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(Almost) no trend in brown frog (*Rana arvalis* and *Rana temporaria*) breeding phenology in southern Sweden 1990-2010

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Breeding phenology of *Rana temporaria* and *R. arvalis* was studied during 1990-2010 and analyzed for trends. Two approaches were used. One, that put equal weight on data from all years, found no trend for either species. The other approach gave more weight to those years when more ponds were studied. This found no trend for *R. temporaria* but a trend towards earlier breeding for *R. arvalis*. Both species tended to breed earlier in years with high February and March temperatures. These temperatures were both correlated to the NAO (North Atlantic Oscillation) index which thus in turn was negatively correlated to date of frog breeding. A lack of a long term trend could indicate no change in climate or that frogs are more governed by date than by spring temperature. The present study supports only the first hypothesis as there was no temperature trend during the study years.

INTRODUCTION

Phenology has always fascinated people (Ekström, 1826; Terhivuo, 1988). Recent findings and debate concerning global warming (Serreze *et al*., 2000) has given the subject further relevance (Hughes, 2000; Parmesan, 2006). Phenology data can serve as bioindicators, reflecting yearly variations in spring weather and trends. However, studying biotic trends, that are likely to be linked to climate, also helps us to understand what effects climate change may have in the future (Neveu, 2009). A much noted phenological phenomenon is the breeding of frogs in spring (Savage, 1961). Apart from the interest per se, this has been deemed relevant in the recent debate concerning amphibian declines (Carey & Alexander, 2003). The issue is particularly relevant for those amphibians that breed explosively early in spring in temperate areas (Richter-Boix *et al*., 2006; Saenz et al., 2006). This should make their response to climate change of particular interest, as warming in northern Europe has been documented during recent periods, e.g. 1966-1995 (Serreze *et al*., 2000). Other species have their breeding more timed to rainfall pulses (Jakob *et al*., 2003; Richter-Boix *et al*., 2006). For these, direct effects of climate warming are less intuitively obvious.

This study concerns the start of breeding for *Rana temporaria* Linnaeus, 1758 and *R. arvalis* Nilsson, 1842 in southern Sweden 1990-2010. The purpose is to respond to the question: Has there lately been a trend to earlier breeding in these species? I also analyse the connection of their phenology to some suspected proximal (spring temperature) and preproximal (NAO) causes.

MATERIALS AND METHODS

Since 1990, I have monitored breeding time for the common frog *Rana temporaria* and the moor frog *R. arvalis* in a varying number of ponds in southern Sweden (the province Scania). The furthest distance between ponds has been 60 km (fig. 1). Over the years, the number of ponds has varied between 43 and 84 (*R. temporaria*) and 7 and 46 (*R. arvalis*)

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Table 1. Number and turn-over of ponds used in the study. "*n*" is the number of ponds actually yielding data in the year. This does not include ponds visited but lacking breeding frog because of draught or for unknown reasons. "New" is number of ponds include in the study for the first time in the year. "Lost" are ponds visited for the last time the year before. There is no consistent relation between these three numbers because of missing data within the different ponds suites.

	Rana temporaria			*Rana arvalis*		
	n	New	Lost	*n*	New	Lost
1990	43			12		
1991	45	9	2	13	3	0
1992	56	12	3	15	5	2
1993	50	2	6	16	3	2
1994	49	30	24	13	8	7
1995	51	13	11	21	7	1
1996	66	16	0	28	10	0
1997	78	7	2	31	3	0
1998	77	2	0	38	4	0
1999	84	7	1	37	3	0
2000	81	1	3	41	4	0
2001	80	3	0	46	4	1
2002	77	1	3	42	0	4
2003	68	2	3	34	2	4
2004	70	0	3	39	1	2
2005	75	0	7	45	4	3
2006	36	1	31	19	0	21
2007	47	0	0	26	2	1
2008	44	0	4	21	1	7
2009	28	0	15	6	0	12
2010	30	0	0	7	0	2

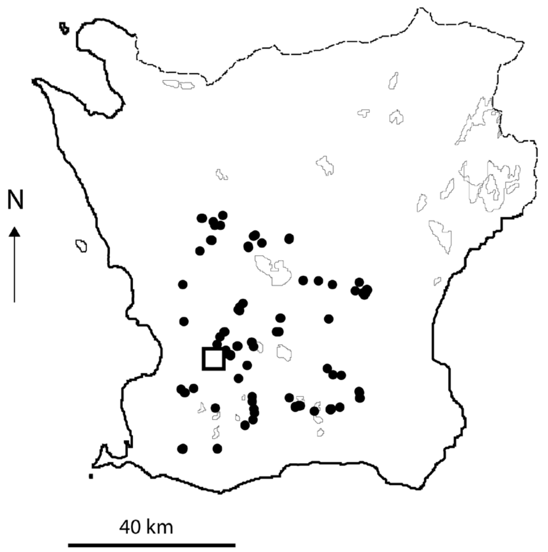

Figure 1. Map of south Sweden with the study ponds. All indicated ponds were not studied in all years. The meteorological station in Lund is shown as an open square.

