The Canadian Field-Naturalist

Wood Frog (*Lithobates sylvaticus*) skeletogenic plasticity in anthropogenic habitats

Laurent Houle[1], Jacinthe Beauchamp[1,2], Luc Sirois[2], Alain Caron[3], Jacques Trottier[1,2], Kathleen Sévigny[1], Olivier Larouche[1,4,5], and Richard Cloutier[1,*]

[1]Laboratoire de Paléontologie et Biologie évolutive, 300 Allée des Ursulines, Rimouski, Quebec G5L 3A1 Canada
[2]Chaire de Recherche sur la Forêt Habitée, 300 Allée des Ursulines, Rimouski, Quebec G5L 3A1 Canada
[3]Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, 300 Allée des Ursulines, Rimouski, Quebec G5L 3A1 Canada
[4]Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204 USA
[5]Current address: Biology Department, Western Carolina University, Cullowhee, North Carolina 28723 USA
[*]Corresponding author: richard_cloutier@uqar.ca

Houle, L., J. Beauchamp, L. Sirois, A. Caron, J. Trottier, K. Sévigny, O. Larouche, and R. Cloutier. 2024. Wood Frog (*Lithobates sylvaticus*) skeletogenic plasticity in anthropogenic habitats. Canadian Field-Naturalist 138(2): 163–174. https://doi.org/10.22621/cfn.v138i2.3133

Abstract

Habitat loss and landscape fragmentation are major causes of numerous amphibian population declines. Although logging activities have been related to serious effects on growth rate and size at metamorphosis in several species, less is known about skeletal developmental modifications associated with disturbed habitats. We studied the effects of forest canopy modifications caused by logging activities on the skeletal development of a pond-breeding anuran, Wood Frog (*Lithobates sylvaticus*). Biotic and abiotic factors were collected for 30 semi-permanent ponds located in three habitat categories (regenerated forest, along skidding trails, and logged areas). A sample of 58 cleared and double-stained tadpoles were analyzed to compare developmental trajectories among habitats. Water temperature and pond morphometric characteristics, which were correlated with logging-related habitat alteration, had a major impact on tadpole developmental differences among pond categories. Developmental plasticity was evident in both absolute and relative timing of chondrification and ossification between regenerated forest ponds and disturbed ponds (i.e., along skidding trails and in logged areas). Ossification and chondrification patterns had a different response to environmental factors. Notably, we observed the early onset of skeletogenesis in the disturbed ponds, which may result in deleterious effects on the fitness of post-metamorphosed juveniles.

Key words: Logging activities; developmental plasticity; pond-breeding amphibians; skeletogenesis; Wood Frog; *Lithobates sylvaticus*

Introduction

Vertebrates can be critically affected by abiotic factors throughout their lifespan (Noble *et al.* 2018; Singh *et al.* 2020). Among these factors, temperature and humidity are known to affect phenology and the timing of breeding activities of several amphibian and reptile species (Bachmann 1969; Beebee 1995; Reading 1998; Lannoo and Stiles 2017; Noble *et al.* 2018; Singh *et al.* 2020). In aquatic environments, water temperature is known to influence the development of cartilage (chondrification) and bone (ossification) in fishes and amphibians (Fuiman *et al.* 1998; Haas 1999; Mabee *et al.* 2000; Gomez-Mestre *et al.* 2010). Skeletal elements that form later in the development have been shown to be more strongly influenced by environmental constraints and were therefore more susceptible to express a plastic response (Hallgrimsson *et al.* 2007; Cardini and Elton 2008; Young and Badyaev 2010; Grünbaum *et al.* 2012). Ossification patterns have also been suggested to be more sensitive to functional requirements during development in comparison to chondrification (Grünbaum *et al.* 2003; Campinho *et al.* 2004). However, abiotic factors in breeding ponds that affect skeletal development in amphibians remain poorly documented (Hanken and Hall 1984; Gomez-Mestre *et al.* 2010).

Logging activities can tremendously change the habitat characteristics, such as soil properties and plant species distribution and abundance within a forest (Krag *et al.* 1986; Aust *et al.* 1993; Kariuki *et al.* 2006). These changes can also have significant effects on animal populations that use these habitats (Potvin

1999). Impacts on the abundance, diversity, and distribution of species, species interactions (e.g., predation), and habitat selection have been documented for numerous animal species (Ganey and Balda 1994; Darveau *et al.* 1997; Potvin 1999; Werner and Glennemeier 1999; Trottier 2006). By removing the forest canopy near aquatic habitats, logging activities contribute to increasing exposure to sun radiation leading to higher water temperature and modifying the duration a pond holds water (hydroperiod; DeMaynadier and Hunter 1995; Waldick *et al.* 1999).

Characteristics of both aquatic and terrestrial microhabitats that are modified by logging activities can influence the distribution and growth of amphibians (DiMauro and Hunter 2002; Houlahan and Findlay 2003; Trottier 2006; Beauchamp 2007). Amphibians are particularly sensitive to habitat modifications that can be linked to some of their physiological and ecological characteristics (such as skin permeability, low dispersal capacity, small home-range size, philopatry; Blaustein 1994; Duellman and Trueb 1994; Demaynadier and Hunter 1998). Such abiotic modifications of ponds can change the availability of tadpole food resources (Skelly *et al.* 2002; Skelly and Golon 2003; Baldwin *et al.* 2006), which can affect the reproductive effort and distribution of pond-breeding amphibians. The creation of temporary ponds along skidding trails and in cutover areas because of logging activity can also negatively affect amphibian development (Waldick *et al.* 1999; DiMauro and Hunter 2002). These temporary ponds are likely to dry out before tadpoles can reach metamorphosis and consequently induce high mortality (DiMauro and Hunter 2002), potentially reducing population fitness. Thus, multiple factors interact with amphibian survival, and logging effects can differ greatly among life stages within conspecifics (Semlitsch *et al.* 2009).

Amphibians can demonstrate phenotypic plasticity during their development in response to different environmental pressures (Newman 1992; Dahl *et al.* 2012). Developmental plasticity in anurans has been observed in several tissues and morphological traits (Udin and Keating 1981; Dieringer 1995; Touchon and Warkentin 2008). For example, the length and thickness of tail muscles of Wood Frog (*Lithobates sylvaticus*) increase in size when predators are present (Relyea 2002). Gomez-Mestre *et al.* (2010) showed that for both Red-eyed Tree Frog (*Agalychnis callidryas*) and African Clawed Frog (*Xenopus laevis*), low temperatures induced a lower degree of ossification and longer legs when compared to those that developed at higher temperatures. Habitat modifications can have observable effects not only on external morphology of pond-breeding amphibians, but also on internal anatomy during development.

The development of the amphibian skeleton is sensitive to pollution and habitat degradation (Ouellet *et al.* 1997; Lajmanovich *et al.* 2003; Taylor *et al.* 2005). Here, we provide an innovative approach to address the effects of logging activities on the skeletogenetic plasticity of amphibians. Our objective was to evaluate the impact of forest canopy modifications from logging activities on the skeletal development of Wood Frog in semi-permanent ponds in eastern Canada. Ponds in anthropogenically disturbed habitats (i.e., near skidding trails and in logged areas) were compared with ponds in regenerated forests. The most discriminating factor among pond categories is expected to be water temperature. Because of the absence of the forest canopy, higher water temperature in disturbed ponds should promote faster Wood Frog development compared to regenerated forest ponds. Environmental contrasts among pond categories are also likely to influence the absolute and relative timing of chondrification and ossification during development. While there is obvious coordination between cartilage and bone formation, the magnitude of their response to environmental constraints may differ. Given the results from previous studies in fishes (Grünbaum *et al.* 2003; Campinho *et al.* 2004), we predict that environmental conditions will have a greater effect on ossification than chondrification patterns.

