

Environmental Factors Influencing Wood Frog (*Lythobates sylvaticus*) Tadpole Size

Amanda Smith and Shelby Kilibarda

Environmental Science

ABSTRACT

Body size variation among individuals and populations within a species is important to study due to the ecological implications, specifically life history population dynamics. Variation in growth among individuals has been attributed to both inherent biological and environmental factors affecting an organism. Several models have been proposed to describe life history variation and tradeoffs. Understanding the controlling influence of body size variation in amphibians requires consideration of all factors. We evaluated which environmental factors have an impact on *Lythobates sylvaticus* tadpole body size. Our study site consisted of 57 wetlands (41 ephemeral and 16 permanent) in Chippewa County, Wisconsin. Tadpole body sizes, measured as snout vent lengths (SVLs), were surveyed in each wetland using minnow traps collected after 24 hours of being submerged. Environmental factors, including dissolved oxygen, canopy cover, pH, temperature, hydroperiod, and chlorophyll-a were quantified. We also considered possible size variations occurring in permanent versus ephemeral wetlands. Tadpoles in permanent ponds were significantly larger ($F_{1,18} = 7.34$, $p\text{-value} = 0.014$) than those in ephemeral ponds. Out of the environmental factors, pH resulted in a positive correlation with tadpole size (Adjusted $R^2 = 0.38$, $F_{2,17}$, $p\text{-value} = 0.006$). The results of our research provide evidence for a relationship between amphibian body size and environmental characteristics. The pH of wetlands may serve as a proxy for the relationship between peat accumulation, or immediate upland landscapes, most often acidifying Pinaceae family and the SVL of *L. sylvaticus*.

Keywords: body size, ephemeral ponds, *Lythobates sylvaticus*

INTRODUCTION

Body size variation among individuals and populations within a species is important to study due to the ecological implications, specifically life history population dynamics. Variation in growth among individuals has been attributed to both inherent biological and environmental factors affecting an organism. Several models have been proposed to describe life history variation and tradeoffs. Life history traits such as age at reproduction, length

of reproduction capabilities, and quantity of offspring, including size and number, all dictate success of species survivorship (Charnov 1993). Furthermore, reproductive value (RV) models the tradeoffs between survivorship, quantity of offspring, and growth (Charnov 1993). The RV model states that depending on the ecological situation organisms will adapt to reproductive outcomes, including size of offspring. The "island rule" offers another size variation theory based on the segregation of one population from another. In the case of populations, segregation means a physical barrier is preventing contact of the two groups. This rule suggests that when segregated, larger organisms will become miniaturized and vice versa at a faster rate and larger scale than non-segregated organisms (Millien 2004). Variation in body size may be due to limited food resources, which restrict or promote growth. Size variation has also been found to be dependent on available food resources according to numerous studies on organisms ranging from mammals to gastropods. Hutchinson (1959) suggested that differentiation in body size may be associated with a reduction in interspecific competition. Research studies investigating adaptation to competitive interactions have noticed a shift in body size, perhaps associated with resource utilization (Brown & Wilson 1956; Adams 2007; Slatkin 1980). A study on *Tarebia granifera* (quilted melania) demonstrated that level of competition, in terms of reaction speed to a food source, was dependent upon body size, which dictated the level of success among individuals (Snider 2008). Thus, competitive dynamics over a limited resource can influence body size variation among species.

Amphibian growth rates and body size are ultimately related to survival. In accordance with the food resource model, tadpoles were observed selectively foraging high protein diatoms that supported rapid growth (Kupferberg 1997); however, some studies have found tadpole growth rates to be independent of food availability (Bardsley 2000). The hatching time of tadpole larvae may influence body size. Wood frog (*Lithobates sylvaticus*) tadpoles that hatch earlier than others may initially be smaller, but may grow the most and at a faster rate. These tadpoles also metamorphosed earlier than the younger tadpoles, suggesting an advantage to early hatching times (Warne 2013). Hydroperiod has to be taken into consideration as well. The survival rate and size of tadpoles is greatly affected by the length of time a pond holds standing water (Amburgey 2012). While many hypotheses have been proposed for the variation of body size, it remains unclear as to which factors. Physiological or environmental are most important.