(tab. 1). The average number has been 59 and 26, respectively. In the first few years the number was gradually increased. In 1994 and 1995 there was part shift of ponds when the financing of the project changed. In the last few years (when financing ceased) the number again decreased.

There is data for the full span of years 1990 to 2010 (with single years of missing data) for 8 ponds (*R. temporaria*) and 2 ponds (*R. arvalis*). Starting after the shift of pond focus, there is data from 1995 to 2005 for 45 and 20 ponds, respectively. Out of these, data continued until 2008 for 27 and 11, and until 2010 for 12 and 3 ponds, respectively. Missing data within ponds suites was usually because the pond was dry in that year so there was no breeding to record.

Within ponds, "breeding sites" were identified. A breeding site consists of all spawn with spawn clumps separated by no more than 1 m. Usually all clumps at a site were actually in physical contact. For each site and species (both species sometimes bred at a common site) the first date of breeding was recorded. This date usually was a good approximation of the time of breeding for all frogs because at any one site most frogs bred in the first two days (pers obs, Loman & Håkansson, 2004). Ponds were visited about every five days during the breeding period. Time for the earliest spawn at a site could therefore be extrapolated from the condition of the spawn at the time of visits. This can usually be done with a certainty of one or at most two days. Another reason for the frequent visits was the need to distinguish spawn from the two species, after more than five days this tends to be difficult.

Meteorological data were taken from the official station (Swedish Meteorological and Hydrological Institute) at Lund in the western part of the study area.

Analysis

For each pond, breeding time was computed as the average breeding time for all sites in the pond, weighted by the number of female frogs breeding (equal to number of spawn clumps) at each site. Thus the measure approximated the actual average breeding time for all frogs at each pond. For ponds with several breeding sites, this measure should be more stable than overall date (first breeding of any frog in the pond) which is usually what is recorded in frog phenology studies. Most ponds had only one or two breeding sites but the number was in some cases much larger. For *R. arvalis* the number of breeding sites per pond ranged from 1 to 28, with a median of 2 and for *R. temporaria* the corresponding numbers were 1, 22 and 2.

Looking for trends in breeding phenology sounds like a simple question to pose but the outcome of the analysis may differ depending on the method used. This is especially so with an unbalanced data set as the present. I thus provide alternative analyses.

Yearly breeding times (one data point per year, representing all ponds in the study) were calculated in two ways. (1) As a straightforward method, using all available data, I computed for each years the average breeding time for all ponds studied in that year, weighted by the number of frogs breeding at each pond.

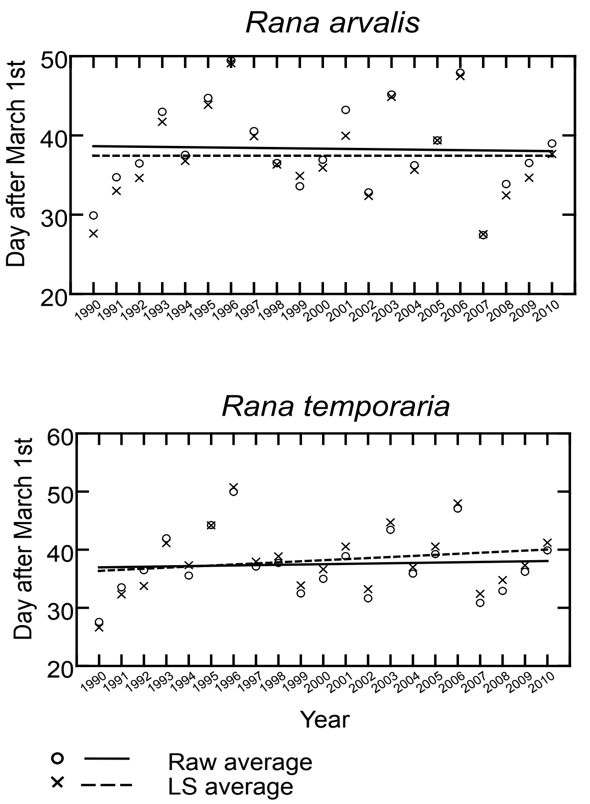

Figure 2. Average breeding time for *R. arvalis* and *R. temporaria* in 1990-2010. Slopes based on the two different measures (see Methods) are also shown.