Methods

Study area

Surveys were conducted in the Eastern Balsam Fir - Yellow Birch bioclimatic domain (Saucier 1998) at the interface between the northern hardwood and boreal biomes of eastern Canada. This region has 88% forest cover that has been exploited by logging companies since 1901 (Boucher *et al.* 2009). Thirty ponds in the Duchénier Wildlife Reserve (eastern Quebec, Canada; Figure S1) were sampled in recently cut and regenerated forests. The area is dominated by coniferous species and is a transition area between the boreal and deciduous forest ecosystems (Rowe 1972).

We selected 30 ponds that: (1) contained at least one amphibian species, (2) had a semi-permanent hydroperiod, (3) were <0.2 ha, (4) lacked a stream connection, (5) were devoid of fishes, and (6) were <500 m from a road. We sampled 10 ponds for each habitat category: logged areas, skidding trails, and regenerated forest areas (Figure S1).

Biotic and abiotic environmental characteristics

Global positioning system coordinates for each pond and distances from the nearest skidding trails, logged area, and regenerated forest were recorded at the beginning of summer 2004. Pond length and width, aquatic substrate composition, vegetation cover around the pond, and vegetal water coverage were recorded on three separate occasions between May and August 2004.

Nine morphometric (length, width, maximum depth, and surface area) and physicochemical parameters (water temperature, transparency, dissolved oxygen concentration, pH, and conductivity) were measured in each pond. The maximum depth and physicochemical characteristics were measured weekly. Physicochemical characteristics were measured between 0800 and 1700 with a YSI 650 MDS portable terminal (Yellow Springs, Ohio, USA) equipped with a YSI 600QS probe. These measurements were made 10 cm below the water surface at the deepest point of each pond. Water transparency was visually estimated using three categories (1 = clear, 2 = semi-opaque, 3 = opaque).

Developmental plasticity

Between 10 May and 19 August 2004, 100 Wood Frog tadpoles and transforming froglets per pond were collected, one pond per habitat category (*n* = 300). The specimens were euthanized in MS-222 (Fisher Scientific, Ottawa, Ontario, Canada), fixed in neutrally buffered formalin (5–10%), and preserved in 70% ethanol.

A sample of 58 Wood Frog tadpoles provided a fairly comprehensive sequence of skeletogenic development for all the pond categories: 20 specimens from a skidding trail pond (D01), 18 from a logged area pond (D101), and 20 from a regenerated forest pond (D57). The developmental stage of each specimen was determined using methods described by Gosner (1960) that refers to a simplified staging table based on external anatomy. Tadpole soft tissues were digested with 1% trypsin in a saturated sodium borate solution (Dingerkus and Uhler 1977). Acid-free double staining protocol (Walker and Kimmel 2007) was used. Cartilaginous structures were stained using acid-free Alcian blue and bones were stained with ethanol/Alizarin red S solution.

Cleared and stained specimens (Figure 1) were observed using a Leica MZ16A stereomicroscope (Heerbrugg, Wahlkreis Rheintal, Switzerland). A skeletal element was considered to be chondrified when the Alcian blue stain was observed. Alizarin red S stain indicated that an element was ossified. A total of 126 skeletal (cranial and postcranial) elements were scored for three possible developmental states: absent (0), chondrified (1), and ossified (2). Cranial nomenclature follow Cannatella (1999) and Duellman and Trueb (1986). Postcranial nomenclature largely follows Duellman and Trueb (1986). The absolute timing (number of days post-hatching [dph]) of the first appearance of cartilage and bone was compared among pond categories. In some analyses, endochondral elements were further separated into six anatomical systems: skull, vertebral column, forelimbs, pectoral girdle, pelvic girdle, and hindlimbs. We define the mean onset as the average number of dph for the onset of all endochondral elements belonging to an anatomical system.

Gosner stage classification is useful to visually establish a relative developmental stage (Gosner 1960), but the time to reach each stage can vary among individuals. Gosner stages were not used as a comparative proxy because they were considered insufficiently accurate to compare development among three pond categories with different developmental durations. Furthermore, because Gosner stages are based on external morphology (Gosner 1960), they are too coarse to describe internal anatomy. Morphometric measurements, including snout–vent length (SVL), head length, and head width, are inappropriate as a size proxy because of the parabolic relationship between developmental duration and all the morphometric measurements considered (Figure S2). We developed a novel age proxy to compare specimens within and among pond categories. An approximate dph was estimated from the mean hatching period in the three ponds from where the specimens were collected. Zero dph was considered the sampling date when the first specimen reached Gosner stage 25 in each pond, the first tadpole stage after hatching.

Statistical analyses

For the 30 surveyed ponds and, individually for the three ponds from where frogs were collected, we used permutation one-way analyses of variance (ANOVA, 10 000 permutations) to describe biotic and abiotic factors among pond categories (Herve 2018). We performed a canonical discriminant analysis (CDA) using the 30 studied ponds to validate the morphometric and physicochemical characteristics responsible for differences among pond categories. Pond lengths and widths were excluded from the CDA to avoid redundancy with surface area. Data were not transformed. The multivariate normality assumption was validated by Mardia's test (Mardia 1985), homoscedasticity was validated using Box's M test (Morrison 1976), and multicollinearity was tested using Pearson correlations (Becker *et al.* 1988). The accuracy of the classification rule was tested by the division of the sum of unconfused predictions on the prediction total, which was 30 (one per pond).

As an indicator of the absolute timing of chondrification and ossification, we compared the onset of all skeletal elements among pond categories (Figures S3 to S7). Permutation one-way ANOVAs (10 000 permutations) complemented by permutation *t*-tests (10 000 permutations) were performed to compare the mean onset of chondrification and ossification events

