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Migratory behaviour during autumn and hibernation site selection in common frogs (*Rana temporaria*) at high altitude

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Aquatic and terrestrial hibernation have both been reported for *Rana temporaria*, but it is as yet unclear which factors influence hibernation site choice. To investigate the hibernation behaviour of common frogs in alpine regions, we fitted 15 adult *R. temporaria* with radio transmitters in September 2011, and located them once a week over a 10 week period to their hibernation sites. The mean distance animals travelled between relocations was 19.4 m (SE=4.1). There was no difference in migratory behaviour between males and females. Distance moved between relocations was significantly influenced by season. Aquatic hibernation prevailed in our study population. One frog hibernated in a pond, seven in streams or springs and none in terrestrial microhabitats. Our results suggest that the onset of hibernation in common frogs is regulated by a combination of seasonal factors rather than temperature alone.

Key words: amphibians, hibernation site selection, migratory behaviour, radio telemetry, *Rana temporaria*

INTRODUCTION

The common frog (*Rana temporaria*) is a widely distributed European amphibian (Gasc et al., 1997). In large parts of its distribution, air temperature drops below zero for prolonged periods of time during winter. Since the body temperature of ectotherms depends largely on ambient temperature, exposure to sub-zero temperatures during hibernation bears the risks of lethal cell damage by ice crystals. In general, amphibians have evolved three ways to cope with this problem (Pinder et al., 1992): (i) freezing tolerance of the body fluids like for example in the North American wood frog *Lithobathes [Rana] sylvaticus* (Storey & Storey, 1984); (ii) production of cryoprotectants to prevent cells from freezing (Storey & Storey, 1988) and (iii) choice of hibernation sites where temperatures do not drop below zero, for example underwater (Boutilier et al., 2000) or in burrows beneath the ground (e.g., *Epidalea [Bufo] calamita* and *Pseudepidalea [Bufo] viridis*, Sinsch & Leskovar, 2011). It is unknown which of these strategies common frogs use to hibernate in alpine areas. Across the species' distribution range, both aquatic (ponds: Koskela & Pasanen, 1974; rivers: Elmberg, 1990; wells: Hagström, 1982) and terrestrial hibernation (wintering dens of snakes: Viitanen, 1965; under logs: Pasanen & Sorjonen, 1994) have been reported, and individuals have been found hibernating alone as well as in groups. Common frogs are also able to tolerate mild sub-zero temperatures by using glucose as a cryoprotectant (Pasanen & Sorjonen, 1994; Voituron et al., 2009). They are also able to meet their metabolic oxygen demand during hibernation purely through cutaneous respiration (Tattersall & Ultsch, 2008; Jackson & Ultsch, 2010).

Severe climatic conditions during hibernation at high altitudes present logistical and physiological challenges. Hibernation lasts 6–8 months compared to 3–4 months at low elevation (Laugen et al., 2003; Ryser, 1996). Low sub-zero temperatures result in the complete freezing of shallow ponds (Pasanen & Sorjonen, 1994), the freezing over of streams and permanent snow cover throughout

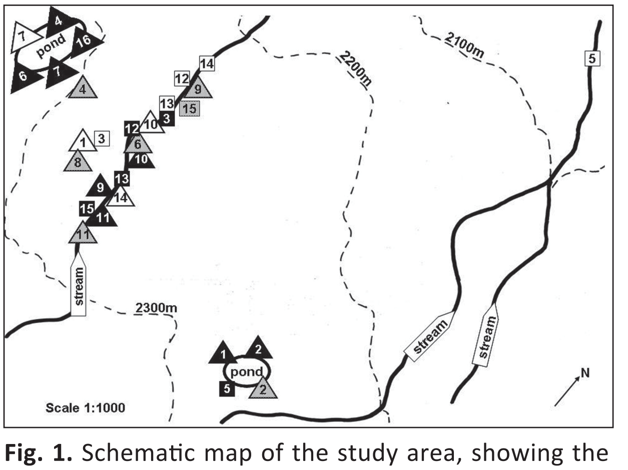

Fig. 1. Schematic map of the study area, showing the approximate locations of first capture (filled symbols) and hibernation site (open symbols) and last location of animals that perished during the study (shaded symbols). Triangles represent males, squares represent females.

