

# ECOGRAPHY

## Research

### Phenological delay despite warming in wood frog *Rana sylvatica* reproductive timing: a 20-year study

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Across all taxa, amphibians exhibit some of the strongest phenological shifts in response to climate change. As climates warm, amphibians and other animals are expected to breed earlier in response to temperature cues. However, if species use fixed cues such as daylight, their breeding timing might remain fixed, potentially creating disconnects between their life history and environmental conditions. Wood frogs *Rana sylvatica* are a cold-adapted species that reproduce in early spring, immediately after breeding ponds are free of ice. We used long-term surveys of wood frog oviposition timing in 64 breeding ponds over 20 yr to show that, despite experiencing a warming of 0.29°C per decade in annual temperature, wood frog breeding phenology has shifted later by 2.8 d since 2000 (1.4 d per decade; 4.8 d per °C). This counterintuitive pattern is likely the result of changes in the timing of snowpack accumulation and melting. Finally, we used relationships between climate and oviposition between 2000 and 2018 to hindcast oviposition dates from climate records to model longer-term trends since 1980. Our study indicates that species can respond to fine-grained seasonal climate heterogeneity within years that is not apparent or counterintuitive when related to annual trends across years.

Keywords: amphibian, breeding phenology, climate change, seasonality, snow water equivalent, spring onset

#### Introduction

As global temperatures and seasonal climatic variability continue to increase, so does interest in understanding how climate change will impact biodiversity. One major concern is that the extent of environmental change may outpace the limits of biological responses with negative effects leading to population declines, changes in species distributions and potentially extinction for species with limited response capacity (Pimm et al. 1995, Thuiller et al. 2004, Urban 2015). These biological changes could eventually affect not just global diversity patterns, but also entire ecosystems and the services that they provide to humans (Bongaarts 2019).



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Many organisms exhibit an annual cycle of life history events, or phenology, that progress in concert with environmental conditions such as seasonal variation in temperature, precipitation or photoperiod (Scranton and Amarasekare 2017). As the global climate continues to shift, phenological events cued by temperature are expected to advance as long-term average temperatures increase and warming occurs earlier in the year (Root et al. 2003, Thackeray et al. 2016). In fact, many species have shown considerable phenological shifts, usually to earlier in the season (Walther et al. 2002, Parmesan and Yohe 2003, Cohen et al. 2018). These shifts are most pronounced for populations in the temperate zone and for ectotherms (Cohen et al. 2018).

If important components of a species' community and ecosystem all advance at similar rates, then these phenological shifts may benefit the species by allowing it to track shifting climates. However, different species respond to different phenological cues with different sensitivities, which can result in asynchronous shifts among interacting species (Ovaskainen et al. 2013). For example, the average flowering date of northeastern North American plants is advancing under climate change and wild bees that rely on pollen show similarly advancing phenology, maintaining synchrony in the mutualistic relationship (Bartomeus et al. 2011). As a counter example, great tits *Parus major* rely on seasonal availability of caterpillar food resources during nesting; however, climate change is causing an advance in the caterpillar phenology. While great tits exhibit advances in laying dates over the same period, the change is not enough to take advantage of peak caterpillar food resources (Visser et al. 1998).

Amphibians exhibit the largest phenological shifts of any vertebrate taxa, which may make this group particularly vulnerable to phenological asynchronies (Parmesan 2006, While and Uller 2014, Thackeray et al. 2016). Disconcertingly, climate change and phenological mismatch have been suggested to contribute to the trend in global amphibians declines (Stuart et al. 2004). Life history and reproductive behavior are regulated primarily by temperature for most temperate amphibians, including emergence from hibernation, initiation of reproductive migration, oviposition timing, etc. (Reading 1998). Amphibian life cycle timing is expected to advance with warming climates as organisms emerge, breed and initiate development earlier in the spring (Hughes 2000). However, at least some amphibians use photoperiod cues in combination with other environmental cues to time their breeding (Canavero and Arim 2009).

We analyzed climate and oviposition timing over 20 yr in a metapopulation of wood frogs *Rana sylvatica* in northeastern Connecticut, USA to evaluate long-term patterns in reproductive phenology relative to changes in key climate variables. Climate data suggest that this region has rapidly warmed in concert with the rest of the northern hemisphere. Given the sensitivity of wood frog breeding phenology to temperature (Benard 2015), we hypothesize that wood frog breeding will have advanced to occur earlier in response to increasing temperatures. Alternatively, wood frogs might also

use fixed cues such as photoperiod, which would mitigate phenological changes in response to climate.

## Methods

### Natural history

In this study, we consider reproductive phenology of the wood frog, a wide-spread North American anuran (Layne and Lee 1995, Lee-Yaw et al. 2008, Larson et al. 2014). The phenology of the wood frog is closely tied to seasonal climate and selection favors the earliest possible reproduction and oviposition, balanced by the threat of egg mortality (if ponds freeze after oviposition) and pond desiccation at the end of the larval period. Most mortality (92–99%) occurs in pre-metamorphic life stages (Dodd 2013) and is often associated with predation or drying of breeding ponds (Camp et al. 1990, Berven 1995, DiMauro and Hunter 2002), which is, itself a function of climate (Brooks 2004). Early reproduction extends the larval period and increases the time available for development before ponds dry. In other amphibians, early oviposition also allows larvae to reach a critical body size threshold that provides refuge from gape-limited predators (Urban 2007). Intraspecific competition for mates also favors males that arrive earliest to breeding ponds (Waldman 1982).