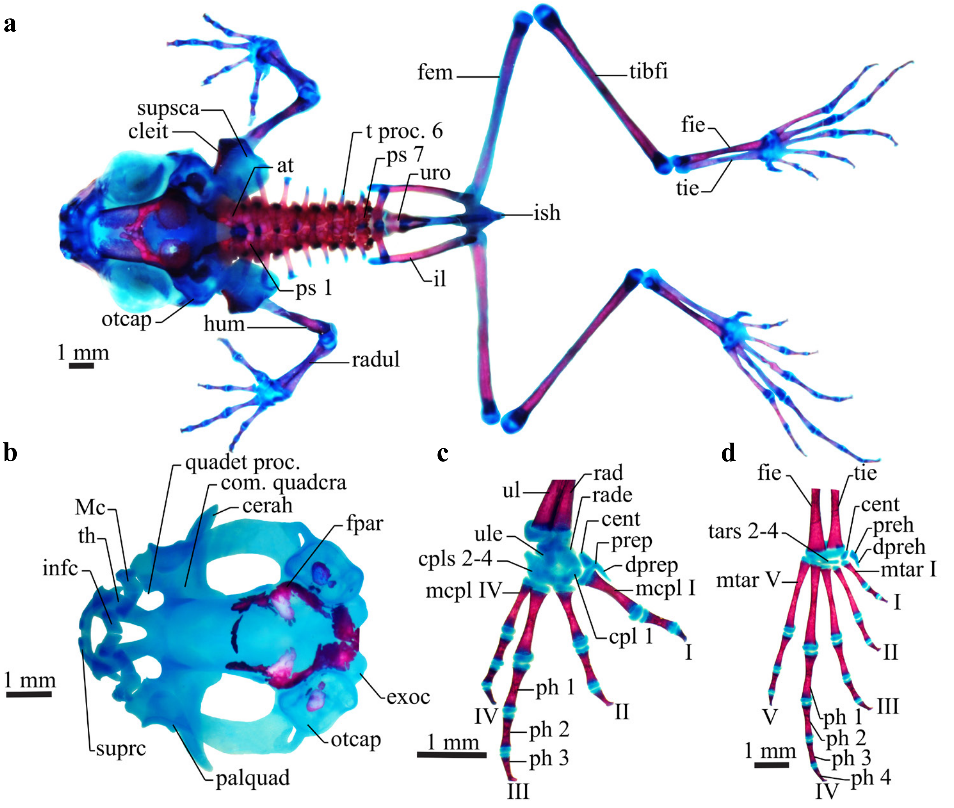

FIGURE 1. a. Dorsal view of the entire skeleton of a fully metamorphosed (Gosner stage 46) Wood Frog (*Lithobates sylvaticus*). b. Skull of a tadpole (Gosner stage 35) and the forelimb (c) and hindlimb (d) of the individual in a. Abbreviations are as follows: otcap = otic capsule; supsca = suprascapular; cleit = cleithrum; at = atlas; ps = presacral vertebra; t proc. = transversal process; uro = urostyle; il = ilium; ish = ishium; hum = humerus; radul = radio-ulna; fem = femur; tibfi = tibiofibular; fie = fibulare; tie = tibiale; suprc = suprarostral cartilage; infc = infrarostral cartilage; Mc = Meckel's cartilage; th = trabecular horn; quadet proc. = quadratoethmoidal process; com. quadcra = commissura quadratocranialis; cerah = ceratohyal; palquad = palatoquadrate; fpar = frontoparietal; exoc = exoccipital; ul = ulna; rad = radius; ule = ulnare; rade = radiale; cent = centrale; cpl(s) = carpal(s); prep = prepollex; dprep = distal prepollex; mclp = metacarpal; ph = phalanx; tars = tarsals; preh = prehallux; dpreh = distal prehallux; mtar = metatarsal. Photos: Laurent Houle.

of endochondral bones within anatomical systems (i.e., skull, vertebral column, forelimb and hindlimb, and pectoral and pelvic girdles; Herve 2018). Almost all skeletogenic event onsets were identified using their first appearance. We used logistic regressions to estimate the onset of six skeletogenic events, considering the cutting point (dph_{50}) as their onset (Grünbaum *et al.* 2012). Considering one skeletogenic event for a given element, the cutting point is defined as the dph when 50% of the individuals had a state of absence of the event and 50% had a state of presence. Logistic model suitability was tested using receiver operating characteristic (ROC) curves (Wright 2005) determined by calculating the area under the curves (AUC). The AUC represents a measure of overall accuracy for a diagnostic test and can be used as a test for a ROC curve (Park *et al.* 2004). Area under the curve values under 0.5 are not considered to be accurate while values of one are perfectly accurate (Park *et al.* 2004).

As an indicator of the relative timing of chondrification and ossification, we compared trajectories of a cumulative number of formed skeletal elements among pond categories. Congruence tests were performed using permutation Spearman's rank correlation tests (10 000 permutations; Dagnelie 1973), conducted using 64 endochondral bones (Table S1). A rank value was given to each chondrification and ossification event using Dagnelie's method to adjust equal ranks and to calculate Spearman's rank coefficient (r_s; Dagnelie 1973). The appendicular endochondral bones were also tested separately using the same method. Multiple comparisons of the r_s were calculated by bootstrapping (10000 iterations).

It was not possible to use a multiple regression/general linear model to compare the onset of skeletal element chondrification or ossification with the pond characteristics from where the frogs were collected because collections were only made in three ponds, one per category.

Results

Biotic and abiotic environmental characteristics

The canopy cover at the 30 ponds studied differed significantly among the three pond categories ($F_{2,27}$ = 77.3, $P < 0.001$; Table S2). All pond morphometric factors differed significantly between disturbed and regenerated forest ponds. Regenerated forest ponds were the largest (length, width, and surface area) and deepest. Water temperature was significantly higher in disturbed ponds ($F_{2,27}$ = 6.9, P = 0.004; Table S2). The substrate composition differed among pond categories. A higher proportion of dead leaves was found in regenerated forest ponds ($F_{2,27}$ = 13.3, $P < 0.001$; Table S2). Skidding trail ponds contained a smaller proportion of humus ($F_{2,27}$ = 8.9, P = 0.001; Table S2) and mosses ($F_{2,27}$ = 4.6, P = 0.011; Table S2) and a higher proportion of fine sediments (i.e., fine sand and silt; $F_{2,27}$ = 14.7, $P < 0.001$; Table S2). Water temperature ($F_{2,33}$ = 10.8, P = 0.01; Table S3) and surface area ($F_{2,6}$ = 10.0, P = 0.003; Table S3) were significantly different among the pond categories from where the frogs were collected.

Pond categories were used as the grouping factor to determine which morphometric and physicochemical parameters were the most discriminating to differentiate ponds. The first dimension of the CDA estimated 86.6% of the optimal linear combination of the seven variables, whereas the second dimension estimated 13.4%. Water temperature, surface area, depth, and transparency have the greatest impact on pond discrimination (Figure 2). The three pond categories were well separated, despite a slight overlap. The classification rule has an accuracy of 89.3%.

Absolute and relative timing of chondrification and ossification

The complete sequences of chondrification and ossification for the 126 Wood Frog skeletal elements (Figures S3–S7) showed congruence and differences in terms of absolute and relative timing among pond categories. The general sequence of formation (chondrification and ossification) for the six anatomical systems was fairly conservative independent of pond categories (Table S4).

The onset of chondrification and ossification varied minimally among individuals for an element within a pond category. The only inter-individual differences were observed for (1) the onset of chondrification for the transversal processes of vertebrae 1 and 2 in both logged area and skidding trail ponds; (2) the ossification of the atlas in the logged area ponds; and (3) the chondrification of the urostyle in skidding trail ponds (Table S5).

Within all anatomical systems, we found similarities in the observed pattern of the mean chondrification and ossification onsets among pond categories (Figure 3a). There was at least one significant difference among pond categories for all the anatomical systems except the skull and pelvic girdle (Table S6, Figure 3a). Chondrification and ossification of vertebral elements were delayed (i.e., longer dph) in regenerated forest ponds (Table S6, Figure 3a). Mean onsets of chondrification of forelimbs and hindlimbs were significantly longer in regenerated forest ponds (Table S6, Figure 3a). Intragroup variation associated with the mean onset of limbs was higher for chondrification than ossification. Mean onset of limb ossification differed significantly among the three pond categories (Table S6, Figure 3a). The mean onset of pectoral girdle chondrification in regenerated forest was longer than in logged area ponds. The mean onset of pectoral girdle ossification in regenerated forest pond was longer than in disturbed ponds (Table S6, Figure 3a). Mean onsets of chondrification and ossification of skull and pelvic girdle did not differ significantly among pond categories.

