, 73°56'W), about 4km. south of the municipality of Ubaque, where manmade ponds in pasture used for cattle farming and bordered by agave plants, shrubs or small stands of pine are the biotope. The mid-mountain population (MIDM) occurs at 2750 m. (4°43'N, 73°59'W), about 3km. south-west of the municipality of La Calera. Here a single pond is surrounded by grassland used to graze cattle and by patches of native secondary forest. The high-mountain populations (HIGM) occur at 3450-3550m. in the Páramo de Sumapaz, near Laguna de Chisacá (4°18'N, 74°12'W), and Páramo de Chingaza, near Lagunaseca (4°42'N, 74°49'W), where the native vegetation around large ponds is typically composed of grasses interspersed with moss cushions, rosette plants and few shrubs. The species occurs most commonly in open areas and individuals breed at permanent or semi-permanent ponds **Lüddecke** (1997).

Between 1992 and 1999, in the course of other studies on life history and behavior of *H. labialis*, both adult and metamorphosing individuals were captured while calling, diving, swimming, walking, or basking. Although I did not record the number of individuals captured under each of the conditions above, the very most of them were not located visually, but net-captured from the bottom of ponds or located acoustically while calling. Once an individual was captured, its snout-vent length was measured and its color pattern was described and noted. This procedure led to 51 initial descriptive categories of color pattern. Some individuals captured in the field were brought to the laboratory for other purposes. From them, we confirmed that, although temperature and reproductive activity seem to affect color brightness, dorsal patterns remain easily recognizable.

Laboratory study

In 1991 and 1992, metamorphosing larvae, in developmental stages between 42 and 45 Gosner (1960), were captured by netting in the bottom of ponds at each study site. Larvae were immediately carried to the laboratory and allowed to complete metamorphosis there. Each cohort (one per year and population) was reared in the laboratory within a 50 x 30 x 25cm. (length x width x height) glass terrarium under common garden conditions Amézquita (1995). Briefly, the bottom of each terrarium was covered with a 3cm. thick water-saturated foam pad, which kept relative humidity inside the terrarium above 80%. Food was offered *ad libitum* and consisted mainly of adult flies (*Musca aff. calliphora*) and mealworms (*Tenebrio molitor*). Juveniles had the opportunity to thermoregulate by moving along a thermal gradient (20–35°C) generated by an incandescent bulb conditioned within each terrarium. We were unable to determine the sex of any of the recently captured metamorphs but 53 of them attained sexual maturity within the 300 days duration of the experiment.

For each individual, color pattern was noted and classified at the end of metamorphosis (postmetamorphic age of 0 days) and then once a month until 300 days of postmetamorphic age. I also calculated Specific growth rates = $(\log \text{SIZE}_2 - \log \text{SIZE}_1) / (\text{AGE}_2 - \text{AGE}_1)$, where SIZE_x refers to the body size at the postmetamorphic age X, that is AGE_x (Andrews, 1982).

Results

Color patterns and ontogenetic variation

Information obtained in the laboratory on short term (month to month) changes in color pattern was used to

reduce 51 initial descriptions to six more consistent categories, that were easy to recognize by rapid inspection of any individual. The categories varied according to relative amount of light brown, green, and dark (near black) tones (Fig. 1).

The categories were consistent and discrete when considering either adult or metamorphic phases but they still changed during postmetamorphic development. A correspondence matrix of juvenile color pattern (0 days of postmetamorphic age) by adult color pattern was built with the information of 53 individuals (8 from LOWM, 29 from MIDM and 16 from HIGM) reared in the laboratory and, to examine relationships between rows and columns, a correspondence analysis was carried out on the data matrix. The analysis maximized data variance in two main dimensions that explain 89% of variation in correspondence matrix. Results are depicted in the form of Euclidean distances between color pattern categories in both juvenile and adult individuals (Fig. 2).

Five groups of color patterns that relate metamorphic and adult phases are apparent from Figure 2. Considering approximate distances between groups' centroids, they can also be arranged ordinally, leading to a gradient from completely brown to completely green individuals. These new categories (hereafter color morphs) are much more consistent across postmetamorphic development (Fig. 3) and were used for subsequent analyses.

Color patterns and geographic variation

To know whether relative occurrence of color morphs vary between the studied populations, we counted their frequency in the samples of individuals captured in the field. Chi-squared analyses revealed interpopulational differences considering both adult ($n=267$, $DF=8$, $P<0.00001$) and recently metamorphosed ($n=315$, $DF=6$, $P<0.00001$) individuals.