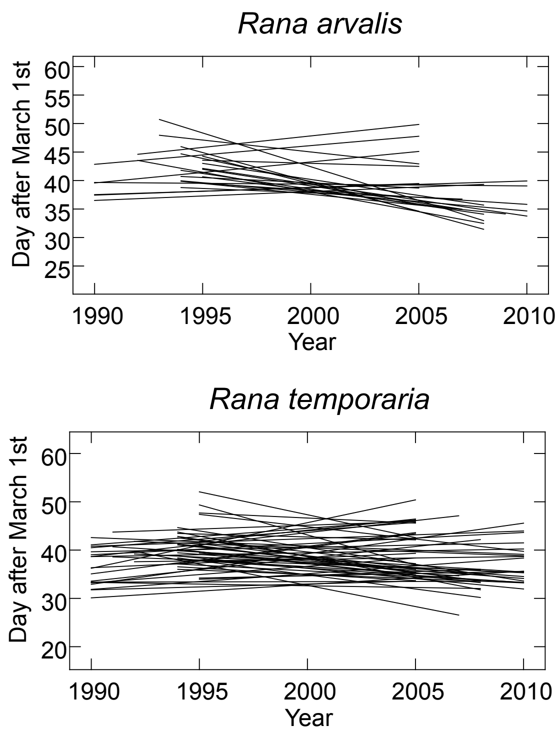

Figure 3. Pond specific regressions. All ponds studied from 1995 to 2005 (at least) are included. For details see methods.

This values suffered from the fact that different ponds were used in different years. (2) To base the yearly values on the same set of ponds I alternatively used a restricted pond set, only including those ponds monitored from (at least) 1995 to (at least) 2005 (but including all available years in the period 1990-2010 for each of these ponds). Even so, there were missing data for some pond by year combinations. To account for this, the ponds were subject to an ANOVA (with ponds and year as categories). Data was weighted by number of frogs breeding at a pond and the least square mean was used to represent each year.

The data was statistically evaluated for trends in two ways. (1a and 1b) I used the yearly estimates (two alternatives as explained above) to compute the Pearson correlation between breeding date and year number. (2) I also used the restricted set, described above, and performed an ANCOVA with ponds as categories and year as co-variate. In this case there was no weighting based on number of breeding frogs but each pond and year represented one data point. This analysis tested for year and pond effects on breeding date, accounting for the fact that different sets of ponds were used in different years (fig. 3).

NAO (North Atlantic Oscillation) is an atmospheric circulation pattern that has a profound impact on north western Europe winter temperature (Hurrell *et al.*, 2003). The impact on winter temperatures tends to be related to the pattern of low pressure cells over the North Atlantic. This can be characterized by an index. One commonly employed index is based on the difference between sea level pressure at Lisbon, Portugal and Reykjavik, Iceland (Hurrell *et al*., 2003; http://www.cgd.ucar.edu/cas/jhurrell/indices.html). This index is here used and labelled "NAO". Because air on the northern hemisphere circulates clockwise around high pressure cells, a high value of the index means that relatively warm westerly winds influence the west European winter. Contrary, a low, negative, value of the index is related to cold winter and a late spring.

RESULTS

Trends

There was no significant correlation between average breeding date and year. This was true for both species and for both raw means and least square means (tab. 2, fig. 2).

Also the approach using an ANCOVA failed to find any effect of year on breeding time for *R. temporaria* (df = 1:715, F = 0.26, P = 0.61) (fig. 3). However, there was a significant pond effect (df = 53:715, F = 4.03, P < 0.001). In contrast, for *R. arvalis* there was a significant year effect (df = 1:289, F = 8.41, P = 0.004) and also a pond effect (df = 23:289, F = 1.90, P = 0.009). The year effect for *R. arvalis* was negative; earlier breeding in the more recent years. In both ANCOVAs the interaction was not significant and removed before final analysis.

