

THE EFFECTS OF CAUDAL SCOLIOSIS ON SWIMMING POTENTIAL AND SURVIVABILITY OF
WOOD FROG (*RANA SYLVATICA*) TADPOLES

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ABSTRACT

Caudal scoliosis is a spinal malformation recognized in larval anurans as a lateral curvature of the tail. Although several reports characterize caudal scoliosis in anurans, no studies have examined the effects of this malformation on swimming potential and survivability of the afflicted tadpoles. I measured the burst-swimming speeds and survival from predation by *Dytiscus* spp. larvae of both normal and scoliotic *Rana sylvatica* tadpoles in order to determine the impact of scoliosis on tadpole fitness. Scoliotic tadpoles had significantly slower burst-swimming speeds than normal tadpoles ($P < 0.001$) and were significantly more susceptible to predation by dytiscids ($\chi^2 = 12.98$, $df = 1$). My results indicate that scoliotic tadpoles suffer a fitness cost and a reduced burst-swimming speed that likely contributes to their increased mortality. This study serves as a basis for future experimentation regarding the causes and implications of scoliosis in anurans.

INTRODUCTION

Caudal scoliosis, characterized in tadpoles as a lateral curvature of the tail, has been formally recognized in several species of North American frogs including the wood frog (*Rana sylvatica*; Hisaoka and List 1957), the northern leopard frog (*Rana pipiens*; Hardy Jr. 1964, Underhill 1966), and the American bullfrog (*Rana catesbeiana*; Hedein 1976). This malformation is distinguished by two curves in the tail of the tadpole,

involving bends in both the spinal chord and notochord (Hisaoaka and List 1957). The primary curve is located at the point of the tail's attachment to the body, with a second curve located farther down the tail axis, twisting the tail in the opposite direction of the first curve (Hisaoaka and List 1957).

Although it has been recognized in anurans, specific causes of this tail malformation have yet to be identified. Underhill (1966) has collected evidence suggesting that scoliosis may be a genetic trait. Other studies have indirectly identified ultraviolet-B (UVB) radiation as a possible cause of scoliosis in tadpoles. Van Uitregt et al. (2007) found that embryonic exposure to UVB radiation caused embryos to develop spinal malformations, even at intensity levels below that of their natural environment. A recent study by Croteau et al. (2008) found that spinal malformations, including scoliosis, were recurring deformities in tadpoles exposed to UVB radiation.

The literature provides inconclusive evidence as to the extent at which this malformation affects swimming potential and performance. In some observations, the scoliotic tadpoles had difficulty swimming rapidly (Hardy Jr. 1964, Underhill 1966) and in other observations, the scoliotic individuals swam normally except in cases of extreme scoliosis (Hisaoaka and List 1957).

Since this deformity modifies the tail, a tadpole's primary agent of locomotion, I was interested in studying whether or not caudal scoliosis negatively impacts a tadpole's swimming speed and thus influences its survivability. The central goal of my study was to utilize several experiments to assess to what extent caudal scoliosis affects the survival of afflicted wood frog tadpoles in the presence of a natural predator. I

measured burst-swimming speeds of normal *Rana sylvatica* tadpoles and those afflicted with scoliosis. Contrary to endurance swimming, burst swimming is an anaerobic process (Arendt 2003) that is a valuable survivable mechanism as a means for escaping predation (Dayton et al. 2005). Measuring burst swimming rather than other forms of endurance swimming allows for a more accurate estimate of tadpole fitness levels (Arendt 2003). I also examined the intraspecific survivability between afflicted and normal *Rana sylvatica* tadpoles in the presence of a natural predator, larvae of the diving water beetle (*Dytiscus* spp). I expected to find reduced burst speeds in scoliotic tadpoles and therefore, increased susceptibility to predation by dytiscids.

METHODS

Organisms: *R. sylvatica* egg masses were collected from ten open canopy ponds within the UNDERC property from early to middle May 2008. The tadpoles were raised until May 27 in wading pools under 60% shade cloth. *Dytiscus* spp. (Dytiscidae) larvae were collected from six vernal ponds within the UNDERC property from June 2-8 2008. The dytiscids were maintained in plastic jars containing water from the pond of collection and a strand of polypropylene rope. Dytiscids were fed one *R. sylvatica* tadpole daily while in captivity.