Wood frogs possess a number of traits that facilitate early spring breeding and make the species particularly sensitive to climate. Adult wood frog males overwinter immediately adjacent to breeding ponds where they select hibernacula beneath forest litter (Regosin et al. 2003, Groff et al. 2017). Proximity to ponds enables breeding to commence as soon as the climate is favorable. Similarly, proximity to the ground surface enables individuals to emerge from hibernation as soon as possible, but simultaneously increases exposure to freezing temperatures (O'Connor and Rittenhouse 2016). Physiological adaptations for freeze-tolerance (Storey and Storey 1984, Storey 1990, Layne and Lee 1995, Costanzo et al. 2013), rapid reanimation post-freezing (Layne and First 1991) and fine-tuned microhabitat selection (O'Connor and Rittenhouse 2016) ameliorate this problem. As a result, wood frogs are generally the earliest breeding amphibians, emerging long before ice and snow have diminished (Dodd 2013).

Seasonal timing of oviposition across the wood frogs' range varies positively with latitude and elevation (Berven 1982, Guttman et al. 1991). Thus, one would expect wood frog breeding and oviposition timing to similarly advance with increasing average temperatures. This is the case for the common frog *Rana temporaria*, a related species found in Europe. Carroll et al. (2009) analyzed more than 70 000 records of oviposition dates for common frog populations across the UK between 1998 and 2007 and found an average advance of 10 days, although this did not correspond to earlier hatching (Scott et al. 2008).

The wood frog populations included in this study breed in vernal pools ranging from 190 m to 296 m in elevation across

0.06 degrees of latitude (8 km). Canopy cover, which also impacts local climate, ranges from nearly completely open (6.5%) to completely closed (98.3%) (see methods for estimating canopy cover below).

## Climate

We extracted daily meteorological data, including maximum and minimum temperature, precipitation and snowpack (quantified as snow water equivalent) from the DayMet database (ver. 3 (Thornton et al. 2016)). The Daymet algorithm uses daily meteorological observations from ground stations to interpolate estimates at 1 km square cells across North America between 1980 and 2018. We extracted estimates for the cell centered nearest the geographical center of our site (tile: 11 754, N 41.9198 W -72.1604). These data were indistinguishable from estimates extracted for tiles encompassing the northernmost and southernmost extent of our site. We computed average daily temperatures as the midpoint between daily minimum and maximum temperatures.

In all cases, we transformed calendar dates into two variables, Year and Day-of-Year values, to enable modeling long-term trends versus annual variability and to account for leap years. We tested for long-term trends in meteorological variables over time by regressing the annual averages against Year. We confirmed that linear models were appropriate, first, by visually comparing linear and loess regression lines. Annual trends may mask important shifts in seasonal climate at shorter temporal scales. So, secondarily, we assessed non-linear, intra-annual trends by regressing the linear function of Year and penalized spline functions of Day-of-Year against each meteorological variable using generalized additive models implemented with ‘mgcv’ (ver. 1.8.26 (Wood 2011)). For each model we include the interaction between the smoothing terms and Year. The interaction term allows the seasonal shape of the curve to vary across year while no interaction would indicate a shift in the mean but no seasonal change. All statistical analyses were performed in R ver. 3.5.2 (R Core Team).

## Oviposition phenology

We used records of annual egg-mass surveys collected between 2000 and 2019 for 64 wood frog breeding ponds at Yale Myers Forest, a 3213 ha research forest in northeastern Connecticut, USA (Fig. 1). During surveys, the entire pond was closely searched by observers. If eggs were found, the observers independently counted and then averaged the estimates (or, in cases of surveys by a single observer, egg masses were counted twice and averaged).

Wood frogs are explosive, aggregate breeders (reviewed in Dodd 2013). Breeding commences shortly after females emerge from hibernation and oviposition occurs in synchrony over a short timespan (reviewed in Dodd 2013). Within a pond, oviposition lasts just a few days (~ 2–8, as reviewed in Dodd 2013) and, among proximal ponds, oviposition is generally concurrent (Petranka et al. 2004). At our site, eggs