In both ontogenetic stages it was easy to recognize the population whose color morphs distribution deviated from the general trend (Fig. 4). Among adults, individuals from the HIGM population exhibited highest frequencies of spotted patterns (brown green spots and brown to green). Excluding this population from chi-square analysis results in non-significant differences in color morphs frequencies between LOWM and MIDM populations ($n=243$, $DF=4$, $P=0.132$). Likewise, among recently metamorphosed individuals, spotted patterns (brown to green and brown green spots) were much more frequent in the lowest elevation population (Fig. 4). Again, excluding this population from chi-square analysis results in no

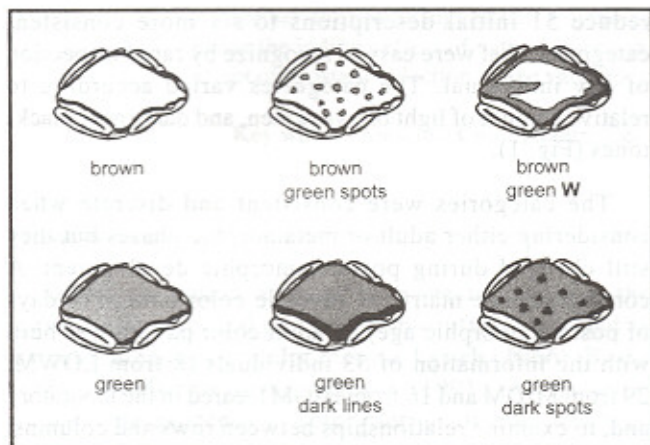


Figure 1. General categories of dorsal color patterns of adult and juvenile *Hyla labialis* at the study sites.

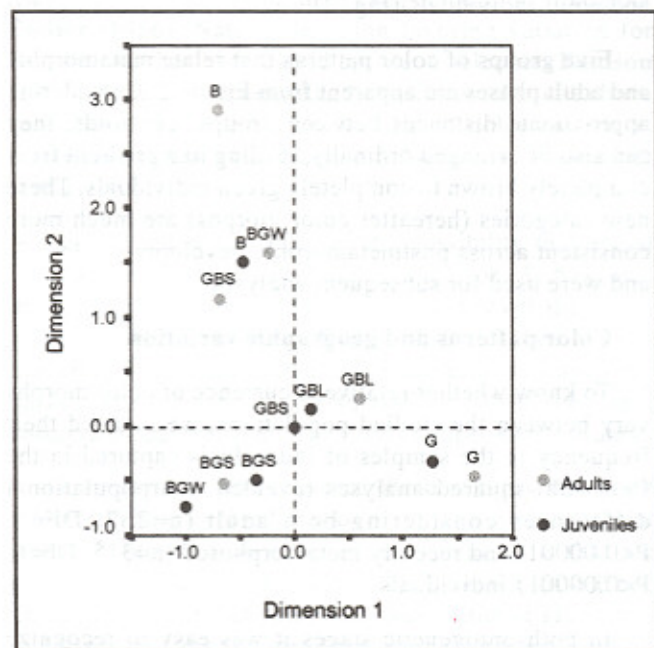


Figure 2. Results of correspondence analysis for the matrix (juvenile color pattern by adult color pattern) describing ontogenetic changes in dorsal coloration of *Hyla labialis*. See text for further explanations.

significant differences in color morph frequencies between MIDM and HIGM populations ($n=223$, $DF=3$, $P=0.168$).

Body size and color patterns

To know whether adult size is related to color morphs of individuals captured in the field, I arranged these data