Generation of scoliotic tadpoles: Scoliosis was induced in *R. sylvatica* tadpoles through exposure to natural UV radiation. Five outdoor pools were set in direct sunlight and filled with tapwater. To each pool, 1L of filtered pond water and 5g of crushed rabbit food were added as sources of algae and nutrients. Fifty zooplankton were

added to each pool to prevent the growth of bacteria and excess phytoplankton. A fiberglass window screening lid of approximately 20% cover topped each pool. Following water stabilization, fifty tadpoles were transferred to each pool. Following four weeks of growth in the outdoor pools, tadpoles were sorted into three categories based on tail morphology: normal, intermediate (showing a very slight degree of scoliosis), and scoliotic. Only those tadpoles in the normal and scoliotic categories were used in experimentation.

Burst speed measurements: To quantify burst speeds of individual tadpoles, an indoor swimming test chamber was constructed. A 35cm by 7.5cm plastic aquarium was filled with water retrieved from the scoliosis-inducing wading pools to a depth of 2cm. A 1cm by 1cm grid covered the bottom of the chamber. The trials were recorded with a Sony digital camcorder (29.97 frames s^{-1}) placed 0.4m directly above the water surface. Tadpoles were weighed before the start of each test. Once placed in the aquarium, each tadpole was given a one-minute acclimatization period. Predator attacks were simulated by swiftly flicking the base of the tail with a dissecting probe. Three trials were recorded per individual. Burst speeds were calculated in $mm s^{-1}$ by measuring the distance traveled in the first 0.3-0.6s following the stimulated attack. The maximum burst speed potential was recorded as the fastest burst speed of the three trials and was used in data analyses. Burst speed measurements were taken over the course of two days.

Predation: To examine the effects of scoliosis on tadpole survival in the presence of a natural predator, ten 0.5m diameter experimental tubs were filled to a

depth of 0.3m with well water. Identical artificial vegetation cover consisting of 14 green polypropylene ropes was added to each tub to provide perching stems for the predator. A fiberglass window screening lid of approximately 20% cover was secured to each tub. Five normal and five scoliotic tadpoles of similar body masses and age (six weeks) were added to each tub. Dytiscid larvae of similar sizes ($\bar{x}=0.964$ g, $SE=0.068$) were starved for twenty-four hours prior to the start of experimentation. Twenty-four hours following the addition of tadpoles, a dytiscid larvae was added to each tub. Tubs were checked for the total number of remaining tadpoles every 3h following the start of experimentation and a predator was removed when there were only five surviving tadpoles in its tub.

Statistical analysis: Data were analyzed using the SYSTAT 12.0 software package (SPSS, Chicago, IL). Initially, ANCOVA tests were run to assess whether body size or test day influenced burst speed. Body mass was determined to influence burst speed and burst speeds were subsequently adjusted to body mass with a residual analysis. Residuals from the ANCOVA test were added to the mean swimming speeds for each phenotype to generate size-adjusted burst-swimming speeds. The adjusted burst speeds were then analyzed with a t-test. Data from the predation experiment was subjected to Pearson's chi-square test and the generated chi-square statistic was compared to a table of critical values of the chi-square distribution.

RESULTS

Burst speed experiment: Normal tadpoles had significantly greater burst speeds than scoliotic tadpoles ($t=3.808$, $df=98$, $P<0.001$), with an average burst speed adjusted for body mass of 107.61 mm s^{-1} ($SE=2.93$) as compared to 92.24 mm s^{-1} for scoliotic tadpoles ($SE=2.78$; Figure 1). The test day did not influence burst speeds (Figure 2).

Predation experiment: Scoliotic tadpoles were significantly more prone to predation than normal tadpoles ($\chi^2=12.98$, $df=1$, $P<0.001$). In nine of the ten replicates, fewer scoliotic tadpoles survived predation than normal tadpoles; 66% of normal tadpoles survived predation as compared to 30% of scoliotic tadpoles (Figure 3). One predator was removed 9h after introduction, six predators were removed 15h after introduction, and the remaining 3 predators were removed 18h after introduction. Predator mass, length of time a predator was present, and tadpole mass were not correlated to the proportion of scoliotic tadpoles that survived predation.

DISCUSSION

Results from this study confirm my initial hypothesis that caudal scoliosis negatively impacts afflicted *R. sylvatica* tadpoles. As predicted, scoliotic tadpoles showed significantly slower burst speeds and were significantly more susceptible to predation than tadpoles displaying a normal phenotype. The lower burst speed of scoliotic tadpoles is likely an important factor in their susceptibility to predation. Normal tadpoles may have more easily evaded attempted predator attacks because they exhibited significantly faster burst speeds.