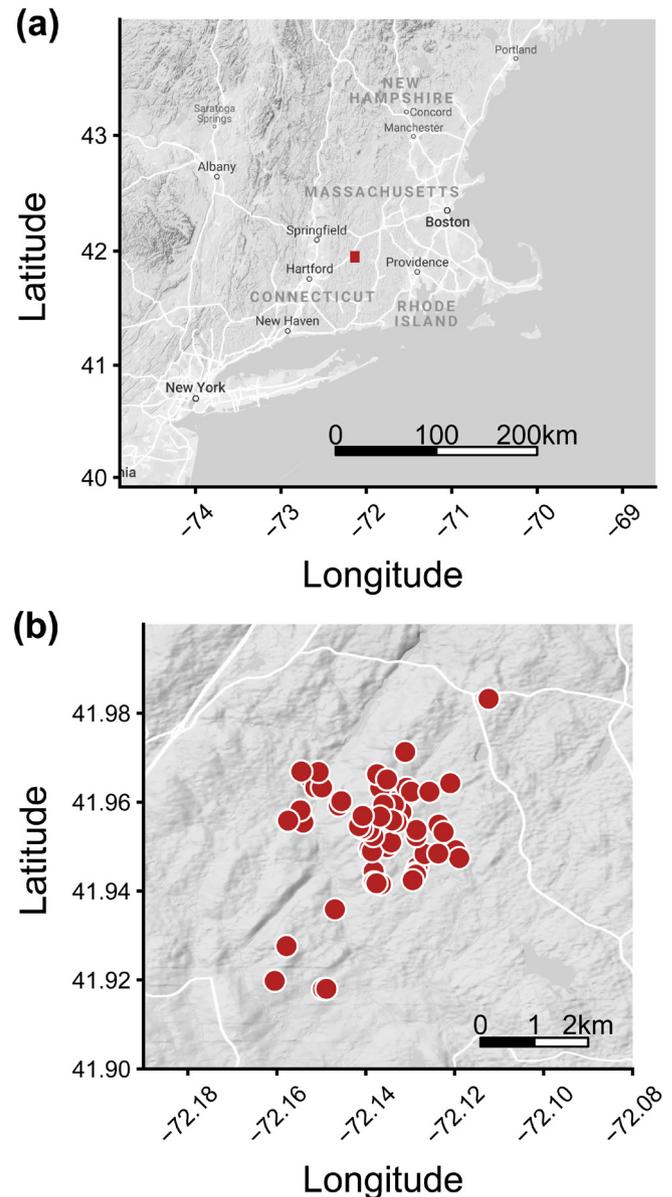


Figure 1. Location of Yale Myers Forest research site in northeastern Connecticut, USA (red square) (a). Locations of 64 wood frog breeding ponds at the site (red circles) (b).

hatch between 9 and 18 d after oviposition, depending on water temperature (Skelly 2004). Surveys were conducted approximately 7 d after oviposition each year to ensure oviposition had concluded and before egg masses swelled too much to be individually distinguished. Thus, the survey dates closely correspond to oviposition timing and we use the survey dates as a proxy for mean annual oviposition date within a pond population.

While survey dates are an imperfect proxy, other measures of oviposition timing, such as records of first or last appearance or calling, are similarly subject to both methodological and biological error (Tryjanowski et al. 2003). In ten years with protracted breeding, multiple surveys were conducted

at each pond and cumulative egg mass counts were recorded. On average, counts differed for 3–4 ponds. In these cases, we estimated the oviposition date for each pond as the average survey day weighted by the number of new egg masses recorded in subsequent resurveys. We excluded observations in which no eggs were recorded in ponds.

Our oviposition data include 1103 observations across 64 ponds between 2000 and 2019. On average, we have records for 17.2 yr (SD = 5.0, median = 19) for each pond, a total of 86% data coverage across all pond-year combinations. We excluded years for which we surveyed ponds but found no eggs ( $n = 278$ ), leaving 825 oviposition date observations for further analysis. We tested the long-term change in wood frog phenology over time by fitting a linear mixed model in ‘lme4’ (ver. 1.1.21 (Bates et al. 2015)) predicting oviposition date by Year, including the site ID from the pond of observation as a random intercept both with and without allowing the slopes of the relationship with Year to vary among ponds as a random effect. We calculated 95% confidence intervals for this estimate with 1000 bootstrap iterations. This approach provided an estimated change in site-wide oviposition.

### Hindcast modeling long-term phenology

Although the 20-yr duration of our dataset is one of the longer continuous oviposition datasets available, it is a short window compared to the local ecological and evolutionary history of wood frogs. We tested the similarity between the short-term trend in our observed dataset and putative long-term change by comparing our results to a hindcast (or ‘retro-diction’ sensu Green 2017) dataset wherein we used our extant oviposition dates to train a random forest model to predict past oviposition dates.

Random forest is a powerful and flexible model-averaging technique that leverages many individual regression trees built with random subsets of the data and random subsets of predictor variables to estimate the outcome resulting from complex relationships among independent variables. Because these models can accommodate many correlated independent variables, we built our random forest from a robust set of pond-wise and year-wise predictors in order to capture the natural variability of pond micro-habitats at our site.

As pond-wise variables, we included latitude, elevation, aspect and a suite of canopy metrics estimated from repeated hemispherical photos. Briefly, five hemispherical photographs were taken along the shore at each cardinal point and at the center of each pond during leaf-off and leaf-on seasons. We used Gap Light Analyzer (Frazer et al. 1999) to estimate average leaf-on and leaf-off global site factor (GSF; the ratio of above-canopy radiation to under-canopy radiation (Anderson 1964)) and a weighted GSF value integrated over the duration of wood frog larval and embryonic life cycle (Halverson et al. 2003). Additionally, we included the within-pond variance in GSF values, which captures important components of the canopy shape and structure above and surrounding the ponds. Point values for aspect and elevation were estimated from the USGS National Elevation Dataset (U.S. Geological

Survey 2002). In addition, we included site-wide daily average temperature, precipitation and SWE between day-of-year 0 and 120 (1 Jan–29 April) from the DayMet dataset. This range spans the earliest and latest observed oviposition dates (day-of-year 74 and day-of-year 113) with a margin. Finally, we included a random noise variable in order to assess rank importance of all our included variables.