Wood Frog cartilaginous trajectories were separated into three phases (Figure 3b): (1) an initial phase between 10 and 20 dph, (2) a gradual increase between 20 and 50 dph, and (3) a final phase after 50 dph, indicating its completion. Ossified trajectories were also separated into three phases (Figure 3c): (1) an initial phase between 10 and 30 dph, (2) a threshold between 30 and 55 dph, and (3) a final phase after 55 dph. Both cartilaginous and ossified trajectories differed among pond categories and occurred faster in disturbed ponds compared to regenerated forest. The cartilaginous trajectory for the regenerated forest differed significantly (25–50 dph) from that of disturbed ponds, indicated by non-overlapping CI. Ossified trajectories showed a similar pattern between disturbed and regenerated forest ponds from 35 to 50 dph.

The correlation between chondrification and ossification of 64 endochondral bones did not differ significantly within and among pond categories (r_s = 0.89–0.92; Table S7). Comparison among pond categories showed slightly higher r_s for the chondrification patterns compared to the ossification between regenerated forest ponds and disturbed ponds. The direction of the correlation between the chondrification and ossification in the skidding trail and logged area apparently reversed, although the difference

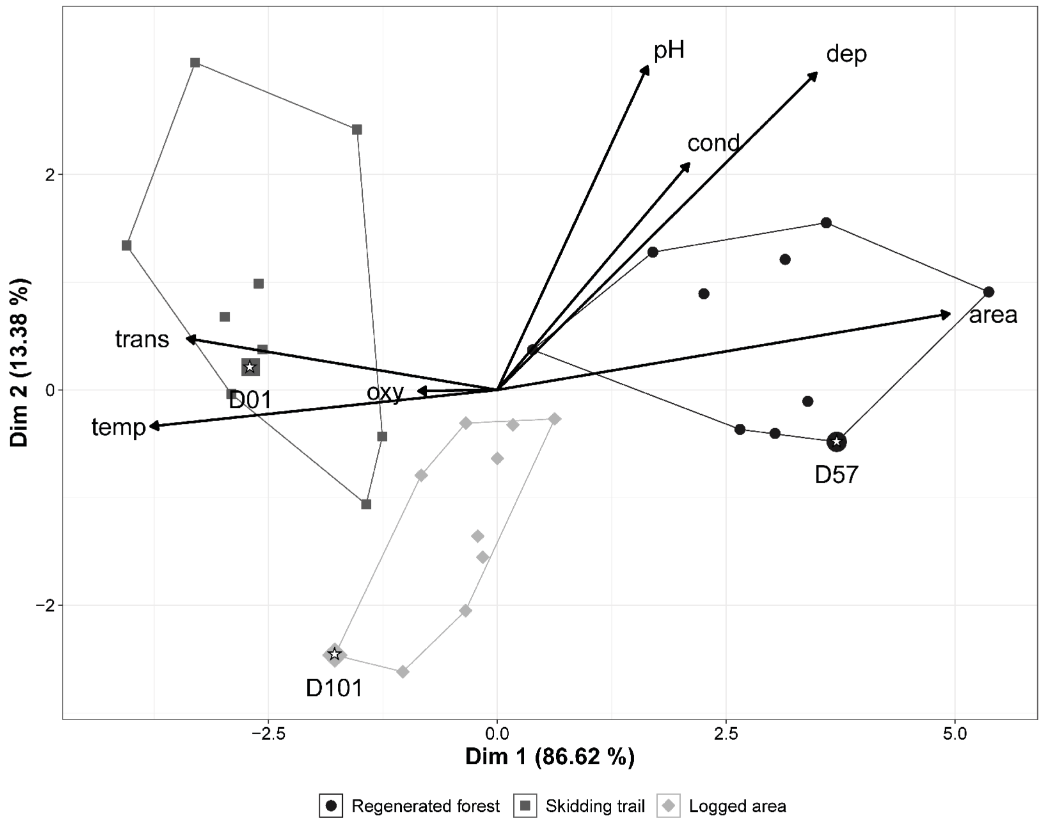

FIGURE 2. Canonical discriminant analysis showing environmental factors explaining differences among pond categories in the 30 studied ponds, Duchénier Wildlife Reserve, eastern Quebec, Canada. Vector lines represent discriminant coefficients for the first (Dim 1) and second (Dim 2) dimensions. The stars identify the ponds from which Wood Frog (*Lithobates sylvaticus*) specimens were sampled. Cond: conductivity; area: surface area; dep: depth; oxy: dissolved oxygen; temp: water temperature; trans: transparency.

was not significant. The appendicular endochondral bones (representing 59.4% of the total number of elements; Table S1) were tested separately. Significant differences in r_s were found in ossification patterns of limbs among pond categories (Table S8). Ossification r_s in both forelimb and hindlimb between the two disturbed ponds were highly congruent and also significantly different from the r_s comparing regenerated forest ponds to skidding trail ponds and r_s comparing regenerated forest ponds to logged area ponds (Table S8). This difference is because of small shifts in the order of the sequences for seven elements in forelimbs and for 11 elements in hindlimbs (mainly distal elements in both anatomical systems) between disturbed and regenerated forest ponds.

Discussion

Amphibians that reproduce in ponds can exhibit plasticity in developmental and growth rates induced by the environment (Amburgey *et al.* 2016; Orizaola and Laurila 2016; Székely *et al.* 2017). To our knowledge, our study is the first to describe amphibian developmental plasticity of skeletogenesis in a field study using absolute and relative timing of skeletogenic (chondrification and ossification) events and developmental trajectories. In our investigation, (1) two canopy-related (water temperature and transparency) and two pond morphometric (surface area and depth) variables were found to be the most important factors explaining differences among pond categories, (2) changes were observed in absolute and relative timing of skeletogenic events, and (3) limb ossification was more responsive to environmental conditions than chondrification. We considered these results indicators of developmental plasticity in Wood Frogs among pond categories. Forest canopy removal by logging accelerates the timing of skeletogenesis

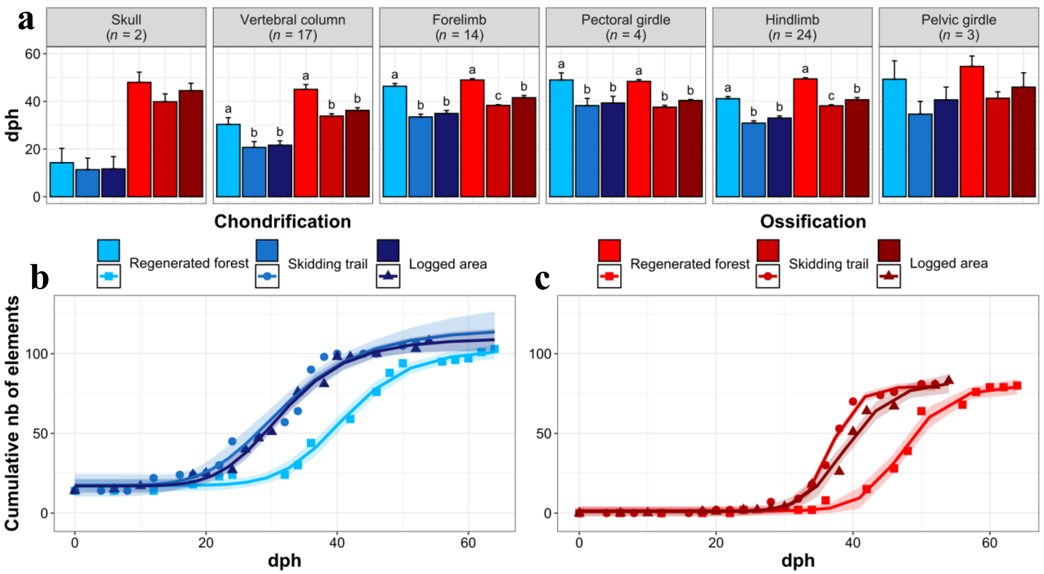

FIGURE 3. a. Mean onset of chondrification (blue bars) and ossification (red bars) of Wood Frog (*Lithobates sylvaticus*) endochondral bones among pond categories in the Duchénier Wildlife Reserve, eastern Quebec, Canada. Letters indicate grouping results of permutation *t*-tests. Wood Frog b. cartilaginous and c. ossified developmental trajectories among pond categories. Non-linear regressions are from dose-response models that use four parameters log-logistic function. Shaded areas correspond to 95% CI associated with each curve.

and seems to have a larger effect on processes that occur later during development than those that occur earlier. Furthermore, we provide the first detailed sequence of Wood Frog chondrification and ossification.