Table 2. Trends (correlation with year number) in average breeding date, and climate. Raw means are based on all ponds studied in the respective year, Least square means are from an ANOVA, accounting for the fact that different ponds were studied in different years. r is Pearson correlation coefficient. Temperatures are measured as the month's mean of average diel temperature. All n are 21 (number of years 1990-2010).

		r	P
Rana arvalis	Raw means	-0.03	0.88
	LS means	0.00	0.99
Rana temporaria	Raw means	0.06	0.79
	LS means	-0.21	0.37
Temperature	Feb	-0.22	0.33
	March	-0.25	0.28
	April	0.25	0.27
NAO		-0.48	0.029

Temperature effects

Both species tended to breed significantly earlier in years with warm February or March weather (tab. 3). This was also true for *R. arvalis* (but not *R. temporaria*) in relation to April weather. Also the NAO index was correlated to February and March, but not April, temperatures. NAO was correlated to breeding time of both species.

DISCUSSION

Interpretation

A likely global trend for warmer climate (Serreze *et al*., 2000; Jones *et al*., 2001), makes us expect a change for earlier breeding of temperate zone frogs. However, there was no clear support for this in the present data although one of the alternative analyses for *R. arvalis* did indeed suggest a change to earlier breeding. Possibly, the different outcome of this analysis (based on an ANCOVA) and the direct correlation between mean date and year is because the former included few ponds studied in the warm first years 1990, 1991 and in the cold years 2009, 2010. These years tended to push the trend towards a more positive slope (later breeding in recent years) for the analyses based on a direct correlation (that gave equal weight to all years) than for the alternative. Which analysis is more relevant is not obvious.

Table 3. Correlation between NAO index and temperature and between date of breeding and temperature. Temperature, raw means and LS means are explained in tab. 2. All *n* are 21 (number of years 1990-2010).

		NAO	*Rana arvalis*		*Rana temporaria*	
			Raw mean	LS mean	Raw mean	LS mean
February	*r*	0.60	-0.48	-0.51	-0.52	-0.54
	P	0.004	0.028	0.019	0.017	0.011
March	*r*	0.62	-0.84	-0.84	-0.82	-0.84
	P	0.003	< 0.001	< 0.001	< 0.001	< 0.001
April	*r*	0.22	-0.5	-0.48	-0.42	-0.34
	P	0.33	0.02	0.029	0.061	0.131
NAO	*r*		-0.48	-0.47	-0.5	-0.57
	P		0.028	0.032	0.02	0.007

Overall, there is no obvious trend in breeding date during the years 1990-2010. Both species tend to breed earlier in years with high March and February temperatures. Therefore the lack of a trend in breeding date is consistent with the lack of a significant trend in spring temperatures over the studied range of years. Actually, there was for February and March a non significant trend for cooler spring temperatures! In this context the meaningfulness of using "significance" can actually be discussed as we are not dealing with a sample.

It can be noted that during these years there has also been a trend to a lower value for the NAO index (tab. 2) and that there is a significant correlation between low values of this index and low temperatures in February and March (tab. 3).

The fact that breeding phenology is correlated to both February and March, but not clearly toApril, temperatures does not prove that two former both causally affects breeding. Because the NAO is mainly a winter phenomenon, there is a tendency that February and March temperatures (but not April) are correlated. What actually governs frog breeding is outside the scope of this report. In the present context it is sufficient to say that winter and/or spring temperatures seem to be involved and for this reason breeding of these two frogs is a good index of NAO.

The usefulness of frog breeding trends as an indicator of climate change is based on an assumed relation between frog breeding and spring temperature. A possible alternative could have been that the breeding of frogs more strongly linked to calendar date. In the present study there was indeed a correlation between breeding and temperature. This has also been documented by others in *Bufo bufo* (Linnaeus, 1758) (Reading, 1998; Tryjanowski et al., 2003 and *R. temporaria*; Tryjanowski *et al*., 2003; Neveu, 2009). Also the correlation between NAO and R.