We grew our random forest from 1000 regression trees with package ‘randomForest’ (ver. 4.10 (Liaw and Wiener 2002)) and assessed the predictive accuracy of our model through 5-fold cross validation. We then used the model fit to all our observations to hindcast oviposition dates between 1980 and 1999. We fit an ordinary least squares linear model to the combined datasets of hindcasted and observed site-wide average oviposition dates to test for long-term phenological change and compared these to the short-term trend displayed by our egg mass surveys. Our hindcast predictions assume that every pond hosted an oviposition event in every year which is not true. Thus, we consider the average oviposition date each year as an approximation of the metapopulation-wide oviposition date and use bootstrap resampling to estimate 95% confidence intervals.

Although random forests are useful for prediction, they are ‘black-box’ models that are not easily interpretable. So, in order to assess the relative importance and direction of the relationship of climatological variables on oviposition dates, we computed 10-, 20-, 30- and 40-day moving averages for daily temperature, precipitation and snow water equivalent between 2000 and 2019 between day-of-year 0 and 160 (1 Jan–9 June). For each variable, we calculated the Pearson’s correlation between each window-average and observed oviposition timing averaged across all ponds for the 20-yr overlap. We plotted the correlation coefficients over time to determine at what point in the season each variable is most likely to influence oviposition timing, determined by the span for non-overlapping windows with the greatest absolute correlation to oviposition timing greater than a 95% confidence threshold ( $r = 0.45$ ).

## Results

### Climate

There has been a substantial increase in annual temperature over time (Fig. 2a). Annual temperature increased at a rate of  $0.29^{\circ}\text{C}$  per decade, or an estimated total increase of  $1.1^{\circ}\text{C}$  since 1980 ( $F_{1,36} = 10.19$ , slope = 0.029,  $p = 0.003$ ). This rate is consistent with estimated increases in the northeast region of the US (Hartfield et al. 2018). There is no evidence of net change in annual precipitation ( $F_{1,36} = 0.000$ , slope =  $-0.000$ ,  $p = 1.0$ ) or in annual snow water equivalent ( $F_{1,36} = 0.002$ , slope =  $-0.009$ ,  $p = 0.97$ ) from 1980 to 2018 (Fig. 2b–c). Although loess curves suggest a quadratic fit for precipitation and snowpack change, second order polynomial fits were also non-significant.

We do see seasonal shifts in daily temperatures and snowpack, but not in precipitation across years (Table 1). The

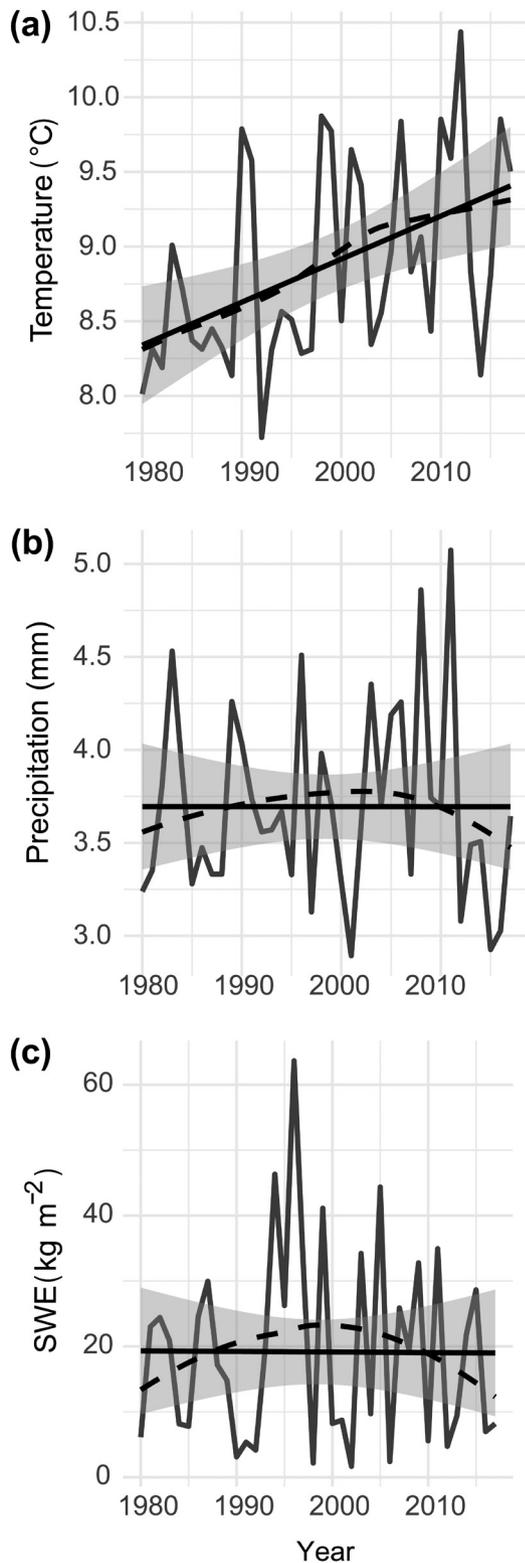


Figure 2. Trends in mean daily temperature (a) precipitation (b) and snow water equivalent (c) between 1980 and 2018 from DayMet meteorological database. Black solid lines and grey bands indicate linear regression line and 95% confidence intervals, respectively. Dashed lines indicates LOESS regression with 90% smoothing span for comparison.  $n=39$  for all models.