One of the morphological traits of scoliotic tadpoles is a disproportional distribution of muscle fibers in the tail (Hisaoaka and List 1957). This anatomical difference may account for the lower burst-swimming speeds in scoliotic *R. sylvatica* tadpoles. Because of their asymmetrical musculature, scoliotic tadpoles may have difficulties turning or rapidly changing directions. Such a handicap would also contribute to lower survivability in the presence of a natural predator. Further experimentation should analyze burst-swimming speeds of both phenotypes in addition to escape angles as factors contributing to differences in survivorship between normal and scoliotic tadpoles.

This study can be improved upon for future replications by altering the method of measuring burst-swimming speeds. In this study, I used a long rectangular aquarium as a test chamber, but often found tadpoles lingering in the corners and remaining along the edges of the tank, even after stimulation. Tadpoles may have displayed slower burst-swimming speeds if swimming along the edge of the tank hindered them. A larger circular or square aquarium may be better suited for filming burst speeds. Reproductions of this study should also measure burst speeds of individuals as the distance a tadpole travels in a uniform time after stimulation of a predator attack. In this study, I measured the burst speeds of different individuals over different time lengths (between 0.3 and 0.6s), which may have introduced some bias in the burst speeds. Also, variations in tadpole morphology that were not quantified in this study can influence swimming speeds. Future replications should consider additional measurements, such as snout-vent length measurements or other morphometric analyses.

Research into the causes of caudal scoliosis in anurans may further explain the results of this study. If UVB radiation can be established as a cause of anuran scoliosis, tadpoles with higher activity rates and therefore greater exposure to UVB radiation may have a higher probability of developing scoliosis. Tadpoles with higher rates of activity also tend to encounter predators more frequently (Dayton et al. 2005). Consequently, the predation experiment of this study may have found that tadpoles with higher activity rates are more susceptible to predation, not necessarily scoliotic tadpoles with reduced burst speeds. Also, if UVB radiation is a factor in the development of scoliosis, this study confirms a previous study by Van Uitregt (2007) of the effects of UVB radiation on embryonic and larval anurans. The study found that UVB radiation had significant effects on the speeds of tadpoles. Tadpoles exposed to higher levels of radiation were significantly slower than tadpoles exposed to lower levels of radiation. If UVB radiation is a cause of scoliosis, this reduction in speed with increasing levels of radiation would be consistent with the findings of my study. Scoliotic tadpoles were likely exposed to greater levels of radiation, a factor that may have reduced their burst-swimming speeds.

Although the fitness costs of malformations have been extensively studied in adult frogs, this research is one of few studies that have examined the fitness cost of a malformation in tadpoles. Any costs to tadpole fitness subsequently influence the levels and composition of frog populations. Malformations such as scoliosis are examples of such factors that negatively affect the fitness of afflicted individuals and can thereby influence frog populations. The findings in this study, in conjunction with experiments

by Van Uitregt (2007), may have implications for anuran populations worldwide, especially those exposed to high levels of UVB radiation. Further experimentation determining specific causes of caudal scoliosis in anurans can determine what risk, if any, frog populations would be at following an increase in scoliotic tadpoles.

Future research should replicate the predation experiment of this study, but while varying factors additional to phenotype, such as burst-swimming speeds and activity rates among experimental tadpoles. The mortality of scoliotic tadpoles in the presence of natural predators is most likely the result of multiple, interrelated factors, including burst speeds and possible differences in activity rates. Future experimentation in the causes of anuran scoliosis, scoliotic tadpole morphology, and survivability of scoliotic tadpoles is necessary to fully comprehend the implications of this study.