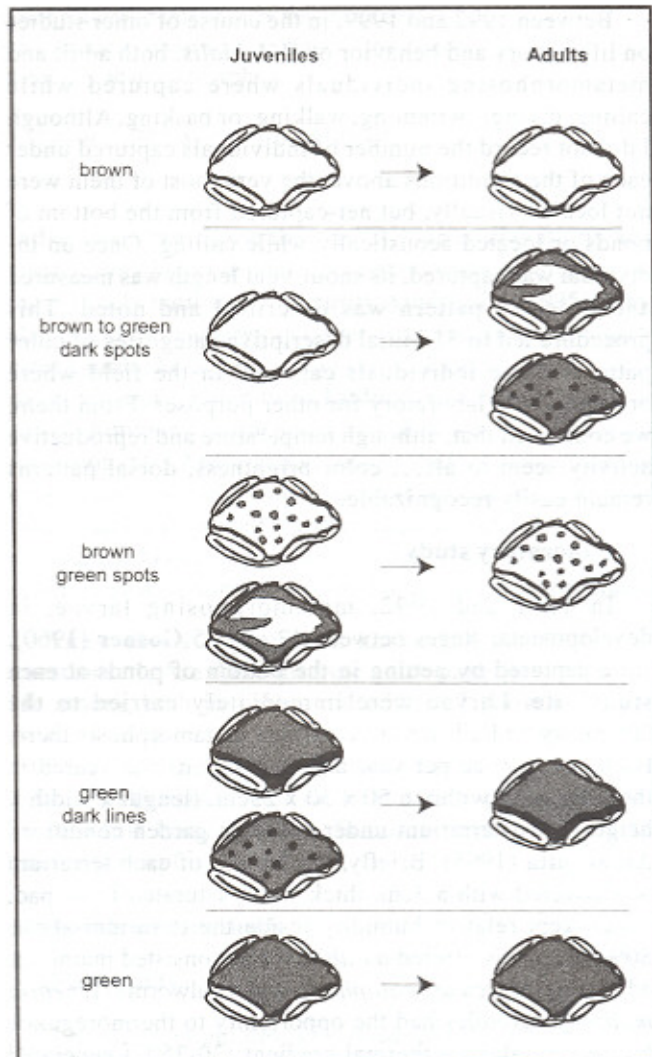


Figure 3. Color morphs of *Hyla labialis* and their most probable changes across postmetamorphic development according to correspondence analysis depicted in Fig. 2. Brown morph of juveniles is assumed to be the origin of brown morph in adults but information for the former was not available.

in box plots on an ordinal scale of color morphs, from only brown to only green (Fig. 5). This information was only available in adequate numbers for males and females from the MIDM population, and for males from the LOWM population. In the MIDM population, analysis of covariance revealed a significant effect of color morph on adult size (ANCOVA, $n=171$, $r^2=0.81$, color morph: $F=51.1$, $P<0.001$). Interestingly, green-related morphs were shorter in snout-vent length than brown-related morphs and the magnitude of this relationship varied between the sexes (color morph by sex: $F=29.4$, $P<0.001$). Differences

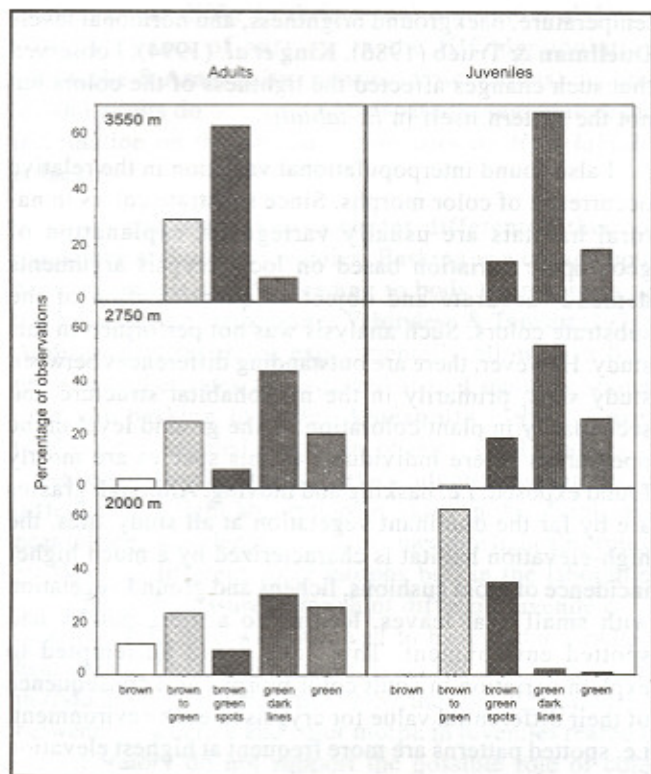


Figure 4. Altitudinal variation in the relative occurrence of color morphs in both juvenile and adult *Hyla labialis*. Sample sizes for the 2000m. site are 43 adults and 92 juveniles, for the 2750m. site, 200 adults and 110 juveniles, and for the 3550m. site, 24 adults and 113 juveniles.

in adult size between the sexes confirmed sexual size dimorphism (sex: $F=193.2$, $P<0.001$). In males from the LOWM population, adult size was also negatively correlated with color morphs scale (linear regression: $n=27$, $r^2=0.653$, $P<0.0001$). Again, green-related morphs were shorter in snout-vent length than brown-related morphs (Fig. 5).