greatest seasonal shift in daily temperatures is seen in the latter portion of the year, after the annual peak temperature around day-of-year 200 (19 July) (Fig. 3). Daily temperatures shifted the least in the colder portion of the year from day-of-year 1 (1 Jan) to approximately day-of-year 100 (~10 April) (Fig. 3). Although there is no evidence of an overall increase in the snowpack, the timing of accumulation and melt is delayed. The models estimate that snowpack accumulates approximately 28 d later, and persists approximately 27 d longer into spring, while the total snow water equivalent of the snowpack in mid-winter and early spring, between day-of-year 13 (13 Jan) and day-of-year 105 (~15 April) remains stable (Fig. 3). The seasonal oviposition window coincides with a period of relatively low shifts toward warmer temperatures, minimally decreased precipitation and increased snowpack (Fig. 3). However, the magnitude of the change in air temperature (0.62–2.67 SD) is much larger than either precipitation (0–0.19 SD) or snowpack (0–0.50 SD) (Fig. 3).

### Oviposition phenology

The earliest and latest oviposition dates on record are day-of-year 74 (~15 Mar) and day-of-year 113 (~23 Apr), respectively, with a median of day-of-year 96 (~6 Apr). In some years, ponds at the southern portion of the site or ponds with more open canopies breed earlier. On average, oviposition across ponds within a given year took place within a short period of 6 d (SD=6.1 d). Fitting a mixed model allowing slopes to vary by pond did not increase predictive power (with (AIC=5939.6) and without (AIC=5937.6) random slopes). The multi-level mixed model fit to survey dates between 2000 and 2019 with random intercept by pond shows a delaying trend of 1.4 d per decade (4.8 d per °C) in site-wide oviposition timing ( $n=825$ , slope=0.139, SE=0.05, 95% CI=0.03, 2.44) (Fig. 4). This pattern contradicts our hypothesis and the general trend seen in most amphibians of phenological advancement in spring reproductive timing in response to warming temperatures.

### Hindcast model

Our random forest model fit well (mean out-of-bag  $R^2=0.94$ ) and yielded high predictive accuracy (RMSE=2.34). On average our predicted oviposition dates were with 1.6 d of the true oviposition date of our hold-out cross-validation observations. The slope of the regression fit to observed oviposition dates (2000–2019) and hindcast predicted dates (1980–1999) provides no support for long-term directional change in phenology ( $n=40$ , slope=-0.069, SE=0.093,  $p=0.45$ , 95% CI=-0.26, 0.17) with a 95% confidence interval from 1000 iterations that includes delay up to 1.7 d/decades and advance up to 2.4 d/decades (Fig. 5). Temperature was the most important site-wide variable in the random forest model, followed by precipitation and snowpack (Supplementary material Appendix 1 Fig. A1). The maximum temperature on day-of-year 69, just prior to oviposition in most years, was the most important variable accounting for model accuracy,

Table 1. Seasonal trends in meteorological variables (temperature, precipitation and snow water equivalent) 1980–2018 fit by penalized general additive models to Year and Day-of-Year (DOY) in the form:  $Y = \beta_0 + \text{Year} + f(\text{DOY}) + \text{Year} \times f(\text{DOY}) + \varepsilon$ , where  $Y$  is the meteorological variable of interest.

Dependent variable	n	Parametric coefficients				Smoothed functions		
		Parameter	Estimate	SE	p-value	Parameter	Est. df	p-value
Temp (°C)	13 870	Intercept	-48.75	6.24	<< 0.001	$f(\text{DOY})$	1.635	< 0.001
		Year	0.01	0.003	0.005	$f(\text{DOY}) \times \text{Year}$	9.181	<< 0.001
Precip (mm)	13 870	Intercept	3.71	13.28	0.780	$f(\text{DOY})$	1.402	0.804
		Year	-0.000	0.003	0.999	$f(\text{DOY}) \times \text{Year}$	1.529	0.940
SWE (kg m <sup>-2</sup> )	7752	Intercept	65.47	78.29	0.403	$f(\text{DOY})$	7.993	<< 0.001
		Year	-0.008	0.020	0.690	$f(\text{DOY}) \times \text{Year}$	1.500	0.018