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FIGURES

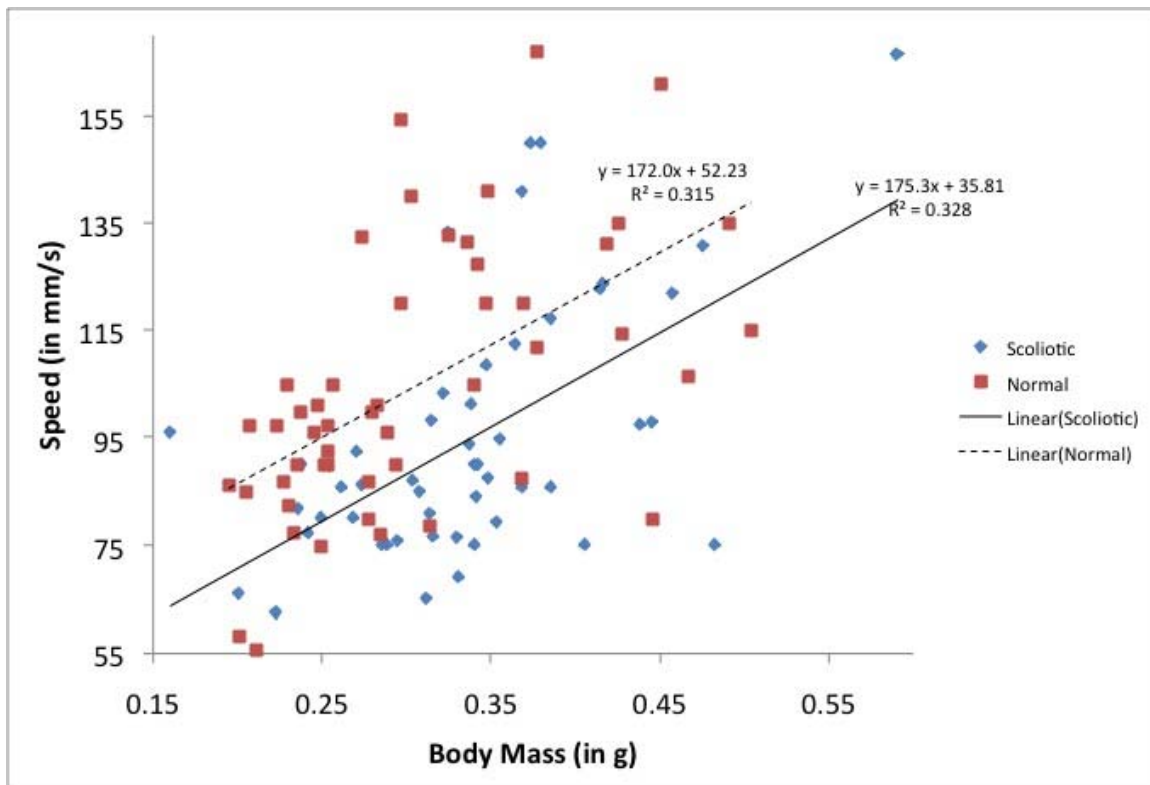


Figure 1: Speed as a function of body mass comparing normal and scoliotic tadpoles. Burst-swimming speeds of both normal ($R^2=0.325$) and scoliotic ($R^2=0.328$) tadpoles linearly increases with body mass.