Combining information from all populations, size at metamorphosis was also negatively related to color morphs scale (ANCOVA: $n=245$, $r^2=0.524$, color morph: $F=10.5$, $P=0.001$) and the magnitude of the relationship varied significantly between populations (color morph by population: $F=8.4$, $P<0.001$). The analysis also confirmed interpopulational differences in size at metamorphosis (population: $F=15.3$, $P<0.001$). In spite of differences in the distribution of color morphs between populations, individuals with more green and less brown are again shorter in snout-vent length, as it is apparent from medians of categories with more than two individuals in Fig. 6.

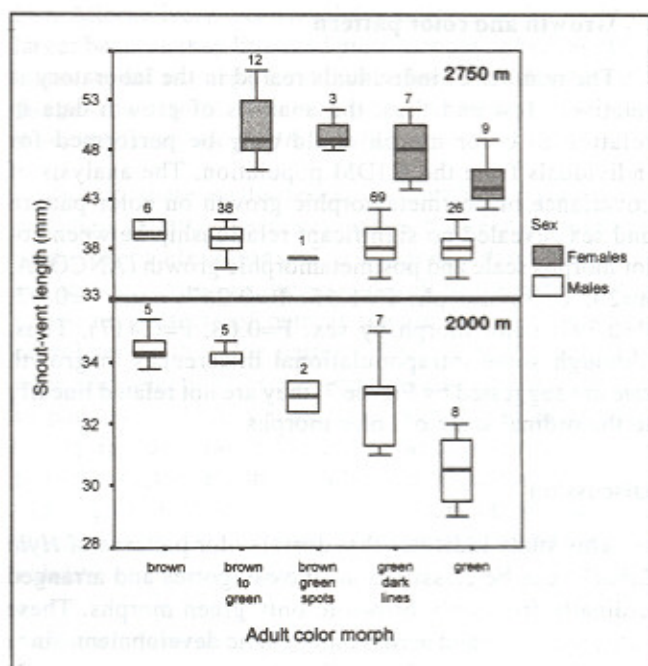


Figure 5. Relationship between adult body size and color morph of *Hyla labialis* from populations at different altitude. The horizontal bar represents the median, the upper and lower ends of the box are the upper and lower quartiles and the middle 50% of data lie between them, the vertical line represents the range of observations, and N is given above the range.

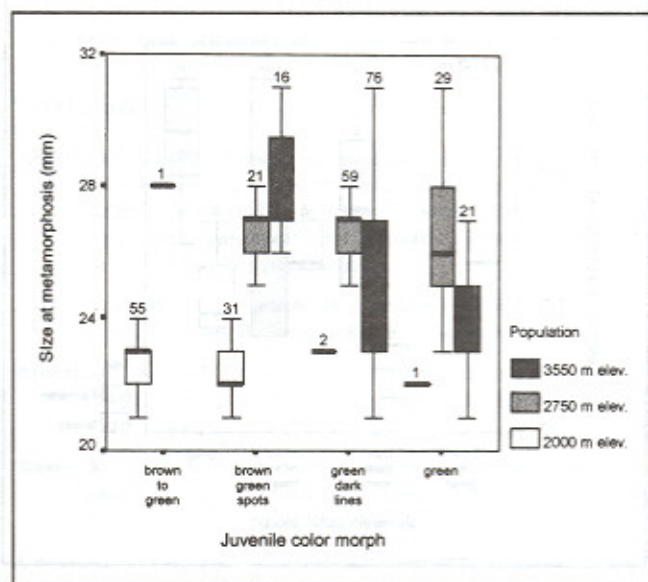


Figure 6. Relationship between size at metamorphosis and juvenile color morph of *Hyla labialis* from populations at different elevations. The horizontal bar represents the median, the upper and lower ends of the box are the upper and lower quartiles and the middle 50% of data lie between them, the vertical line represents the range of observations, and N is given above the range.