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most of the hibernation period. To avoid getting trapped in ice or snow, timely migration to a hibernation site is crucial, and hibernation sites must be frost-resistant and buffered against fluctuations of ambient temperatures. To obtain insights into the hibernation behaviour of common frogs at high altitudes, we fitted adults with radio transmitters in autumn and tracked them to their hibernation sites.

MATERIALS AND METHODS

The study was conducted in an alpine basin in the Fotsch Valley in the northern Stubai Alps, Austria (11°12'535"E, 47°08'587"N) at an elevation of 2300 m (Fig. 1). The vegetation is dominated by alpine grassland, dwarf shrubs, moss and lichens as well as swampy areas. Several small lakes and ephemeral ponds serve as breeding habitats for a large population of common frogs. Based on egg mass counts during the 2011 and 2012 breeding periods, the effective population size was estimated to be around 700 adults. Alpine newts (*Ichthyosaura [Triturus] alpestris*) are the only other amphibian species occurring in this area.

We attached radio transmitters (Holohil Systems Ltd, BD-2H, 17 x 8 x 4mm, 3.4g) to 15 adult *R. temporaria* to record their movements (line-of-sight distances between two successive locations) between September 11 and November 28, 2011. In September, 7 adult frogs (1 female, 6 males) were retrieved from the two main breeding ponds, and a further 8 frogs (5 females, 3 males) were found along nearby streams. Transmitters were attached externally with a waistband of silicone rubber tubing. A fine cotton thread was passed through the tubing and tied to fix the package to the frog (Rowley & Alford, 2007). After fitting the transmitters in the field, frogs were released at the point of capture and relocated once per week using a receiver (Followit, RX 98) and a hand-held three elements Yagi antenna. When frogs were relocated we recorded their position using GPS (Garmin, eTrex).

We used the X-Y latitude-longitude GPS data points to calculate the line-of-sight distance each frog had travelled between two observations using the Haversine formula (Sinnott, 1984). Distribution of distances was normalized by $\log_{10}$ transformation. Records from a nearby weather station run by the Tyrolean Avalanche Advisory Service were used to calculate average temperatures between two successive relocations. Since there was no significant difference between the average distance travelled by males and females (one-way ANOVA $F_{1,81}$=0.007; p=0.935), all data were pooled and the effect of temperature and other seasonal changes on the distance moved was estimated using multiple linear regression models. To account for general seasonal changes we numbered the weeks of the study period from 1 to 10 and included it in the regression model as the independent variable "week". Significance was accepted for p<0.05. The software package PASW Statistics v.18 (SPSS, Chicago) was used for all analyses.

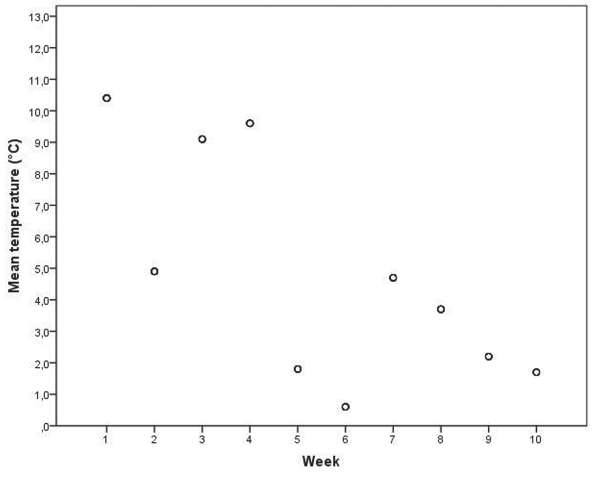

Fig. 2. Weekly mean temperature (°C) plotted against the week of study, beginning on September 11 and ending on November 28, 2012.