Amphibian growth rate has previously been related to several biotic and abiotic factors in breeding ponds (Amburgey *et al.* 2016; Freidenburg 2017). One such important factor is the forest canopy (Skelly *et al.* 1999; Renken *et al.* 2004; Semlitsch *et al.* 2008). Closed canopy ponds are normally associated with slower growth of anurans than open canopy ponds because of the lack of light, cooler water temperature, lower dissolved oxygen concentration, lower primary productivity, lower periphyton abundance, and different types of food resources (Skelly *et al.* 1999, 2002; Werner and Glennemeier 1999). Because all selected ponds in our study had a semi-permanent hydroperiod, hydroperiod was assumed to be relatively constant among pond categories. For this reason, we did not intensively monitor water level variation. However, Székely *et al.* (2017) showed that Pacific Horned Frog (*Ceratophrys stolzmanni*) can exhibit plastic reactions, such as an accelerated developmental rate and a reduced duration of some metamorphic stages, because of a declining water level. Gomez-Mestre *et al.* (2013) also showed similar results for Western Spadefoot (*Pelobates cultripes*) using a different method. In our study, the greater depth found in regenerated forest ponds is likely to have slowed Wood Frog developmental rate. Significant differences observed in developmental trajectories between disturbed and regenerated forest ponds provide some evidence of a shift in developmental rate. Based on previous studies (Skelly *et al.* 2002; Gomez-Mestre *et al.* 2013; Dittrich *et al.* 2016; Székely *et al.* 2017), we strongly suspected that water temperature and depth had an impact on developmental rates we observed among pond categories.

Development and growth differences can affect survival

Wood Frog is known to breed in open and closed canopy ponds (Werner and Glennemeier 1999). Growth has been reported to be faster in open canopy ponds (Skelly *et al.* 1999; Werner and Glennemeier 1999; Halverson *et al.* 2003), but Skelly *et al.* (2002) reported that some biotic factors (such as food availability) can partially mitigate depressed growth rate in closed canopy ponds.

Faster development in disturbed ponds in comparison to regenerated forest ponds could be a fitness advantage. In North America, an early metamorphosis means a longer terrestrial feeding and growing period before winter (Semlitsch *et al.* 1988). Berven (1990) noted that Wood Frog juveniles that metamorphosed early and were larger at metamorphosis, had a higher adult survival, and could reach sexual maturity earlier. For many ranids, at least 80% of the growth that leads to adult size occurs during the terrestrial phase (Werner 1986). However, as shown in our study, the entire skeletogenesis occurs during the aquatic growth period; it is known that the pre-metamorphic period affects the morphology and performance of juveniles (Álvarez and Nicieza 2002; Relyea and Hoverman 2003; Richter-Boix *et al.* 2006). Álvarez and Nicieza (2002) showed that a faster growth rate (from high water temperature) can negatively affect the jumping performance and the amount of energy reserves of juvenile Iberian Painted Frog (*Discoglossus galganoi*). A fluctuating water level, a characteristic associated with open canopy ponds, can lead to faster growth rate in Common Parsley Frog (*Pelodytes punctatus*), but also to juveniles with shorter and less muscular hind limbs (Richter-Boix *et al.* 2006). Logging activities are also known to alter terrestrial habitat characteristics in such ways that they can become highly unsuitable for many amphibian species (DeMaynadier and Hunter 1995; Waldick *et al.* 1999). This leads us to conclude that the faster development in disturbed ponds in response to higher water temperature and lower (and more fluctuating) water level could lead to poorer juvenile fitness. This, associated with a harsh terrestrial habitat, could reduce Wood Frog survival rates.

Developmental plasticity

We found that the frogs had a plastic response in the absolute and relative timing of chondrification and ossification between disturbed and regenerated forest ponds. The observed differences among ponds seem to be related to water temperature, transparency, and morphometric characteristics (i.e., surface area and depth). Acceleration of developmental rate associated with higher temperature has also been reported in fishes (Fuiman *et al.* 1998) and birds (Hosseini and Hogg 1991). Developmental plasticity in skeletal anatomy has been studied in fishes exposed to different experimental constraints (Campinho *et al.* 2004; Cloutier *et al.* 2010). Some authors concluded that there was a small impact of plasticity on the order of events within the sequences of chondrification and ossification in Arctic Char (*Salvelinus alpinus*; Cloutier *et al.* 2010; Grünbaum *et al.* 2012).

In our study, we found significant changes in the order of limb ossification between disturbed and regenerated forest ponds. Limb chondrification patterns are very similar when comparing pond categories, but ossification patterns are more congruent between both disturbed pond types than between disturbed ponds and regenerated forest ponds. This suggests that the sequence of bone formation of the appendicular skeleton might be slightly more responsive to environmental differences observed between disturbed and regenerated forest ponds. Although there is coordination between chondrocyte and osteoblast differentiation in the formation of endochondral bone (Cloutier *et al.* 2010), some authors have reported a different response between chondrification and ossification in fishes exposed to temperature treatments (Mabee *et al.* 2000; Campinho *et al.* 2004). Cloutier *et al.* (2010) concluded that the effects of environmental constraints on patterns of chondrification and ossification could be partially independent in fishes. As suggested by our study, this pattern is likely to be similar in amphibians. The ossification pattern of the appendicular skeleton reacts more sensitively than the chondrification pattern. This could be because ossification mostly occurs after chondrification; the accumulation of variant phenotypes during earlier developmental stages leads to a greater likelihood to observe larger scale variation in ossification patterns.

Environmental characteristics (such as canopy-related factors) are well known to influence amphibian richness, distribution, reproduction effort, and the survival of tadpoles (Hecnar and M'Closkey 1998; Kolozsvary and Swihart 1999; Lehtinen *et al.* 1999; Marsh and Trenham 2001). Developmental plasticity is also suggested to affect tadpole and adult survival and reproduction which can have important repercussions on population dynamics. The absolute and relative timing of chondrification and ossification in the frogs in our study showed a plastic response to different environmental conditions.

Author Contributions

Writing – Original draft: L.H.; Writing – Review & Editing: L.H., L.S., O.L., A.C., and R.C.; Conceptualization: L.H., J.B., J.T., L.S., and R.C.; Investigation: J.B. and J.T.; Methodology: L.H., J.B., and K.S.; Formal analysis: L.H., A.C., and R.C.; Funding Acquisition: R.C.