Understanding the factors that may influence body size variation in amphibians requires thorough consideration. In order to further evaluate these hypotheses, we focused on *Lithobates sylvaticus* tadpoles in the glaci-

ated Western Wisconsin landscape to test which environmental factors have an impact on body size. Our study site consisted of 57 wetlands (41 ephemeral and 16 permanent) in Chippewa County, Wisconsin. Tadpole body size, measured as snout vent lengths (SVLs), were surveyed in each wetland using minnow traps collected after 24 hours of being submerged. Environmental factors, including dissolved oxygen, canopy cover, pH, temperature, hydroperiod, and chlorophyll-a were quantified. We also considered possible size variations occurring among permanent and ephemeral wetlands. Knowing permanent wetlands have standing water year round, the tadpole's metamorphosis is not constrained by water duration, but offers the possible risk of more predators.

METHODS

Study Area

This study was conducted in the Chippewa Moraine State Recreation Area in Chippewa County, Wisconsin (45° 13' 13.32" N, 91° 24' 39.7" W) in six large (≥5.0 Hectare) wetland systems. This area is heavily forested and surrounded by many kettle lakes created by the most recent glacial ice age. Within the study area, there are both permanent wetlands and ephemeral ponds.

Amphibian Sampling

Amphibians were surveyed during May 2013 using minnow traps. Amphibians were sampled over a one-week time period using minnow traps with three traps per ephemeral pond and four traps per permanent pond. The minnow traps were dispersed near the perimeter of each pond (distance from dry land ≥ 0.25 m) and submerged half way into the water column, leaving sufficient space for tadpoles to enter. The traps remained in the ponds for 24 hours. After 24 hours, the traps were pulled from the ponds and the tadpoles were identified and measured from the tip of the snout to the vent. The species, visible metamorphosis characteristics, and SVL were recorded. Once data was recorded from each captured tadpole, they were released to the wetland.

Environmental Characteristics

Dissolved oxygen, temperature, and pH readings were obtained three times during the May-August wetland season using a YSI Pro20 dissolved oxygen meter to capture variability. Readings were taken at three locations within each pond (two near perimeter and one in the center). The probe was lowered halfway into the water column and recorded after values stabilized. Tree canopy cover was quantified using a convex spherical densiometer.

Three water samples (1000mL each) were collected from each pond using light impenetrable Nalgene bottles to prevent Chlorophyll A degradation. Two samples were taken near the perimeter of the wetland and one sample from the middle in

order to account for variability. The water samples were chilled in portable coolers with ice packs in order to prevent chlorophyll-a degradation. Chlorophyll-a was quantified according to standard operating procedures.

Statistical Analysis

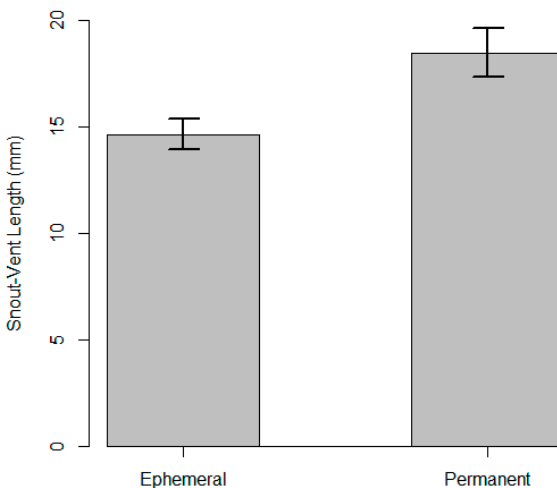
We used multiple regression to assess the relationship between tadpole SVL and the quantified environmental characteristics. We also assessed the variation in mean SVL among ephemeral and permanent wetlands by performing an analysis of variance (ANOVA). Upon finding a significant relationship between body size and wetland classification, we conducted an analysis of covariance (ANCOVA) to assess whether variation in environmental characteristics among the different wetland classes was explaining the variation of tadpole body size among permanent and ephemeral wetlands.

RESULTS

Out of the 40 ephemeral ponds surveyed, only half contained *L.sylvaticus* tadpoles. The mean SVL was 13.7 mm (\pm 8.19). Minimum SVL was 8.33 mm and maximum was 20.2 mm.