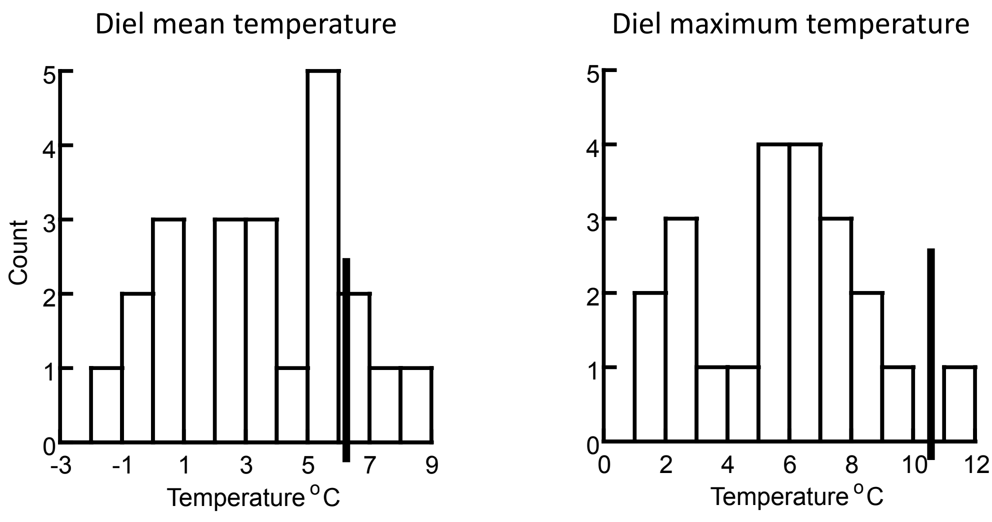

Figure 4. High February temperatures. For each of the 21 years (1990-2010) I have calculated the diel mean and diel maximum temperature for the warmest 7-day period. The temperature distribution of these weeks is shown in the histogram. The thick bar indicates the average (for all 15*22 days) temperature during the period 1-15 April (main frog breeding period) during these years.

temporaria phenology found has previously been noted by Forchhammer *et al*. (1998) but not by Neveu (2009). However, it is not certain one can extrapolate this pattern in time. Also in the present climate, periods with high February temperatures do occur (fig. 4). However, early breeding in February would be very risky because of the possibility of later freezing temperatures, killing laid eggs (Loman & Håkanssons, 2004). This may be the reason why *R. temporaria* and *R. arvalis* are not know to breed before the middle of March in this area, south Sweden. In contrast, *R. temporaria* is know to occasionally breed even in late autumn in southwest England (Chadwick, 2003). If this reasoning is true, frog breeding phenology in south Sweden may not correlate to a warm spring outside the present time span (middle of March and later) and it is not certain that a warmer climate will automatically result in a correspondingly earlier frog breeding. This suggestion does not take evolutionary responses into account.

Other frog phenology studies

Analysing frog phenology is by no means a novel idea. Several long-term studies of frog breeding phenology have been presented. The by far longest published is from Finland where the breeding times for *R. temporaria* during the years 1846 (!) to 1986 all over the country were compiled and analysed by Terhivuo (1988).

Another long term data set from central Finland holds data from 1952 to 2005 (Lappalainen *et al*., 2008). Gibbs and Breisch (2001) compared the breeding times of six frog species in New York, USA during 1900-1912 with that during 1990-1999. For Finnish *R. temporaria* (both studies) and for 4 of the 6 American species there was a tendency for earlier breeding in later years. None was breeding later. Other studies over shorter time intervals have given more mixed evidence. Out of four frog species in North America, studied for various time intervals within the period 1967-1999, Blaustein *et al.* (2001) only found evidence of earlier breeding in one. However, two out of three English frogs studied by Beebee (1995) tended to earlier breeding during 1978-1994. The exception was *R. temporaria*. A more recent study however suggests a trend to earlier breeding since appr. 1995 also for this species (Scott *et al*., 2008). Also, both species (*R. temporaria* and *Bufo bufo*) studied 1978-2002 by Tryjanovski (2003) in Poland tended to breed earlier during later years. However, a later analysis of these data, including three more years (1978-2005), failed to detect a trend for *R. temporaria* (Sparks *et al*., 2007). Also, *B. bufo* studied by Reading (2003) in England 1980-2001 did not show any trend for earlier breeding. Most of these studies also analyse the timing of breeding in relation to spring temperature in individual years; the general picture does suggest a correlation between high spring temperatures and early breeding. The evidence from frog phenology for a trend to warmer springs is thus mixed. However, it should be noted that although several studies fail to find a trend to earlier breeding, several do find it and none finds a trend to later later.

Evaluation

The outcome of this study and the very mixed pattern found by earlier studies of temperate frog breeding phenology stress that these trends, even if when based on substantial number of years, are dependent on the area and the actual suite of years studied. Climate change, if present, does certainly not proceed as an even trend or is uniform over large areas. The obvious relation between frog breeding in this particular area and the NAO temporal patterns also stress that regional trends are not necessarily strongly correlated to global trends. Effects of global warming on spring phenology do not simply mean that everything takes place earlier. The effects are particular to the systems studied and to different regions.

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