Acknowledgements

We thank S. Côté, G. Breton Marceau, and J. Dupont for their help with data acquisition and V. Roy for his help with data analysis. We thank the Duchénier Wildlife Reserve for the access. We also thank the Conseil de bassin de la rivière Rimouski and the Ministère des Ressources Naturelles, de la Faune et des Parcs for donation of aerial photos of the study site. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) with undergraduate (L.H. and J.T.) and graduate (J.B., L.H., and J.T.) scholarships, a discovery grant (R.C.), and the Chaire de Recherche sur la Forêt Habitée (L.S., J.B., J.T., and R.C.). The animal care and sampling protocols followed the instructions of the Comité de protection des animaux (CPA), associated to the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) and the Société de la Faune et des Parcs (ref. 20020604-005-01-S-F). Three permits were obtained for this study: Comité de protection des animaux (CPA), Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ), and Société de la Faune et des Parcs (SFP; ref. 20020604-005-01-S-F). We also thank the reviewers who provided useful comments on the manuscript.

Raw data repository and voucher specimens

Raw data are available freely from the following GitHub repository https://github.com/LaurentHoule03/Raw_data_Lithobates_sylvaticus_skeletogenesis.git. The specimens will be donated to the herpetological collection of the Canadian Museum of Nature.

Literature Cited

Álvarez, D., and A.G. Nicieza. 2002. Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia 131: 186–195. https://doi.org/10.1007/s00442-002-0876-x

Amburgey, S.M., M. Murphy, and W.C. Funk. 2016. Phenotypic plasticity in developmental rate is insufficient to offset high tadpole mortality in rapidly drying ponds. Ecosphere 7: e01386. https://doi.org/10.1002/ecs2.1386

Aust, W., T. Reisinger, J. Burger, and B. Stokes. 1993. Soil physical and hydrological changes associated with logging a wet pine flat with wide-tired skidders. Southern Journal of Applied Forestry 17: 22–25. https://doi.org/10.1093/sjaf/17.1.22

Bachmann, K. 1969. Temperature adaptations of amphibian embryos. American Naturalist 103: 115–130. https://doi.org/10.1086/282588

Baldwin, R.F., A.J.K. Calhoun, and P.G. deMaynadier. 2006. The significance of hydroperiod and stand maturity for pool-breeding amphibians in forested landscapes. Canadian Journal of Zoology 84: 1604–1615. https://doi.org/10.1139/z06-146

Beauchamp, J. 2007. Impacts de l'exploitation forestière sur les communautés d'amphibiens d'étangs semi-permanents du Bas-Saint-Laurent. M.Sc. thesis, Université du Québec à Rimouski, Rimouski, Quebec, Canada. Accessed 5 July 2024. https://semaphore.uqar.ca/id/eprint/138.

Becker, R.A., J.M. Chambers, and A.R. Wilks. 1988. The New S language: a Programming Environment for Data Analysis and Graphics. Wadsworth & Brooks/Cole Advanced Books & Software, Pacific Grove, California, USA.

Beebee, T.J. 1995. Amphibian breeding and climate. Nature 374: 219–220. https://doi.org/10.1038/374219a0

Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the Wood Frog (*Rana sylvatica*). Ecology 71: 1599–1608. https://doi.org/10.2307/1938295

Blaustein, A.R. 1994. Chicken little or Nero's fiddle? A perspective on declining amphibian populations. Herpetologica 50: 85–97.

Boucher, Y., D. Arseneault, and L. Sirois. 2009. La forêt préindustrielle du Bas-Saint-Laurent et sa transformation (1820-2000): implications pour l'aménagement écosystémique. Le Naturaliste Canadien 133: 60–69. Accessed 5 July 2024. https://constellation.uqac.ca/id/eprint/8234/.

Campinho, M.A., K.A. Moutou, and D.M. Power. 2004. Temperature sensitivity of skeletal ontogeny in *Oreochromis mossambicus*. Journal of Fish Biology 65: 1003–1025. https://doi.org/10.1111/j.0022-1112.2004.00505.x

Cannatella, D. 1999. Architecture: cranial and axial musculoskeleton. Pages 52–81*in* Tadpoles: the Biology of Anuran Larvae. *Edited by* R.W. McDiarmid and R. Altig. University of Chicago Press, Chicago, Illinois, USA.

Cardini, A., and S. Elton. 2008. Does the skull carry a phylogenetic signal? Evolution and modularity in the guenons. Biological Journal of the Linnean Society 93: 813–834. https://doi.org/10.1111/j.1095-8312.2008.01011.x

Cloutier, R., A. Caron, T. Grünbaum, and N.R. Le François. 2010. Effect of water velocity on the timing of skeletogenesis in the Arctic Charr, *Salvelinus alpinus* (Salmoniformes: Teleostei): an empirical case of developmental plasticity. International Journal of Zoology 2010: 1–15. https://doi.org/10.1155/2010/470546

Dagnelie, P. 1973. Théorie et méthodes statistiques : applications agronomiques. Les presses agronomiques de Gembloux, Gembloux, Namur, Belgium.

Dahl, E., G. Orizaola, A.G. Nicieza, and A. Laurila. 2012. Time constraints and flexibility of growth strategies: geographic variation in catch-up growth responses in amphibian larvae. Journal of Animal Ecology 81: 1233–1243. https://doi.org/10.1111/j.1365-2656.2012.02009.x

Darveau, M., L. Bélanger, J. Huot, É. Mélançon, and S. DeBellefeuille. 1997. Forestry practices and the risk of bird nest predation in a boreal coniferous forest. Ecological Applications 7: 572–580. https://doi.org/10.1890/1051-0761(1997)007[0572:fpatro]2.0.co;2

DeMaynadier, P.G., and M.L. Hunter. 1995. The relationship between forest management and amphibian ecology: a review of the North American literature. Environmental Reviews 3: 230–261. https://doi.org/10.1139/a95-012

DeMaynadier, P.G., and M.L. Hunter. 1998. Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology 12: 340–352. https://doi.org/10.1111/j.1523-1739.1998.96412.x

Dieringer, N. 1995. 'Vestibular compensation': neural plasticity and its relations to functional recovery after labyrinthine lesions in frogs and other vertebrates. Progress in Neurobiology 46: 97–129. https://doi.org/10.1016/0301-0082(95)80009-W

DiMauro, D., and M.L. Hunter. 2002. Reproduction of amphibians in natural and anthropogenic temporary pools in managed forests. Forest Science 48: 397–406. https://doi.org/10.1093/forestscience/48.2.397

Dingerkus, G., and L.D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology 52: 229–232. https://doi.org/10.3109/10520297709116780

Dittrich, C., S. Drakulić, M. Schellenberg, J. Thein, and

M.O. Rödel. 2016. Some like it hot? Developmental differences in Yellow-bellied Toad (*Bombina variegata*) tadpoles from geographically close but different habitats. Canadian Journal of Zoology 94: 69–77. https://doi.org/10.1139/cjz-2015-0168

Duellman, W.E., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, Maryland, USA.