Growth and color pattern

The number of individuals reared in the laboratory is relatively low and thus, the analysis of growth data in relation to color morph could only be performed for individuals from the MIDM population. The analysis of covariance of postmetamorphic growth on color pattern and sex revealed no significant relationship between color morphs scale and postmetamorphic growth (ANCOVA, $n=29$, color morph: $F=1.35$, $P=0.257$; sex: $F=0.07$, $P=0.797$; color morph by sex: $F=0.68$, $P=0.417$). Thus, although some intrapopulation differences in growth rate are suggested by Figure 7, they are not related linearly to the ordinal scale of color morphs.

Discussion

This study indicates that dorsal color patterns of *Hyla labialis* can be classified in five categories and arranged ordinally from only brown to only green morphs. These categories are valid across ontogenetic development, since they take in account changes in color pattern from recently metamorphosed stages to sexually mature adults. Dorsal coloration in anurans is known to experience physiological changes within hours, depending on factors such as

temperature, background brightness, and hormonal levels (Duellman & Trueb (1986), King *et al.* (1994)). I observed that such changes affected the lightness of the colors but not the pattern itself in *H. labialis*.

I also found interpopulational variation in the relative occurrence of color morphs. Since substrate colors in natural habitats are usually variegated, explanation of geographic variation based on local crypsis arguments demands accurate and objective quantification of the substrate colors. Such analysis was not performed in this study. However, there are outstanding differences between study sites, primarily in the microhabitat structure and secondarily in plant coloration, at the ground level in the open areas where individuals of this species are mostly found exposed, i.e. basking and moving. Although grasses are by far the dominant vegetation at all study sites, the high-elevation habitat is characterized by a much higher incidence of moss cushions, lichens and ground vegetation with small oval leaves, leading to a more patchy and spotted environment. Thus, one could be tempted to explain variation in adult color morphs as a consequence of their differential value for crypsis in each environment, i.e. spotted patterns are more frequent at highest elevation because they are adaptive in the more spotted grounds characteristic of this area. A role of coloration in local crypsis would also help to explain the prevalence of color polymorphism in the species through apostatic selection that favored several locally adapted morphs (Paulson (1972), Endler (1986), Guilford (1992)). Formal testing of this explanatory framework requires further knowledge on the habitat use by individuals of each population, on the kind of predators that mostly prey on this species, and on the perception mechanisms they mostly use to locate the frogs.

Interpopulational variation in color patterns already is evident among individuals at the end of metamorphosis. Color patterns should affect juvenile performance mainly during the postmetamorphic phase, as the trait itself is not expressed before metamorphosis. In juveniles, there is no suggestive relationship between interpopulational variation in color morphs and corresponding variation in background environment. Furthermore, patterns that are more common in adults are not always more common in juveniles of the same population (Fig. 4). Although sample size is low for the adults of the HIGM population ($n=24$), the difference in color patterns between juveniles and adults in the other two populations most probably do not obey to a sample size artifact. Therefore, juvenile color patterns may play a role in crypsis only if juveniles and adults are spatially segregated or if they are attacked by

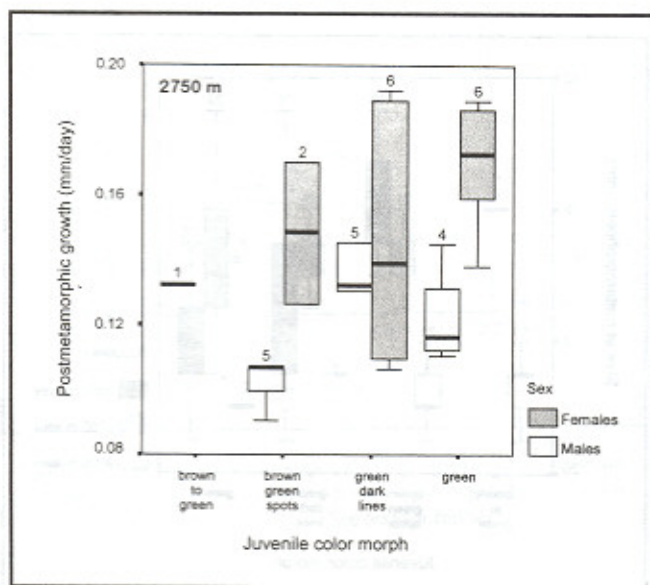


Figure 7. Postmetamorphic growth of juvenile color morphs of *Hyla labialis* from the mid-elevation population, reared in the laboratory during 300 days under common garden conditions. The horizontal bar represents the median, the upper and lower ends of the box are the upper and lower quartiles and the middle 50% of data lie between them, the vertical line represents the range of observations, and N is given above the range.

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