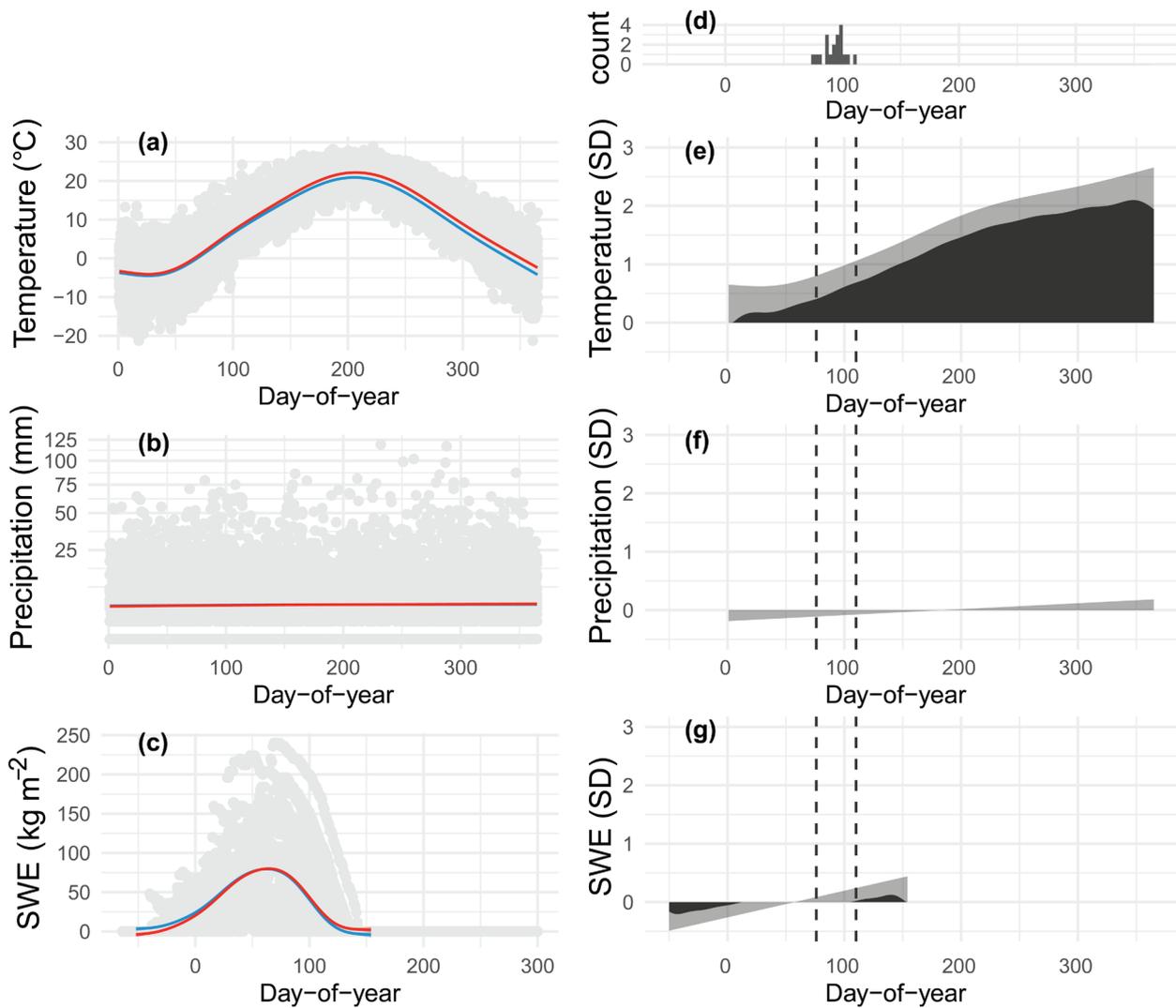


Figure 3. Seasonal trends in daily temperature (a), precipitation (square root scale) (b) and snow water equivalent (c) from 1980 (blue) to 2018 (red) as predicted by generalized additive model with interaction between Year and penalized spline smooth on Day-of-Year with 95% confidence intervals. Points represent daily values ( $n = 13\,869$  for all models). Annual mean oviposition dates (2000–2019) (d) in comparison to relative, seasonal change in temperature (e), precipitation (f) and snow water equivalent (SWE) (g) between 1980 and 2018. Seasonal change is the difference in daily values fit by generalized additive models for 1980 and 2018. All differences are scaled to the standard deviation between annual averages for each variable in order to compare relative magnitude of change that coincides with the oviposition window (dotted lines). Dark bands indicate significant difference between 95% confidence intervals. Light bands indicate total difference. All meteorological observations from Daymet data between 1980 and 2018.

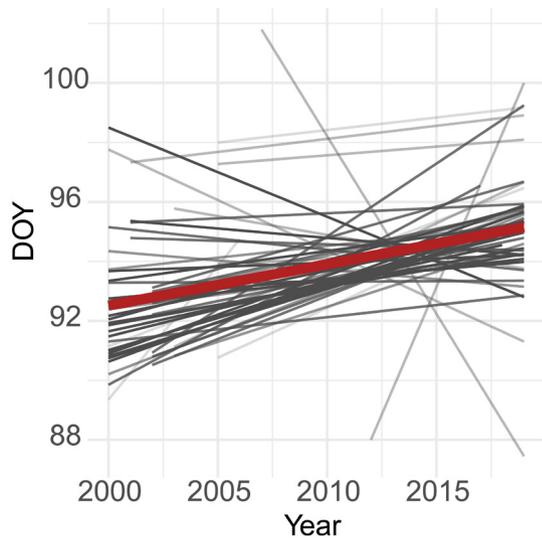


Figure 4. Trends in observed oviposition dates (DOY=Day-of-Year) across years between 2000 and 2019. Grey lines represent trends for individual ponds with transparency indicating number of observations (darker lines=more observations) (excludes ponds with fewer than 4 yr of observation for simplicity). The red line represents the trend across all ponds, estimated as the fixed effect of a multilevel mixed effect model ( $n=825$ , slope=0.139, SE=0.05, 95% CI=0.03, 2.44).

followed by precipitation on day-of-year 56. The importance of daily snowpack values was low but consistent. Only a single pond-wise variable, latitude, was ranked as highly important in the model.