Freidenburg, L.K. 2017. Environmental drivers of carry-over effects in a pond-breeding amphibian, the Wood Frog (*Rana sylvatica*). Canadian Journal of Zoology 95: 255–262. https://doi.org/10.1139/cjz-2016-0080

Fuiman, L.A., K.R. Poling, and D.M. Higgs. 1998. Quantifying developmental progress for comparative studies of larval fishes. Copeia 1998: 602–611. https://doi.org/10.2307/1447790

Ganey, J.L., and R.P. Balda. 1994. Habitat selection by Mexican Spotted Owls in northern Arizona. Auk 111: 162–169. https://doi.org/10.2307/4088514

Gomez-Mestre, I., S. Kulkarni, and D.R. Buchholz. 2013. Mechanisms and consequences of developmental acceleration in tadpoles responding to pond drying. PLoS ONE 8: e84266. https://doi.org/10.1371/journal.pone.0084266

Gomez-Mestre, I., V.L. Saccoccio, T. Iijima, E.M. Collins, G.G. Rosenthal, and K.M. Warkentin. 2010. The shape of things to come: linking developmental plasticity to post-metamorphic morphology in anurans. Journal of Evolutionary Biology 23: 1364–1373. https://doi.org/10.1111/j.1420-9101.2010.02016.x

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190.

Grünbaum, T., R. Cloutier, and P. Dumont. 2003. Congruence between chondrification and ossification sequences during caudal skeleton development: a Moxostomatini case study. The Big Fish Bang: Proceedings of the 26th Annual Larval Fish Conference. *Edited by* H.I. Browman and A.B. Skiftesvik. Institute of Marine Research, Nordnes, Norway.

Grünbaum, T., R. Cloutier, and B. Vincent. 2012. Dynamic skeletogenesis in fishes: insight of exercise training on developmental plasticity. Developmental Dynamics 241: 1507–1524. https://doi.org/10.1002/dvdy.23837

Haas, A. 1999. Larval and metamorphic skeletal development in the fast-developing frog *Pyxicephalus adspersus* (Anura, Ranidae). Zoomorphology 119: 23–35. https://doi.org/10.1007/s004350050078

Hallgrimsson, B., D.E. Lieberman, N.M. Young, T. Parsons, and S. Wat. 2007. Evolution of covariance in the mammalian skull. *In* Tinkering: the Microevolution of Development. *Edited by* G. Bock and J. Goode. Novartis Foundation Symposium, John Wiley and Sons Chichester, United Kingdom. https://doi.org/10.1002/9780470319390.ch12

Halverson, M.A., D.K. Skelly, J.M. Kiesecker, and L.K. Freidenburg. 2003. Forest mediated light regime linked to amphibian distribution and performance. Oecologia 134: 360–364. https://doi.org/10.1007/s00442-002-1136-9

Hanken, J., and B.K. Hall. 1984. Variation and timing of the cranial ossification sequence of the Oriental Fire-Bellied Toad, *Bombina orientalis* (Amphibia, Discoglossidae). Journal of Morphology 182: 245–255. https://doi.org/10.1002/jmor.1051820302

Hecnar, S.J., and R.T. M'Closkey. 1998. Species richness patterns of amphibians in southwestern Ontario ponds. Journal of Biogeography 25: 763–772. https://doi.org/10.1046/j.1365-2699.1998.2540763.x

Herve, M. 2018. Testing and plotting procedures for biostatistics. Package 'RVAideMemoire'. Accessed 5 July 2024. https://cran.r-project.org/web/packages/RVAideMemoire/index.html.

Hosseini, A., and D.A. Hogg. 1991. The effects of paralysis on skeletal development in the chick embryo. I. General effects. Journal of Anatomy 177: 159–168. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1260423/.

Houlahan, J.E., and C.S. Findlay. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences 60: 1078–1094. https://doi.org/10.1139/f03-095

Kariuki, M., R.M. Kooyman, R.G.B. Smith, G. Wardell-Johnson, and J.K. Vanclay. 2006. Regeneration changes in tree species abundance, diversity and structure in logged and unlogged subtropical rainforest over a 36-year period. Forest Ecology and Management 236: 162–176. https://doi.org/10.1016/j.foreco.2006.09.021

Kolozsvary, M.B., and R.K. Swihart. 1999. Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. Canadian Journal of Zoology 77: 1288–1299. https://doi.org/10.1139/z99-102

Krag, R., K. Higginbotham, and R. Rothwell. 1986. Logging and soil disturbance in southeast British Columbia. Canadian Journal of Forest Research 16: 1345–1354. https://doi.org/10.1139/x86-238

Lajmanovich, R.C., M. Sandoval, and P.M. Peltzer. 2003. Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. Bulletin of Environmental Contamination and Toxicology 70: 612–618. https://doi.org/10.1007/s00128-003-0029-x

Lannoo, M.J., and R.M. Stiles. 2017. Effects of short-term climate variation on a long-lived frog. Copeia 105: 726–733. https://doi.org/10.1643/CH-16-449

Lehtinen, R.M., S.M. Galatowitsch, and J.R. Tester. 1999. Consequences of habitat loss and fragmentation for wetland amphibian assemblages. Wetlands 19: 1–12. https://doi.org/10.1007/bf03161728

Mabee, P.M., K.L. Olmstead, and C.C. Cubbage. 2000. An experimental study of intraspecific variation, developmental timing, and heterochrony in fishes. Evolution 54: 2091–2106. https://doi.org/10.1111/j.0014-3820.2000.tb01252.x

Mardia, K.V. 1985. Mardia's test of multinormality. Pages 217–221 *in* Encyclopedia of Statistical Sciences. *Edited by* S. Kotz and N.L. Johnson. John Wiley and Sons, Hoboken, New Jersey, USA. https://doi.org/10.1002/0471667196

Marsh, D.M., and P.C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15: 40–49. https://doi.org/10.1111/j.1523-1739.2001.00129.x

Morrison, D.F. 1976. Multivariate Statistical Methods. Mc-

Graw-Hill, New York, New York, USA.

Newman, R.A. 1992. Adaptive plasticity in amphibian metamorphosis. BioScience 42: 671–678. https://doi.org/10.2307/1312173

Noble, D.W., V. Stenhouse, and L.E. Schwanz. 2018. Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. Biological Reviews 93: 72–97. https://doi.org/10.1111/brv.12333

Orizaola, G., and A. Laurila. 2016. Developmental plasticity increases at the northern range margin in a warm-dependent amphibian. Evolutionary Applications 9: 471–478. https://doi.org/10.1111/eva.12349

Ouellet, M., J. Bonin, J. Rodrigue, J.-L. DesGranges, and S. Lair. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Diseases 33: 95–104. https://doi.org/10.7589/0090-3558-33.1.95

Park, S.H., J.M. Goo, and C.-H. Jo. 2004. Receiver operating characteristic (ROC) curve: practical review for radiologists. Korean Journal of Radiology 5: 11–18. https://doi.org/10.3348/kjr.2004.5.1.11

Potvin, F., R. Courtois, and L. Bélanger. 1999. Short-term response of wildlife to clear-cutting in Quebec boreal forest: multiscale effects and management implications. Canadian Journal of Forest Research 29: 1120–1127. https://doi.org/10.1139/x99-040

Reading, C. 1998. The effect of winter temperatures on the timing of breeding activity in the Common Toad *Bufo bufo*. Oecologia 117: 469–475. https://doi.org/10.1007/s004420050682

Relyea, R.A. 2002. Costs of phenotypic plasticity. American Naturalist 159: 272–282. https://doi.org/10.1086/338540

Relyea, R.A., and J.T. Hoverman. 2003. The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. Oecologia 134: 596–604. https://doi.org/10.1007/s00442-002-1161-8

Renken, R.B., W.K. Gram, D.K. Fantz, S.C. Richter, T.J. Miller, K.B. Ricke, B. Russell, and X. Wang. 2004. Effects of forest management on amphibians and reptiles in Missouri Ozark forests. Conservation Biology 18: 174–188. https://doi.org/10.1111/j.1523-1739.2004.00312.x

Richter-Boix, A., G.A. Llorente, and A. Montori. 2006. Effects of phenotypic plasticity on post-metamorphic traits during pre-metamorphic stages in the anuran *Pelodytes punctatus*. Evolutionary Ecology Research 8: 309–320.

