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## Is body size of the water frog *Rana esculenta* complex responding to climate change?

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**Abstract** Recent studies on climate responses in ectothermic (cold-blooded) vertebrates have been few in number and focussed on phenology rather than morphology. According to Bergmann's rule, endothermic (warm-blooded) vertebrates from cooler climates tend to be larger than congeners from warmer regions. Although amphibians are ectothermic vertebrates, weather and climatic conditions may also impact on their morphology, and thereby affect their survival rates and population dynamics. In this paper, we show, in a unique long-term study during the period 1963–2003 in an agricultural landscape in western Poland, that the body length of two water frog parental species (males of both *Rana ridibunda* and *R. lessonae*) increased significantly. However, their hybridogenetic hybrid *R. esculenta* did not show similar changes. A significant relationship with a large-scale climatic factor, the winter North Atlantic Oscillation index, was found positive for *R. ridibunda* males and *R. lessonae* females, and negative for *R. esculenta* females. Our findings, the first for amphibians, are consistent with other studies reporting that recent climate change has affected the morphology of animals. However, we also show that changes in amphibian phenotype linked to climate may vary independently between (even very similar) species.

### Introduction

Recently, it has been suggested that many observed changes in animal populations result from global warming (Yom-Tov 2001; and review in Walther et al. 2002). Such changes have occurred not only in classical demographical studies of population size and productivity, but also in the timing of breeding and in the morphology of individuals within a population. To date, the majority of studies on climatic influence on animals has been undertaken on birds and mammals, and studies on amphibians are scarce (Laugen et al. 2005; Walther et al. 2002). However, among the various factors affecting global amphibian decline, climatic changes have been identified as having particular importance (Carey and Alexander 2003; Kiesecker et al. 2001). In amphibians, climate mainly affects breeding phenology, change in habitat suitability, spread of infectious diseases and perhaps population size (Kiesecker et al. 2001; Walther et al. 2002). Studies from birds and mammals show that body size and body condition can also fluctuate with large-scale climatological events (Grant and Grant 2002; Post et al. 1997). We suspect a similar reaction in amphibians (for review, see Laugen et al. 2005). Because amphibians are sedentary, ectothermic vertebrates they may be even more sensitive to climatic change than birds and/or mammals (Bull and Burzacott 2002; Tryjanowski et al. 2003). Previous studies on amphibians in the context of climate change focussed mainly on the aquatic phenological phases of their life cycle (Carey and Alexander 2003; Walther et al. 2002). However, new models have predicted that the terrestrial phase of the life cycle may be even more important for population dynamics and survival, but data to confirm or refute these models are scarce Biek et al. (2002).

In this paper, we studied the three taxa of water frogs (*Rana esculenta* complex) living in Central Europe. This complex consists of the widespread and common European edible frog *Rana esculenta*, a natural hybridogenetic fertile hybrid (see below) between the other two, the marsh frog *Rana ridibunda* and the pool frog *Rana lessonae* (Berger 1973b; Rybacki and Berger 1994). The taxa differ in their ecological niches (Berger and Rybacki 1998; Rybacki and

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Berger 1994). *R. ridibunda* inhabits large water bodies in which it hibernates, whereas *R. lessonae* lives in small water bodies and hibernates on land. The hybrid *R. esculenta* inhabits all types of water body and hibernates either in water or on land (Berger and Rybacki 1998; Rybacki and Berger 1994). *R. esculenta* reproduces by hybridogenesis, resulting in their gametes normally containing unrecombined genomes of one parental species (*ridibunda* or *lessonae*; Uzzel et al. 1977) and thus reproduce only hemiclonally (Berger 1973b; Uzzel et al. 1977).

To test the links between phenotype and climate indices, good long-term data are required, and analysis of such data can sometimes explain previously unpredictable evolutionary changes. The main aim of this paper is to 1) describe the changes in body size of two parental species of water frogs and their natural hybrid during a 40-year period, 2) identify possible relationships between morphological and climatological data and 3) answer the question: do hybrids follow parental species in their morphological changes? Finally, we discuss the potential implications of our findings for the evolution of water frogs.

## Materials and methods

**Study area and data sources** European water frogs were studied during 1963–2003 in the Wielkopolska region of western Poland (52° N, 16° E). This is a region of typical farmland, where arable fields occupy nearly 70% of the area. The study taxa are among the most common and most numerous amphibians, both in the study area (ponds often contain hundreds of individuals) and in Poland, in general (Rybacki and Berger 1994). The climate of the region is characteristic of central Europe where two climate types, oceanic and continental, meet. The mean annual temperature is approximately 8°C (sub-zero mean monthly temperatures occur in December–February) with mean annual precipitation approximately 550 mm.

The North Atlantic Oscillation (NAO; Stenseth et al. 2003) measures a standardised pressure difference between the Azores and Iceland. A winter index was calculated as the mean of monthly values for December–March. A high value suggesting milder and wetter winter weather and has proved useful in other studies (e.g., Stenseth et al. 2003).

**Morphological variables** Frogs were caught by hand and net from May through September in water bodies (in total, 1,026 *R. lessonae*, 505 *R. ridibunda* and 1,603 *R. esculenta*) and measured (snout-vent length, SVL) with callipers to an accuracy of 0.1 mm (Berger 1966). In the analysis, we used only measurements of frogs that were sexually mature (Berger 1966; Berger 1973a).

**Statistical analysis** All analyses were undertaken on annual mean values weighted by sample size. Changes in time were investigated by regressing mean annual SVL on year or NAO. Differences in SVL and in changes in SVL between sexes of the same taxa (and changes between taxa of the same sex) were examined in ANCOVA, with year (or NAO) treated as a covariate and either sex or taxa as a factor plus an interaction term between covariate and factor. Because data were not collected in all years, and because individuals of all three taxa were not always caught in the same year, sample sizes differ slightly between analyses. All statistical analyses were conducted using MINITAB version 13 and SPSS for Windows packages with  $p=0.05$  used as a threshold for significance testing.

## Results

Body length of males of both *R. ridibunda* and *R. lessonae* increased significantly between 1963 and 2003 (Table 1). No such change was apparent in the males of *R. esculenta*. Estimated increases in body length for *R. ridibunda* and *R. lessonae* during this period ranged from 6 to 10%, respectively, whilst a (non-significant) decrease of 10% was estimated for female *R. esculenta*. There was a significant difference between taxa for both females ( $F_{2,64}=5.13$ ,  $p=0.009$ ) and males ( $F_{2,61}=4.46$ ,  $p=0.016$ ). There were no significant differences in changes between the sexes for any taxa (Table 1).

Winter NAO increased significantly ( $F_{1,39}=5.72$ ,  $p=0.022$ ) during the study period. The results of weighted regression between body length and NAO are reported in Table 2. There was a significant positive relationship for *R. ridibunda* males and *R. lessonae* females and a significant negative relationship for *R. esculenta* females (Fig. 1). Relationships between body length and NAO between taxa

**Table 1** Mean body lengths in the three frogs and change in body length (SVL) through time (mm/year), together with an assessment of the significance in body size and change in body size between sexes of the same taxa

		SVL		Change		<i>p</i>	Significance of difference between sexes in	
		<i>n</i> years	Mean	SE	<i>b</i> (SE)		size	change in size
<i>R. ridibunda</i>	♀	22	87.35	1.78	0.223 (0.108)	0.051	$F_{1,39}=14.79$ , $p<0.001$	$F_{1,37}=0.07$ , $p=0.792$
<i>R. ridibunda</i>	♂	19	80.53	1.22	0.189 (0.059)	0.005		
<i>R. lessonae</i>	♀	24	64.49	0.95	0.098 (0.066)	0.154	$F_{1,45}=24.37$ , $p<0.001$	$F_{1,43}=0.05$ , $p=0.822$
<i>R. lessonae</i>	♂	23	58.35	1.19	0.117 (0.056)	0.049		
<i>R. esculenta</i>	♀	24	77.41	1.27	-0.180 (0.101)	0.089	$F_{1,47}=34.72$ , $p<0.001$	$F_{1,45}=1.36$ , $p=0.250$
<i>R. esculenta</i>	♂	25	69.09	0.77	-0.037 (0.052)	0.493		

**Table 2** Regression of mean body lengths of the three frogs in relation to the winter NAO index, together with an assessment of the significance (from ANCOVA) of the difference between sexes of the same taxa

		Regression coefficient		<i>p</i>	<i>R</i> <sup>2</sup>	Significance ANCOVA
		<i>b</i>	SE			
<i>R. ridibunda</i>	♀	0.92	1.35	0.501	2.3	$F_{1,37}=0.31, p=0.58$
<i>R. ridibunda</i>	♂	1.78	0.68	0.018	28.8	
<i>R. lessonae</i>	♀	1.86	0.69	0.013	24.9	$F_{1,43}=1.86, p=0.18$
<i>R. lessonae</i>	♂	0.63	0.58	0.291	5.3	
<i>R. esculenta</i>	♀	-2.18	0.95	0.032	19.3	$F_{1,45}=1.53, p=0.22$
<i>R. esculenta</i>	♂	-0.76	0.48	0.127	9.8	

All analyses are based on annual mean values weighted by sample size

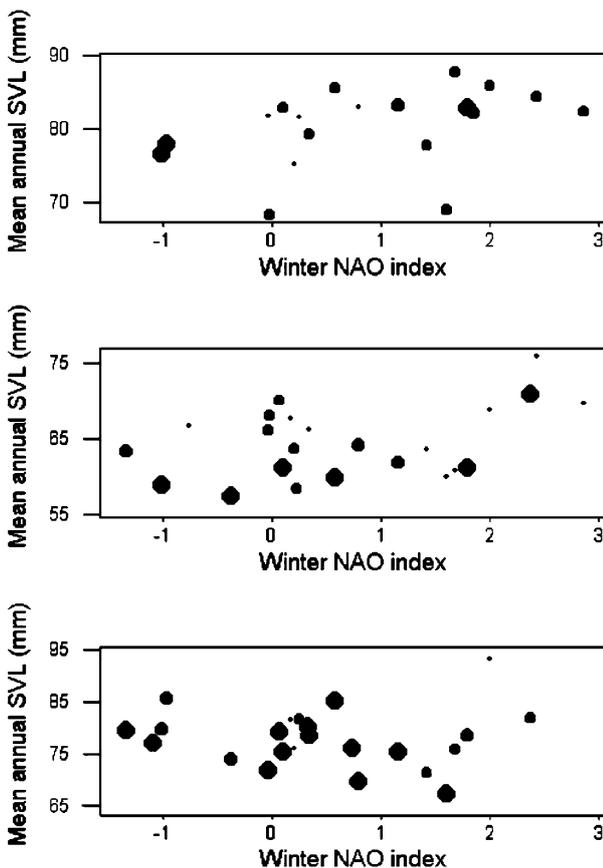
were significantly different for both females ( $F_{2,64}=4.84, p=0.011$ ) and males ( $F_{2,61}=4.91, p=0.011$ ). The non-significant differences in change between sexes within taxa paralleled that of their opposite gender (Table 2).

## Discussion

Body size is one of the key phenotypic traits of animals. It has a profound influence on their morphology, physiology

and ecology (Schmidt-Nielsen 1984; Yom-Tov 2001). This is especially true in endotherms whose basal metabolic rate is related to body mass (Schmidt-Nielsen 1984). On the other hand, data on ectotherms, including amphibians, are generally scarce and there is still a lot of controversy on how changes in temperature could affect amphibian body size (Laugen et al. 2005). It is well-known that body size of amphibians is related to age, population density and effects of predators via survival (Berger 1973a,b; Denton and Beebee 1993). Some of the results found here hover on either side of statistical significance and, in variable taxa such as frogs, can only be confirmed or refuted by additional years of data or larger sample sizes. Despite this, we report that, in a unique long-term study in Poland over four decades, the body length of two water frog species (*R. ridibunda* and *R. lessonae*) increased. However, we did not find a similar directional change in their closely related, hemiclinal hybrid *R. esculenta*. Indeed, there was some suggestion, although not significant, of an opposite change in *R. esculenta* females. The fitness consequences of such changes are not known. We should, therefore, consider two important questions: what is the cause of change in body size of water frogs in the study area and why did the hybrid species change differently from the parental species?

In the study area, all three taxa of water frogs are subject to the same environmental change, although we do not have appropriate data to investigate population density effects (Berger and Rybacki 1998). In terms of ecology, differences in selective factors (breeding habitats, hibernation sites, food resources) vary only between the two parental species (Berger and Rybacki 1998; Lodé 2001; Rybacki and Berger 1994). One reason for an increase in body size may be that after mild winters, there are more insect preys which allow improved growth (Gotthard 2001). If *R. esculenta* tadpoles coexist with the tadpoles of their parental species in good ecological conditions, they metamorphose much earlier and reach a larger body size (70–110 mm as adults). On the other hand, *R. esculenta* tadpoles, in contrast to tadpoles of *R. ridibunda* and *R. lessonae*, are extremely sensitive to poor ecological conditions (Berger 1973a). In poor conditions, their development and growth is slow resulting in a small body size (50–70 mm as adults), i.e., very similar to individuals of *R. lessonae*. However, the studied frogs



**Fig. 1** The relationships between body length and winter NAO for (top) *R. ridibunda* males, (middle) *R. lessonae* females and (bottom) *R. esculenta* females. Increasing symbol size represents sample sizes of 1–5, 6–10, 10–20 and >20 individuals, respectively

differed in their relationship between body size and climatic factors not only between taxa but also between genders. The latter may be driven by a larger investment in reproduction by females. Therefore, we suspect that factors other than environmental ones can play a role in establishing differentiation. The first possible mechanism promoting differences in body size of the hybrid are potential changes in mating strategy. This might involve simply changing to *R. lessonae* or *R. ridibunda* partners in relation, for example, to climatological events (Negovetic et al. 2001) and/or to parental species density. Hemiclonal hybridogenesis constitutes an evolutionary challenge because the hybrids are not able to survive without the use of the non-*ridibunda* genotype. Hybrid $\times$ hybrid matings usually produce *ridibunda* offspring that are not viable. To have successful reproduction and survive, hybrid males should mate with non-*ridibunda* females. However, *R. esculenta* females tend to prefer *R. lessonae* males (Abt and Reyer 1993; Lodé 2001), because females prefer smaller males in the copulation phase (*amplexus*). Therefore, if males of both parental species become larger, the probability of choosing the smaller species (*R. lessonae*) should increase over time. This may explain the contrast in changes in body length between *R. esculenta* and the parental species. In addition, the difference between breeding population patterns has been suggested to be independent for the hybrid and the one parental species (*R. lessonae*) that occurs in Switzerland (Som et al. 2000). The results reported in our paper show strong evidence for differential change in morphology that is driven, at least in part, by large scale climatic change. We do not have the opportunity to identify whether the observed changes in the studied frog taxa result from phenotypical plasticity and/or microevolutionary changes. Previous studies on frogs suggested that both possibilities may occur (Laugen et al. 2005; Tryjanowski et al. 2003), and without experimentation they are not easy to separate (Møller and Merilä 2004). Nevertheless, our findings of body size changes over time may provide opportunities to more fully understand problems of amphibian conservation in the light of rapid climate change.

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