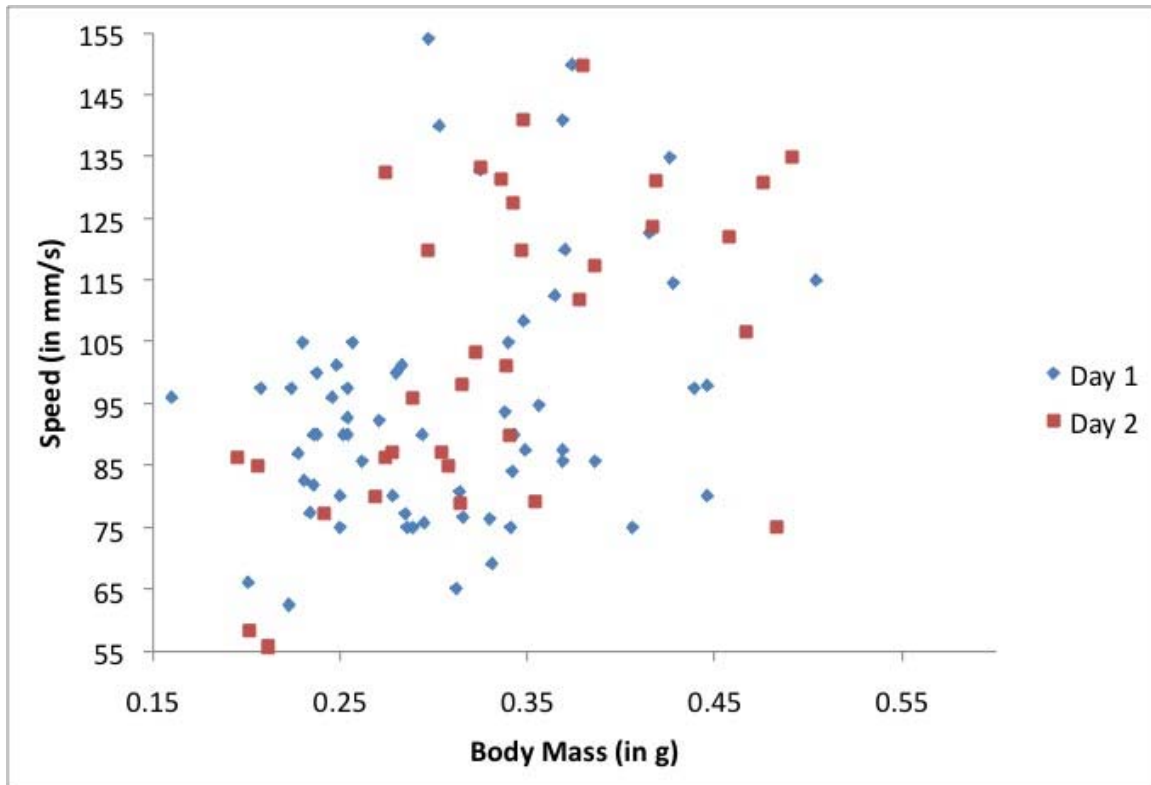


Figure 2: Speed as a function of body mass comparing measurements taken on day 1 and day 2 of swimming trials. The day that swimming trials were recorded on had no effect on burst-swimming speeds.

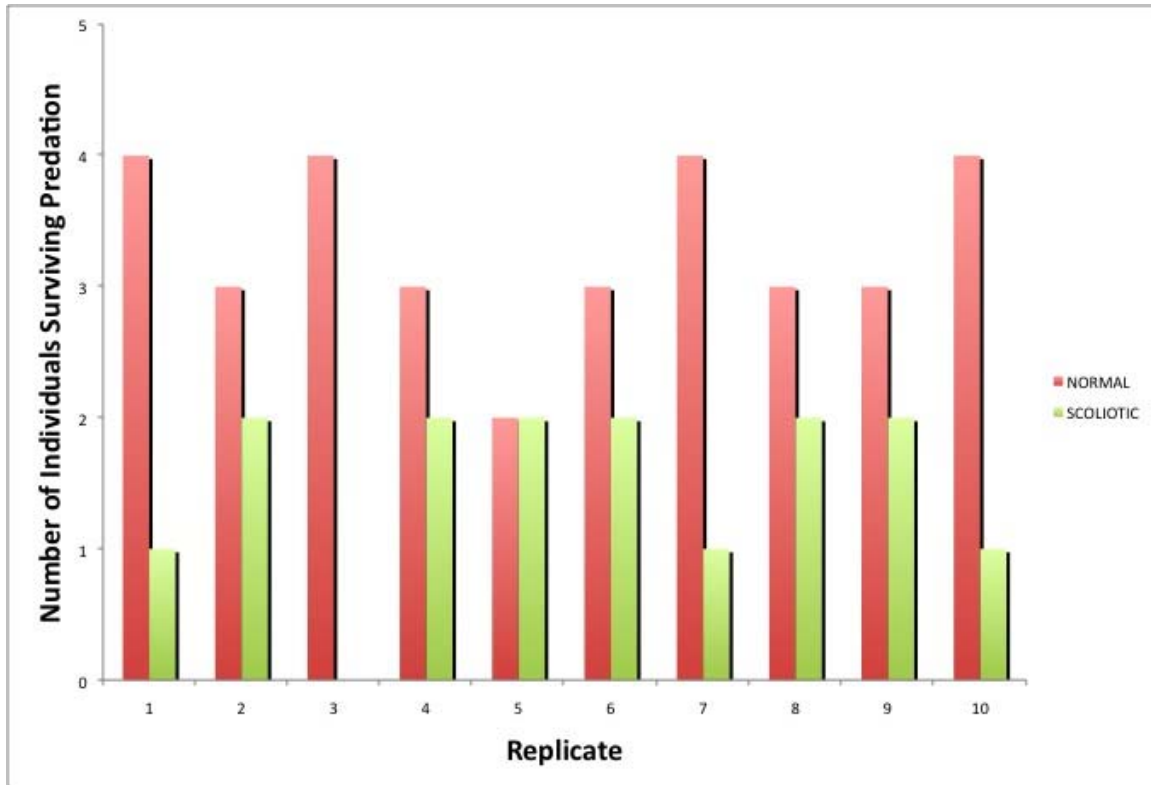


Figure 3: Numbers of normal and scoliotic tadpoles surviving predation. Scoliotic tadpoles were significantly more susceptible to predation than normal tadpoles ($\chi^2=12.98$, $df=1$).