When considered individually, all meteorological variables showed significant bivariate correlations with oviposition dates for at least one seasonal window (Supplementary material Appendix 1 Fig. A2). The periods of strongest correlation between temperature and oviposition timing included a negative relationship at the outset of oviposition from day-of-year 66 to 106 and a weaker but significantly negative correlation earlier in the season from day-of-year 25 to

45. A negative relationship between temperature and oviposition means that warmer temperatures are correlated with earlier oviposition (i.e. lower day-of-year). There is a positive relationship between oviposition and precipitation from day-of-year 93 to 113 which means that greater precipitation in this window is correlated with later oviposition dates. There is a positive relationship between oviposition and snow water equivalent from day-of-year 77 to 87, just prior to a period of significant correlation with radiation from day-of-year 80 to 90. Some climate windows with significant correlation to oviposition occur after the frogs breed, indicating that these are temporally autocorrelated, not causal.

## Discussion

Most studies across taxonomic groups, including all prior studies of amphibians, have documented advancement of breeding phenology in response to changing climate (Carroll et al. 2009, Green 2017). Although phenological delay has been documented in some amphibian species (Todd et al. 2011), these reports involve fall-breeding species in which delay is expected with warmer summers. The present study is one of the first to find a delay in reproductive timing (2.8 d over 20 yr) in a spring-breeding amphibian. As in other studies that have documented advances in timing, we are working in a system in which temperatures have increased in recent decades (0.6°C over 20 yr).

We included a hindcast modelling procedure to evaluate whether estimated long-term patterns of oviposition negate the delaying trend we see in our shorter-term observations. Green (2017) used a similar approach to show that prior estimates of phenological delay in Fowler's toads *Anaxyrus fowleri* were incompatible with long-term estimates from hindcasted breeding dates that suggested advancing phenology. In contrast to short-term estimates of phenological delay in oviposition, our hindcasted dataset of annual metapopulation-wide oviposition show no evidence of directional change. However, confidence

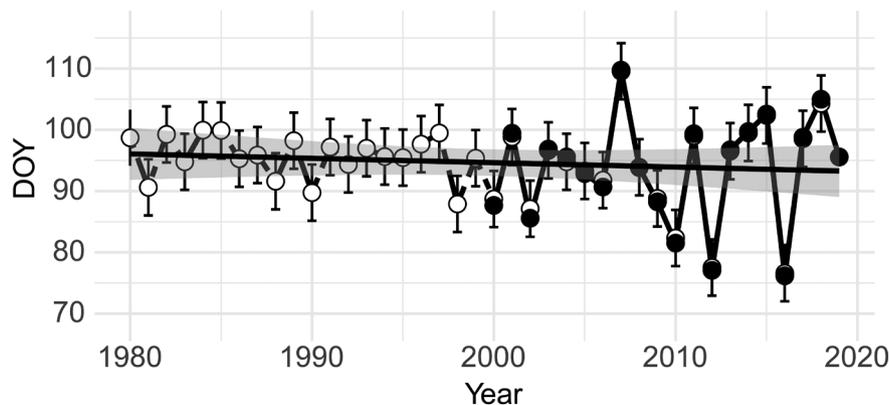


Figure 5. Predicted (open circles (1980–2018)) and observed (filled circles (2000–2019)) oviposition dates. Error bars indicate 95% prediction intervals (estimated as  $\pm 1.96 \times$  cross-validated RMSE). Simple linear regression and 95% confidence intervals fit to predicted (1980–1999) and observed (2000–2019) dates ( $n=40$ ) are shown. Note that meteorological variables for 2019 were unavailable at the time of publishing, so no predictions were made for that year.

intervals for the long-term hindcast model include our slope estimate of 1.4 d/decade. The fact that our model fit to hindcasted data showed no evidence of change in oviposition may be due to lack of power as this model cannot account for intra-annual variation in oviposition dates among ponds nor years when ponds host no breeding events.

Among animal taxa, amphibians exhibit some of the strongest relationships between changing climates and phenological shifts. A meta-analysis across all animals found that amphibians demonstrated an average spring advancement of 7.6 d per decade, almost double the average of all taxa combined (Parmesan 2007). An analysis of United Kingdom taxa found an average advancement of 2.6 d per °C, with amphibians exhibiting an average advancement of 3.4 d per °C, similar to other highly sensitive groups – plants, freshwater phytoplankton and insects (Thackeray et al. 2016). A more recent meta-analysis of global responses estimated an average advancement of 3.23 d per decade for amphibians (Cohen et al. 2018). Similarly, a meta-analysis of amphibian breeding phenology found an average advancement of  $6.09 \pm 1.65$  d per decade (While and Uller 2014). In the northeast, Gibbs and Breisch (2001) found that four species of amphibians now initiate breeding calls 10–13 d earlier than a century earlier.

Although there has been a marked increase in annual average temperatures at our site over the past few decades, the magnitude of the shift varies across the season. Relative warming is greater later in the season while the least warming occurs in the late winter and early spring, coinciding with oviposition. Similarly, we see no evidence of annual change in snowpack, but we do see evidence of seasonal shifts, such that snow accumulates later and persists longer into spring by up to 27 d. The positive correlation between snow, precipitation and low temperatures may explain why snowpack was not ranked as highly important in our hindcast model. Later persisting snow may be driving the counterintuitive phenological delay if snow cover prevents adults wood frogs from emerging and migrating to ponds. Alternatively, snow cover over ponds may insulate the ice, delaying ice-out and preventing breeding, even if frogs emerged earlier. Temperature and precipitation can interact to increase the snowpack when precipitation falls during periods below freezing. However, temperature and precipitation can rapidly reduce snowpack when rain falls and melts snow. While snowpack is certainly an important factor in wood frog reproductive timing, the interaction between temperature and precipitation exhibits a stronger effect.

Our results indicate that phenology is closely tied to complex, intersecting changes in climate across the season. Furthermore, our results highlight the danger in using annual averages or coarse-grained, a priori selection of seasonal windows as predictors of phenology. We show that climatic changes can be heterogeneous within years with or without annual climatic change across years. Indeed, predictive frameworks that rely on mean annual rates of climatic change have come under criticism for masking potentially relevant asynchronous seasonal variation (Straile et al. 2015, Senner et al. 2018). The relative impact of seasonal variations in climate depends on the ecology of the species in question and how

climatic shifts across the season interact with seasonally correlated life-history traits, like phenology. Thus, relating fine-grained, seasonal changes in interacting climatic variables, to trait change, can help resolve idiosyncratic effects of climate change and better relate these unexpected departures to general trends (Muths et al. 2017, Kirk et al. 2019).

Although our study site likely represents a single metapopulation, variation in oviposition timing across ponds illustrates fine-grained spatial heterogeneity in responses to climate change (Senner et al. 2018). In our study, spatial difference in latitude between our ponds was an important variable in predicting oviposition timing. This variation is minimal at the scale of our study, but reflects similar variability at larger spatial scales (Sheridan et al. 2018). For example, Sheridan et al. (2018) tested for relationships between climatic variables and breeding phenology across the wood frog range from museum specimens dating back to 1901. They found that the day of first collection at a site (assumed to indicate breeding timing) was delayed in coastal Alaska and the coastal Northeast in association with either very large or very small proportional increases in frost free days, while average increases were associated with phenological advancement in breeding, indicating high spatial heterogeneity in wood frog responses to climate change. Examining phenological responses to climate change at small scales may help illuminate the processes driving range-wide patterns.

If organismal responses are cued by seasonal climates, variation in climate across space and time may result in life history mismatch. For instance, asymmetric shifts in climate during early life stages may delay or accelerate development and result in suboptimal timing for later life history events, like metamorphosis, reproduction or hibernation (Van Dyck et al. 2015, Hale et al. 2016). Similarly, trophic mismatches can result if predators and prey respond to cues at different times in the season (Straile et al. 2015). Spatial variability in climate cues may affect some populations more or less and could lead to changes in migration rates or source–sink dynamics. Local extinction may increase in ponds that change unfavorably while populations in ponds with favorable change could increase due to immigration or intrinsic population growth (Miller-Rushing et al. 2010). If phenological shifts driven by climate are maladaptive, selection may cause species to compensate with other mechanisms (Root et al. 2003, Hoffmann and Sgrò 2011, Merilä and Hendry 2014). For example, some species exhibit adaptive differences in physiological rates which are both plastic and genetic (Berven 1987, Conover and Present 1990). Thus, the impacts of climate change will vary considerably at temporal and spatial scales not typically considered in global predictions.

In conclusion, we demonstrate that, despite seasonal shifts in temperature that would generally predict advances in breeding phenology, we find the opposite trend of phenological delay, likely due to the interacting effect of more persistent snowpack. Our results belie the strong presumption that global warming will shift phenology in a particular direction and highlight the need to closely investigate the drivers of biotic change, even when gross patterns match expectations.

Otherwise, we may miss importance but unexpected mechanisms. Coupling long-term biological and climatological datasets at fine-spatial scales and temporal resolution aids our understanding of counterintuitive phenological patterns and can help generate predictions for future responses. The next step is to evaluate how shifts in phenology might translate into demographic differences. Such data are necessary to move beyond coarse estimates of species responses and start to generate more mechanistic models that make predictions based on biology rather than correlations (Urban et al. 2016). These insights will be critical for deciding which species to conserve and how to design conservation programs to mitigate the effects of climate change.

## Data availability statement

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.dv41ns1vs>> (Arietta et al. 2020).

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*Author contributions* – AA, LF, SB, AR, MU and DS contributed to data collection and field study. AA performed the analysis. DS conceived of the study. AA and DS wrote the manuscript with input from LF, AR and MU.

*Permits* – Animal handling was approved by Yale Univ. IACUC (2004-10361, 2007-10361, 2010-10361, 2013-10361, 2016-10361, 2019-10361, 2006-11024, 2009-11024, 2009-11040) and field surveys were conducted with permission from Yale Myers Research Committee (SKE01, AND17).

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Supplementary material (available online as Appendix ecog-05297 at <[www.ecography.org/appendix/ecog-05297](http://www.ecography.org/appendix/ecog-05297)>). Appendix 1.