We found conclusive support of tadpole body size variation between ephemeral and permanent ponds. Mean body size was significantly larger in tadpoles sampled from permanent wetlands (Figure 1, $F_{1,18} = 7.34$, $p\text{-value} = 0.014$). Mean body size of tadpoles was 14.65 mm (SE = 0.72) in ephemeral wetlands and 18.48 mm (SE = 1.16) in permanent wetlands.

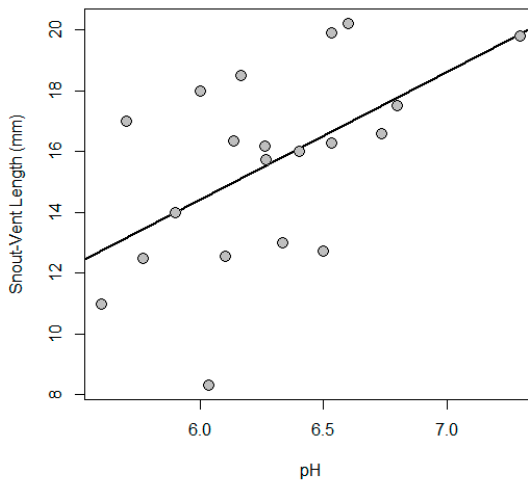
FIGURE 1



Dissolved oxygen, temperature, canopy cover, and chlorophyll-A were not significantly correlated with SVL after conducting stepwise multiple regression analyses on each environmental factor. Water chemistry variables differed greatly as shown by the wide range of values (Table 1). pH was the only environmental factor to be significantly correlated with SVL ($R^2 = 0.30$, $F_{1,18} = 7.56$, $p\text{-value} = 0.013$, Figure 2). The result of the ANCOVA indicated that both wetland classification (permanent and ephemeral) and pH explain a significant proportion of the variation in SVL (Adjusted $R^2 = 0.38$, $F_{2,17}$, $p\text{-value} = 0.006$).

TABLE 1

Variable	Mean	Minimum	Maximum
Dissolved oxygen (mg/L)	1.8	0.3	7.0
pH	5.9	5.9	6.6
Temperature (°C)	17.1	13.2	22.2

FIGURE 2

DISCUSSION

The potential influences of body size variation include: wetland duration, resources, predators, water temperature, and competition. Further studies are needed to conclude which sources have the greatest impact on the body size variance in permanent versus ephemeral ponds. We determined pH was the only significant factor having a correlation with SVL in ephemeral ponds and permanent wetlands. We believe factors affecting pH are most likely litter inputs and peat accumulation, in addition to geological characteristics. Studies examining types of leaf litter have indicated that tadpole size may be related to both the species of amphibian and species of trees supplying leaf litter to the aquatic system (Stoler & Reylea, 2011). Further, leaf litter has an influence on phytoplankton and zooplankton, which will not only be associated with water chemistry, but also food resources for tadpoles. Thus, pH of wetlands may serve as a proxy for the relationship between immediate upland landscapes, most often acidifying Pinaceae family, and the SVL of *L. sylvaticus*. As pH increases, SVL increases; thus more basic wetlands are likely to have sustainable populations due to larger amphibians. Wetland ecosystems are ultimately driven by primary producers, which depend on nutrients and sunlight availability, both of which are provided by the immediate surrounding landscape.

While our study focused on *L. sylvaticus*, it is important to realize the potential implications of our results on other members of the biological community that consume and are consumed by *L. sylvaticus*. Wetlands provide flood protection, filtration of harmful pollutants, and other benefits that are significant economic benefits. The potential relationship between leaf litter, wetland pH, and the basal components of the aquatic food web suggest that these wetlands are functionally linked to the adjacent upland forests. Previous research in this type of wetland also suggests that land use in upland forests have a significant impact on wetland communities (Hanson, Palik, Church, & Miller, 2010). Tree harvesting in upland forests creates changes in permanent and ephemeral ponds, such as reduced invertebrate variability. These results suggest that forested wetlands require significant protection. Future studies designed to evaluate multiple species of animals and plants of wetlands would promote a better understanding of biodiversity functions of wetland ecosystems. With expansive research we believe conservation policy could be greatly beneficial to the greater watersheds.

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