Rowe, J.S. 1972. Forest regions of Canada. Canadian Forestry Service Publication 1300. Canadian Forestry Service, Ottawa, Ontario, Canada.

Saucier, J.-P. 1998. Paysages régionaux du Québec méridional. Gouvernement du Québec, Ministère des ressources naturelles, de la Faune et des Parcs, Sainte-Foy, Quebec, Canada.

Semlitsch, R.D., C.A. Conner, D.J. Hocking, T.A.G. Rittenhouse, and E.B. Harper. 2008. Effects of timber harvesting on pond-breeding amphibian persistence: testing the evacuation hypothesis. Ecological Applications 18: 283–289. https://doi.org/10.1890/07-0853.1

Semlitsch, R.D., D.E. Scott, and J.H.K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69: 184–192. https://doi.org/10.2307/1943173

Semlitsch, R.D., B.D. Todd, S.M. Blomquist, A.J.K. Calhoun, J.W. Gibbons, J.P. Gibbs, G.J. Graeter, E.B. Harper, D.J. Hocking, M.L. Hunter, D.A. Patrick, T.A.G. Rittenhouse, and B.B. Rothermel. 2009. Effects of timber harvest on amphibian populations: understanding mechanisms from forest experiments. BioScience 59: 853–862. https://doi.org/10.1525/bio.2009.59.10.7

Singh, S.K., D. Das, and T. Rhen. 2020. Embryonic temperature programs phenotype in reptiles. Frontiers in Physiology 11: 35. https://doi.org/10.3389/fphys.2020.00035

Skelly, D.K., L.K. Freidenburg, and J.M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology 83: 983–992. https://doi.org/10.1890/0012-9658(2002)083[0983:fcatpo]2.0.co;2

Skelly, D.K., and J. Golon. 2003. Assimilation of natural benthic substrates by two species of tadpoles. Herpetologica 59: 37–42. https://doi.org/10.1655/0018-0831(2003)059[0037:aonbsb]2.0.co;2

Skelly, D.K., E.E. Werner, and S.A. Cortwright. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. Ecology 80: 2326–2337. https://doi.org/10.1890/0012-9658(1999)080[2326:ltddoa]2.0.co;2

Székely, D., M. Denoël, P. Székely, and D. Cogălniceanu. 2017. Pond drying cues and their effects on growth and metamorphosis in a fast developing amphibian. Journal of Zoology 303: 129–135. https://doi.org/10.1111/jzo.12468

Taylor, B., D. Skelly, L.K. Demarchis, M.D. Slade, D. Galusha, and P.M. Rabinowitz. 2005. Proximity to pollution sources and risk of amphibian limb malformation. Environmental Health Perspectives 113: 1497–1501. https://doi.org/10.1289/ehp.7585

Touchon, J.C., and K.M. Warkentin. 2008. Fish and dragonfly nymph predators induce opposite shifts in color and morphology of tadpoles. Oikos 117: 634–640. https://doi.org/10.1111/j.0030-1299.2008.16354.x

Trottier, J. 2006. Impact de l'exploitation forestière sur la richesse et l'abondance des amphibiens de la forêt boréale méridionale du Bas-Saint-Laurent. M.Sc. thesis, Université du Québec à Rimouski, Rimouski, Quebec, Canada. Accessed 5 July 2024. https://semaphore.uqar.ca/id/eprint/134.

Udin, S.B., and M.J. Keating. 1981. Plasticity in a central nervous pathway in *Xenopus*: anatomical changes in the isthmotectal projection after larval eye rotation. Journal of Comparative Neurology 203: 575–594. https://doi.org/10.1002/cne.902030403

Waldick, R.C., B. Freedman, and R.J. Wassersug. 1999. The consequences for amphibians of the conversion of natural, mixed-species forests to conifer plantations in southern New Brunswick. Canadian Field-Naturalist 113: 408–418. https://doi.org/10.5962/p.358609

Walker, M.B., and C.B. Kimmel. 2007. A two-color acid-free cartilage and bone stain for Zebrafish larvae. Biotechnic & Histochemistry 82: 23–28. https://doi.org/10.1080/10520290701333558

Werner, E.E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. American Naturalist 128: 319–341. https://doi.org/10.1086/284565

Werner, E.E., and K.S. Glennemeier. 1999. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. Copeia 1999: 1–12. https://doi.org/10.2307/1447379

Wright, D.B. 2005. Receiver operating characteristics curves. Pages 1718-1721 *in* Encyclopedia of Statistics in Behavioral Science. *Edited by* B.S. Everitt and D.C. Howell. John Wiley & Sons, Hoboken, New Jersey, USA. https://doi.org/10.1002/0470013192

Young, R.L., and A.V. Badyaev. 2010. Developmental plasticity links local adaptation and evolutionary diversification in foraging morphology. Journal of Experimental Zoology Part B 314: 434–444. https://doi.org/10.1002/jez.b.21349

Received 19 December 2022
Accepted 20 January 2025
Associate Editor: W.D. Halliday

SUPPLEMENTARY MATERIALS:

FIGURE S1. Location of the 30 studied ponds in the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S2. Snout–vent length, head width, and head length non-linear relationships during larval period for 45 of the 58 cleared and stained Wood Frog (*Lithobates sylvaticus*) tadpoles and froglets from the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S3. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification and ossification for cranial elements from a regenerated forest, a skidding trail, and a logged area pond in the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S4. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification and ossification for the vertebral column from a regenerated forest, a skidding trail, and a logged area pond in the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S5. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification and ossification for forelimb elements from a regenerated forest, a skidding trail, and a logged area pond in the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S6. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification and ossification for pectoral and pelvic girdle elements from a regenerated forest, a skidding trail, and a logged area pond in the Duchénier Wildlife Reserve, Quebec, Canada.

FIGURE S7. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification and ossification for hind limb elements from a regenerated forest, a skidding trail, and a logged area pond in the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S1. List of endochondral bones of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S2. Mean and 95% CI values of 24 biotic and abiotic parameters among pond categories from 30 ponds in the Duchénier Wildlife Reserve, Quebec, Canada where chondrification and ossification sequences of Wood Frog (*Lithobates sylvaticus*) were studied.

TABLE S3. Mean and 95% CI values of 24 biotic and abiotic parameters among pond categories from the three ponds in the Duchénier Wildlife Reserve, Quebec, Canada where the Wood Frog (*Lithobates sylvaticus*) specimens were collected.

TABLE S4. Chronology of formation for the six anatomical systems of Wood Frog (*Lithobates sylvaticus*) from all three pond categories pooled from the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S5. Logistic models output used to determine onset of chondrification or ossification for six skeletal elements of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S6. Permutation ANOVAs and permutation *t*-tests for the mean onsets of chondrification and ossification of 64 cartilage bones of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S7. Test of congruence within and among pond categories between cartilaginous and ossified trajectories of 64 cartilage bones of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada.

TABLE S8. Test of congruence among and within pond categories between cartilaginous and ossified trajectories of cartilage bones from the appendicular skeleton of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada.