The Canadian Field-Naturalist

Supplementary Materials:

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

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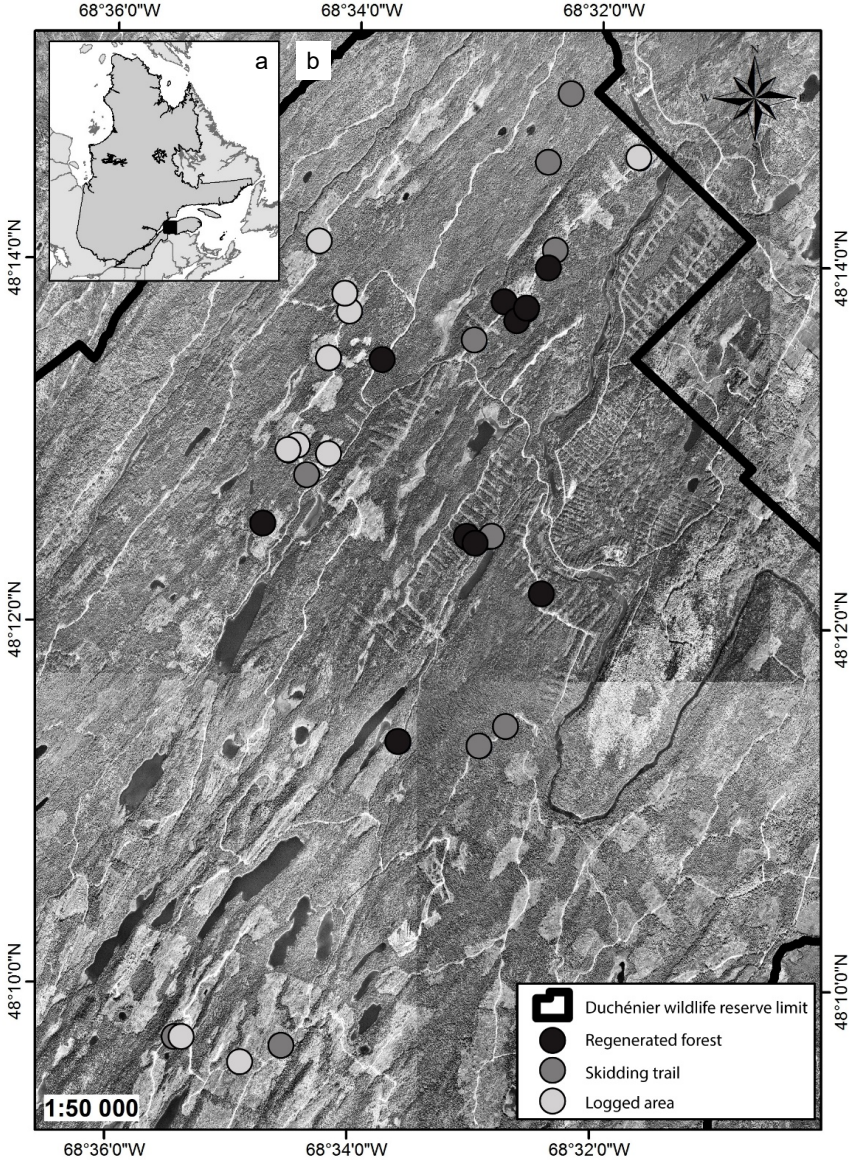

Figure S1. Location of the 30 studied ponds in the Duchénier Wildlife Reserve, Quebec, Canada.

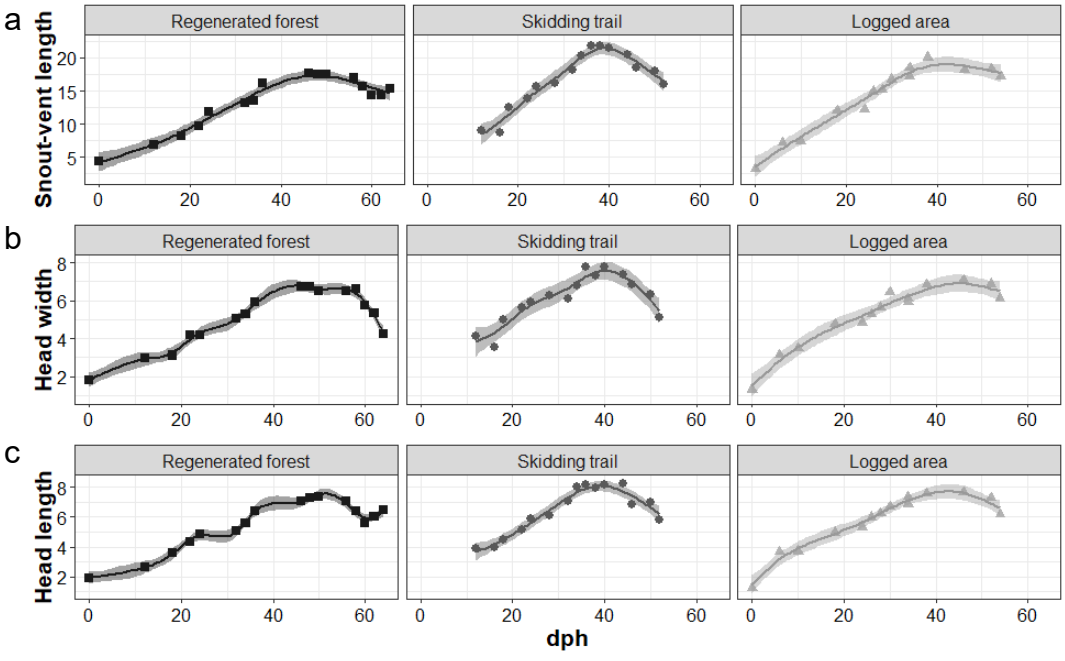

FIGURE S2. Snout–vent length, head width, and head length non-linear relationships during larval period for 45 of the 58 cleared and stained Wood Frog (*Lithobates sylvaticus*) tadpoles and froglets from the Duchénier Wildlife Reserve, Quebec, Canada. Because 13 of the 58 specimens lost their skin integrity due to minor skin perforations, they were not measured. A sample of 16, 15, and 14 specimens were measured from a regenerated forest pond, a skidding trail pond, and a logged area pond, respectively. Generalized additive models were used to represent the global relationship.

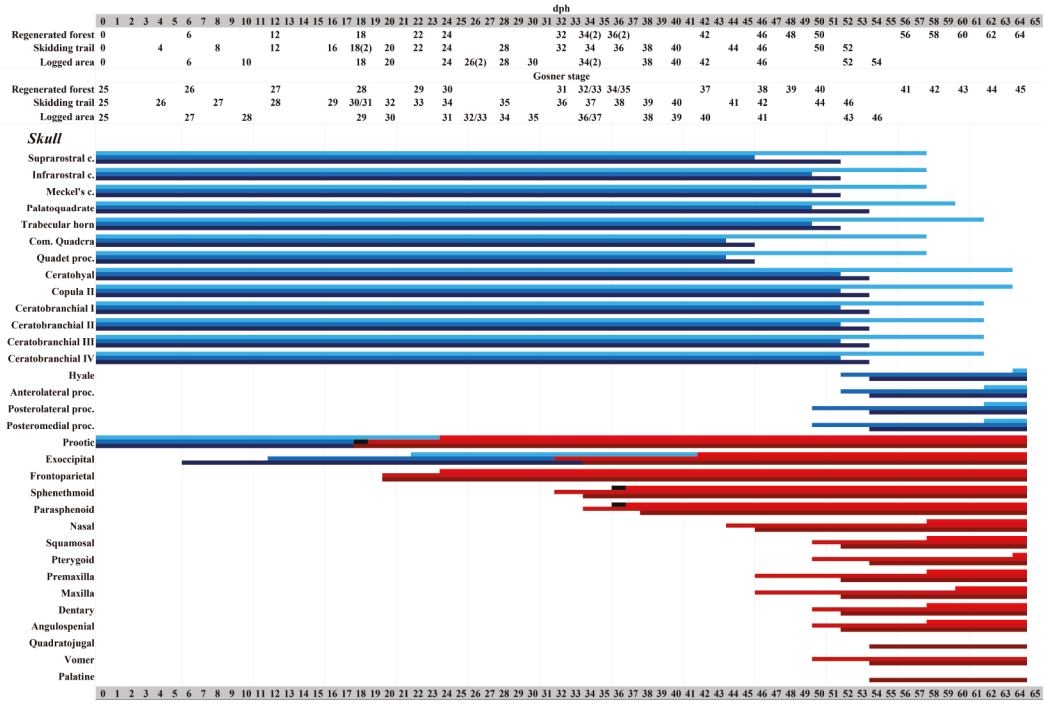

FIGURE S3. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification (blue bars) and ossification (red bars) for cranial elements from a regenerated forest (upper paler bars), a skidding trail (middle intermediate-coloured bars), and a logged area pond (lower darker bars) in the Duchénier Wildlife Reserve, Quebec, Canada. Each black section indicates that Gosner stage is unclear for this skeletogenic event. c. = cartilage; proc. = process; Quadcra = quadratocranialis; Quadet = quadratoethmoidal.

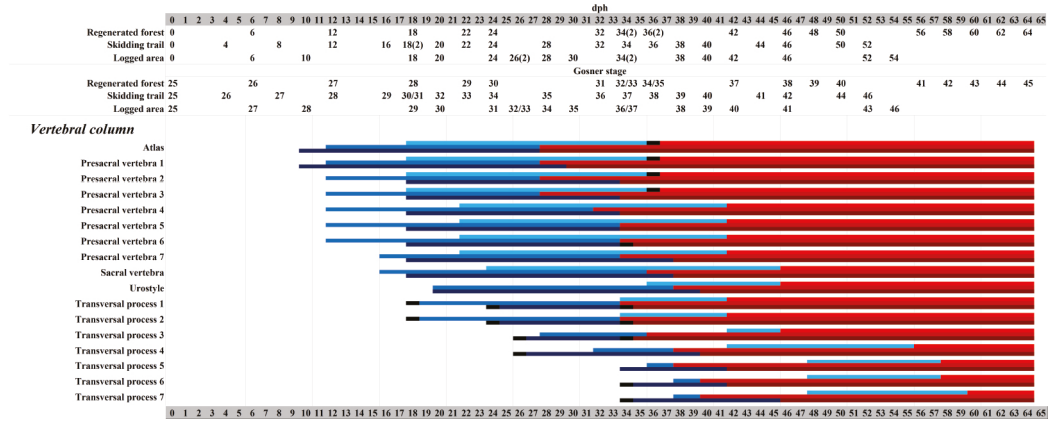

FIGURE S4. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification (blue bars) and ossification (red bars) for the vertebral column from a regenerated forest (upper paler bars), a skidding trail (middle intermediate-coloured bars), and a logged area pond (lower darker bars) in the Duchénier Wildlife Reserve, Quebec, Canada. Each black section indicates that Gosner stage is unclear for this skeletogenic event.

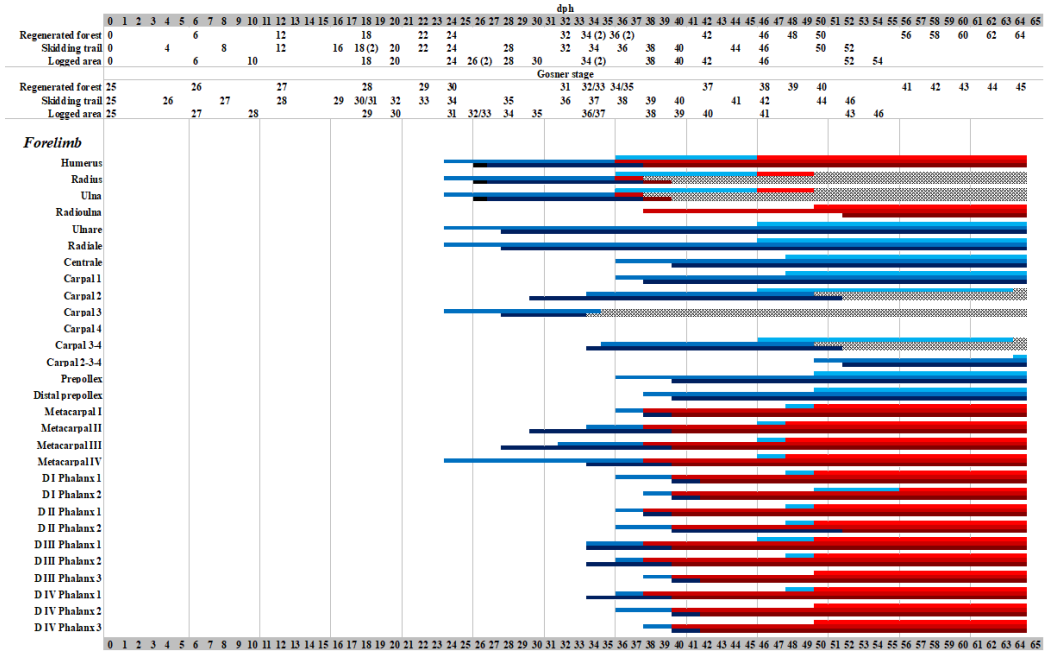

FIGURE S5. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification (blue bars) and ossification (red bars) for forelimb elements from a regenerated forest (upper and paler bars), a skidding trail (middle intermediate-coloured bars), and a logged area pond (lower and darker bars) in the Duchénier Wildlife Reserve, Quebec, Canada. Each black section indicates that Gosner stage is unclear for this skeletogenic event. Each hatched area indicates that an element becomes fused to another. D = digit.

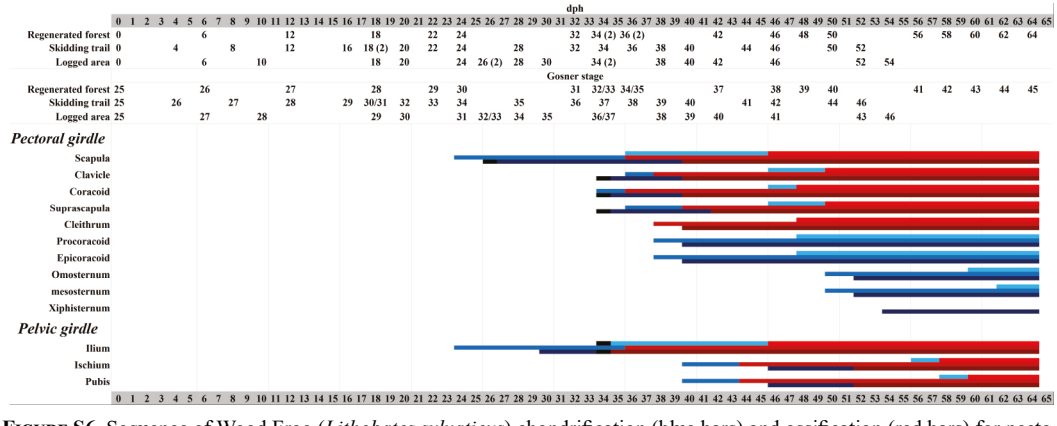

FIGURE S6. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification (blue bars) and ossification (red bars) for pectoral and pelvic girdle elements from a regenerated forest (upper and paler bars), a skidding trail (middle intermediate-colored bars), and a logged area pond (lower and darker bars) in the Duchénier Wildlife Reserve, Quebec, Canada. Each black section indicates that Gosner stage is unclear for this skeletogenic event.

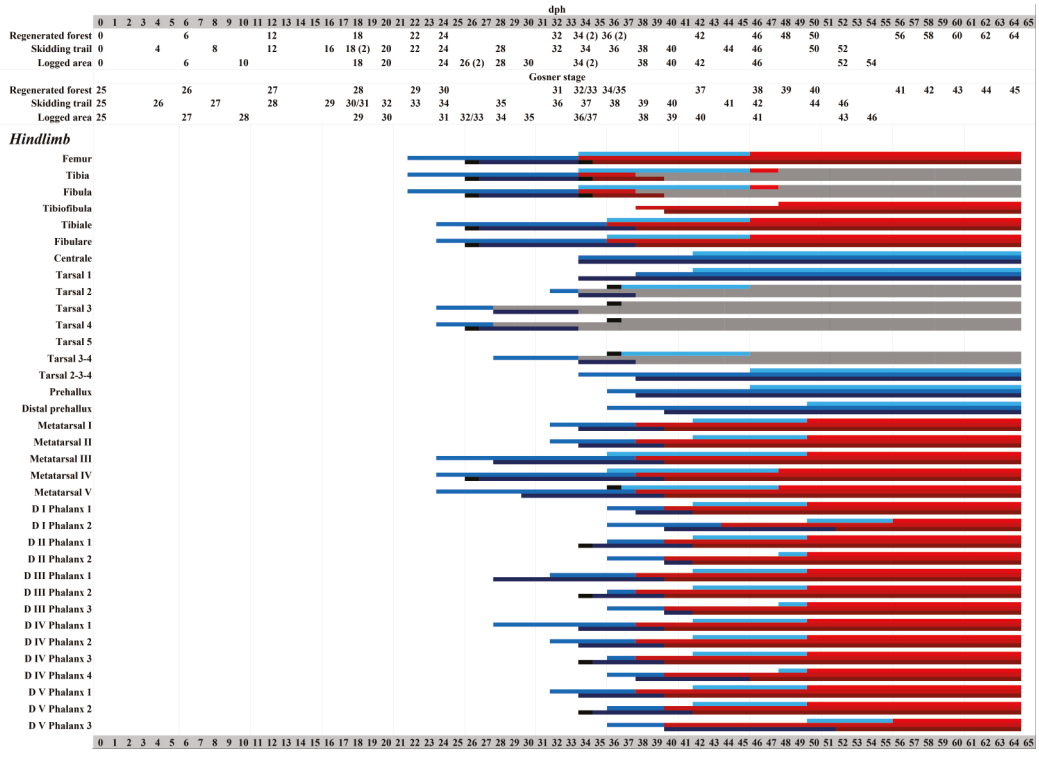

FIGURE S7. Sequence of Wood Frog (*Lithobates sylvaticus*) chondrification (blue bars) and ossification (red bars) for hind limb elements from a regenerated forest (upper and paler bars), a skidding trail (middle intermediate-coloured bars), and a logged area pond (lower and darker bars) in the Duchénier Wildlife Reserve, Quebec, Canada. Each black section indicates that Gosner stage is unclear for this skeletogenic event. Each grey area indicates that an element becomes fused to another. D = digit.

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

Endochondral bones	Anatomical system
Prootic	Skull
Exoccipital	
Atlas	Axial
Presacral vertebra 1	
Presacral vertebra 2	
Presacral vertebra 3	
Presacral vertebra 4	
Presacral vertebra 5	
Presacral vertebra 6	
Presacral vertebra 7	
Sacral vertebra	
Urostyle	
Transversal process 1	
Transversal process 2	
Transversal process 3	
Transversal process 4	
Transversal process 5	
Transversal process 6	
Transversal process 7	
Humerus	Forelimb
Radius	
Ulna	
Metacarpal 1	
Metacarpal 2	
Metacarpal 3	
Metacarpal 4	
Phalanx 1-1	
Phalanx 1-2	
Phalanx 2-1	
Phalanx 2-2	
Phalanx 3-1	
Phalanx 3-2	
Phalanx 4-1	Forelimb
Scapula	Pectoral girdle
Clavicle	
Coracoid	
Suprascapula	
Femur	Hind limb
Tibia	
Fibula	
Calcaneum	
Astragalus	
Metatarsal 1	
Metatarsal 2	
Metatarsal 3	
Metatarsal 4	
Metatarsal 5	
Phalanx 1-1	
Phalanx 1-2	
Phalanx 2-1	
Phalanx 2-2	
Phalanx 3-1	
Phalanx 3-2	
Phalanx 3-3	
Phalanx 4-1	
Phalanx 4-2	
Phalanx 4-3	
Phalanx 4-4	
Phalanx 5-1	
Phalanx 5-2	
Phalanx 5-3	
Ilium	Pelvic girdle
Ischium	
Pubis	

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. Permutation (perm.) *P*-value column shows the significance of results from permutation one-way ANOVA (10 000 permutations) among pond categories.

Characteristics	Pond category						Perm. *P*-value
	Regenerated forest		Skidding trail		Logged area		
	Mean	CI range	Mean	CI range	Mean	CI range	
Vegetal cover							
Bushes (%)	14.50	11.37–17.63	7.00	1.00–13.00	12.17	6.35–17.98	0.076
Trees (%)	14.50	12.18–16.82	0.00	0.00–0.00	3.00	0.50–5.50	<0.001
Herbs (%)	4.00	−4.24–12.24	21.33	6.32–36.35	14.17	4.54–23.79	0.058
Morphometric							
Surface area (m²)	853.88	651.79–1055.96	149.65	65.67–233.78	359.68	116.67–602.73	<0.001
Width (m)	21.18	17.33–25.02	6.91	5.54–8.27	12.13	6.58–17.70	<0.001
Length (m)	54.32	44.77–63.87	27.97	14.10–41.84	32.05	16.21–47.89	0.011
Depth (cm)	103.38	88.37–118.40	72.05	55.26–88.84	65.70	51.82–79.58	0.002
Physico-chemical							
Transparency*	1.17	0.97–1.37	1.98	1.56–2.41	1.59	1.06–2.12	0.019
Conductivity (mS/cm)	147.62	92.48–202.77	99.92	49.88–149.97	84.32	53.66–114.97	0.108
Dissolved oxygen (%)	56.12	47.83–64.41	61.02	47.83–74.22	59.14	47.02–71.26	0.800
pH	6.70	6.50–6.90	6.45	6.24–6.67	6.20	5.71–6.69	0.063
Temperature (°C)	14.64	13.81–15.47	17.53	16.12–18.93	16.57	15.11–18.02	0.003
Water coverage							
Bushes (%)	5.67	1.27–10.06	0.00	0.00–0.00	3.67	−1.06–8.40	0.061
Free water (%)	65.50	52.71–78.29	68.50	54.72–82.28	53.17	39.14–67.19	0.171
Woody debris (%)	12.00	7.80–16.20	5.33	−0.39–11.06	7.00	3.16–10.84	0.082
Emergent plants (%)	9.50	−4.77–23.77	25.67	11.07–40.26	18.67	6.50–30.84	0.172
Floating plants (%)	2.33	−0.49–5.15	0.50	−0.30–1.30	1.00	−0.28–2.28	0.291
Mosses (%)	4.00	0.31–7.69	0.00	0.00–0.00	16.33	3.53–29.13	0.005
Substrate							
Woody debris (%)	10.00	7.08–12.92	5.00	−0.33–10.33	6.83	2.92–10.74	0.171
Plant debris (%)	5.50	−5.74–16.74	16.67	3.04–30.30	4.17	−0.27–8.60	0.123
Dead leaves (%)	16.00	7.95–24.05	1.33	−0.24–2.90	3.33	0.95–5.72	<0.001
Humus (%)	56.83	44.51–69.15	13.00	−3.54–29.54	49.33	26.49–72.17	0.001
Fine sediments (%)	0.00	0.00–0.00	52.00	30.13–73.87	10.67	−7.00–28.33	<0.001
Mosses (%)	11.67	1.55–21.78	0.00	0.00–0.00	25.50	4.61–46.39	0.011

*Clear 1, intermediate 2, opaque 3.

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. Permutation (perm.) *P*-value column shows the significance of results from permutation one-way ANOVA (10 000 permutations) among pond categories.

Characteristics	Pond category						Perm. *P*-value
	Regenerated forest (D57)		Skidding trail (D01)		Logged area (D101)		
	Mean	CI range	Mean	CI range	Mean	CI range	
Vegetal cover							
Bushes (%)	15.00	−9.84–39.84	5.00	−38,03–48.03	20.00	−23.03–63.03	0.142
Trees (%)	11.7	−8.38–31.78	0.00	0.00–0.00	0.00	0.00–0.00	—
Herbs (%)	0.00	0.00–0.00	5.00	−38,03–48.03	25.00	−40.72–90.72	—
Morphometric							
Surface area (m²)	782.31	−1018.84–2583.46	41.70	−105.88–189.28	161.18	−348.23–670.59	0.003
Width (m)	22.33	−56.47–101.13	4.50	−12.71–21.71	3.50	−3.42–10.42	0.060
Length (m)	44.60	−55.61–144.81	11.80	−49.69–73.29	46.05	−53.26–145.36	0.124
Depth (cm)	88.50	−20.59–197.59	58.50	−60.18–177.18	72.33	−22.41–167.07	0.304
Physico-chemical							
Transparency*	1.00	1.00–1.00	1.73	−2.78–6.24	2.18	0.01–4.35	—
Conductivity (ms/cm)	104.00	−46.05–254.05	98.11	−20.51–216.73	34.93	−15.30–85.16	0.200
Dissolved oxygen (%)	80.12	35.53–124.71	63.39	−107.17–133.95	84.84	0.56–169.12	0.607
pH	6.74	3.84–9.64	6.54	5.42–7.66	5.58	1.77–9.39	0.103
Temperature (°C)	15.04	13.75–16.33	17.05	8.86–25.24	20.12	12.03–28.21	0.010
Water coverage							
Bushes (%)	10.00	−14.84–34.84	0.00	0.00–0.00	0.00	0.00–0.00	—
Free water (%)	81.7	−6.84–170.24	80.00	−19.37–179.37	65.00	−24.57–154.57	0.546
Woody debris (%)	5.00	−21.29–31.29	0.00	0.00–0.00	5.00	−26.03–36.03	—
Emergent plants (%)	0.00	0.00–0.00	20.00	−69.57–109.57	25.00	−64.57–114.57	—
Floating plants (%)	3.30	−15.67–22.27	0.00	0.00–0.00	0.00	0.00–0.00	—
Mosses (%)	0.00	0.00–0.00	0.00	0.00–0.00	5.00	−19.84–29.84	—
Substrate							
Woody debris (%)	5.00	−12.21–22.21	0.00	0.00–0.00	5.00	−26.03–36.03	—
Plant debris (%)	0.00	0.00–0.00	10.00	−33.03–53.03	0.00	0.00–0.00	—
Dead leaves (%)	15.00	−50.72–80.72	0.00	0.00–0.00	0.00	0.00–0.00	—
Humus (%)	53.30	−26.55–133.15	0.00	0.00–0.00	63.30	−88.48–215.08	—
Fine sediments (%)	0.00	0.00–0.00	90.00	3.95–176.05	31.70	−87.68–151.08	—
Mosses (%)	26.70	−35.82–89.22	0.00	0.00–0.00	0.00	0.00–0.00	—

* Clear 1, intermediate 2, opaque 3.

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. Ranges of days post-hatching (dph) take into account the earliest and latest elements formed within a system. The sample size (*n*) represents the number of skeletal elements associated with each system.

Anatomical system	*n*	Sequence of chondrification (dph)	Sequence of ossification (dph)
Skull	32	0–64	18–64
Vertebral column	17	10–48	28–60
Hind limb	35	22–50	34–56
Pelvic girdle	3	24–58	35–60
Pectoral girdle	10	24–62	35–60
Forelimb	29	24–64	36–56

Table S5. Logistic models output used to determine the onset of chondrification or ossification for six skeletal elements of Wood Frog (*Lithobates sylvaticus*) from the Duchénier Wildlife Reserve, Quebec, Canada. AUC = area under the curve; CI are 95 %.

Skeletal element	Pond category	Skeletogenesis event	dph_{50}	AUC	CI_{AUC}	Odd ratio	CI_{odd}
Urostyle	Skidding trail	Chondrification	19.618	0.940	± 0.179	1.512	± 1.206
Transversal process 1	Skidding trail	Chondrification	21.282	0.969	± 0.118	1.931	± 3.655
Transversal process 2	Skidding trail	Chondrification	21.282	0.969	± 0.118	1.931	± 3.655
Atlas	Logged area	Ossification	27.558	0.975	± 0.084	1.931	± 3.984
Transversal process 1	Logged area	Chondrification	21.243	0.967	± 0.126	1.493	± 1.078
Transversal process 2	Logged area	Chondrification	21.243	0.967	± 0.126	1.493	± 1.078

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.

Anatomical system		Chondrification (*P*-value)	Ossification (*P*-value)
Skull (*n* = 2)	ANOVA	0.808	0.697
	Regenerated forest - skidding trail	—	—
	Regenerated forest - logged area	—	—
	Skidding trail - logged area	—	—
Vertebral column (*n* = 17)	ANOVA	0.014	<0.001
	Regenerated forest - skidding trail	0.027	<0.001
	Regenerated forest - logged area	0.027	<0.001
	Skidding trail - logged area	0.788	0.134
Forelimb (*n* = 14)	ANOVA	<0.001	<0.001
	Regenerated forest - skidding trail	<0.001	<0.001
	Regenerated forest - logged area	<0.001	<0.001
	Skidding trail - logged area	0.631	0.0018
Pectoral girdle (*n* = 4)	ANOVA	0.019	<0.001
	Regenerated forest - skidding trail	0.089	0.043
	Regenerated forest - logged area	0.029	0.043
	Skidding trail - logged area	0.994	0.110
Hind limb (*n* = 24)	ANOVA	<0.001	<0.001
	Regenerated forest - skidding trail	<0.001	<0.001
	Regenerated forest - logged area	<0.001	<0.001
	Skidding trail - logged area	0.191	0.014
Pelvic girdle (*n* = 3)	ANOVA	0.316	0.167
	Regenerated forest - skidding trail	—	—
	Regenerated forest - logged area	—	—
	Skidding trail - logged area	—	—

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.

Comparison within pond categories		Ossification (r_s)		
		Regenerated forest	Skidding trail	Logged area
Chondrification (r_s)	Regenerated forest	0.89 ($P < 0.001$)		
	Skidding trail		0.92 ($P < 0.001$)	
	Logged area			0.89 ($P < 0.001$)

Comparison among pond categories	Chondrification (r_s)	Ossification (r_s)
Regenerated forest - Skidding trail	0.93 ($P < 0.001$)	0.88 ($P < 0.001$)
Regenerated forest - Logged area	0.92 ($P < 0.001$)	0.89 ($P < 0.001$)
Skidding trail - Logged area	0.92 ($P < 0.001$)	0.96 ($P < 0.001$)

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. Letters refer to the multiple comparison results between r_s from three pond categories.

Forelimb (*n* = 14)

Comparison within pond categories		Ossification (r_s)		
		Regenerated forest	Skidding trail	Logged area
Chondrification (r_s)	Regenerated forest	0.84 ($P < 0.001$)		
	Skidding trail		0.81 ($P < 0.001$)	
	Logged area			0.92 ($P < 0.001$)

Comparison among pond categories	Chondrification (r_s)	Ossification (r_s)
Regenerated forest - Skidding trail	0.81 ($P < 0.001$)	0.84 ($P < 0.001$) [b]
Regenerated forest - Logged area	0.87 ($P < 0.001$)	0.82 ($P < 0.001$) [b]
Skidding trail - Logged area	0.85 ($P < 0.001$)	0.99 ($P < 0.001$) [a]

Hindlimb (*n* = 24)

Comparison within pond categories		Ossification (r_s)		
		Regenerated forest	Skidding trail	Logged area
Chondrification (r_s)	Regenerated forest	0.82 ($P < 0.001$)		
	Skidding trail		0.88 ($P < 0.001$)	
	Logged area			0.88 ($P < 0.001$)

Comparison among pond categories	Chondrification (r_s)	Ossification (r_s)
Regenerated forest - Skidding trail	0.93 ($P < 0.001$)	0.88 ($P < 0.001$) [b]
Regenerated forest - Logged area	0.92 ($P < 0.001$)	0.89 ($P < 0.001$) [b]
Skidding trail - Logged area	0.92 ($P < 0.001$)	0.96 ($P < 0.001$) [a]