RESULTS

The mean distance frogs covered during seven days was 19.4 m (SE=4.1). In week 8, female #5 was found 442.2 m downstream from her previous location (Fig. 1). Since she had to pass over a 100 m high escarpment, we believe that she was carried their by the stream rather actively moved there and thus excluded this data point from all further analyses. Average weekly temperatures ranged from 0.6 to 10.4°C (Fig. 2). The multiple regression analysis yielded a significant model ($F_{2,80}$=6.773, p=0.002, adjusted r^2=0.123) including two variables, with "week" having a significant negative effect on the distance moved (Table 1). However, there was a strong intercorrelation between temperature and week, reflecting the dependence of temperature on season.

The overall mortality rate was 40%. One male released in the pond died halfway between the pond and the stream due to unknown reasons. One female of the stream group also died due to unknown reasons. One male was trapped and killed in pond ice when the pond froze in mid-November, and three males of the stream group were preyed upon by an unknown predator between November 24 and October 6, with their transmitters found in a deserted marmot cave. One transmitter had to be removed from a male after seven weeks, when the antenna was tangled up in grass. None of the frogs lost their transmitters during the study period.

By November 28 all frogs stopped moving, and we assumed that they had reached their hibernation habitat and ceased telemetry work. Aquatic hibernation

Table 1. Multiple regression model relating common frog movement to temperature and seasonal changes during the study period (B=unstandardized coefficients, β=standardized coefficients, *p*=probability value).

Source of variation	B	β	*p*
Intercept	1.694		
Week	-0.111	-0.524	0.001
Mean temperature	-0.045	-0.270	0.072

in streams prevailed. Out of the seven frogs originally found in ponds, three frogs (1 female, 2 males) migrated to the stream (Fig. 1). One male moved from the pond to a small spring, and one male remained in the pond. The radio-tracked frogs found along the stream stayed close to or in the stream while active. Five individuals (4 females, 1 male) also hibernated in the stream and one female moved into a nearby spring towards the end of the study period. Since it was impossible to access hibernacula without destroying them or disturbing frogs, transmitters were retrieved the following spring whenever possible.

DISCUSSION

The distance covered by radio-tracked common frogs between two observations was best predicted by week of the study period. This suggests that factors such as photoperiod or availability of food might play a major role in triggering the onset of hibernation in *R. temporaria*. Despite a large number of laboratory studies on metabolic and physiological changes to thermal acclimatization in anurans (e.g., Feder, 1982; Blem et al., 1986; Dunlap, 1980; 1989; Sinsch, 1991), it remains difficult to assess the actual impact of photoperiod on the occurring physiological changes. However, studies on reptiles have demonstrated the influence of seasonal factors other than temperature on the onset of hibernation. In the horned lizard (*Phrynosoma mcallii*), the onset of hibernation seems controlled by photoperiod rather than temperature (Mayhew, 1965), and in garter snakes (*Thamnophis sirtalis parietalis)* physiological changes seem to be controlled by a combination of endogenous and exogenous seasonal factors (Hoskins & Aleksiuk, 1973; Joy & Crews, 1986).

The high mortality of tagged frogs might be due to the external attachment of transmitters. Although less invasive and easier to accomplish than implantation, it cannot be excluded that the external attachment might reduce the animal's general ability to move and to escape predators. Moreover, it also bears the risk of the antenna getting tangled up in vegetation, and the visibility of tagged frogs to potential predators is increased. The implantation of transmitters (Eggert, 2002; Johnson, 2006; Sinsch et al., 2012) should be considered in future studies.

Although aquatic as well as terrestrial hibernation has been previously reported for common frogs (Koskela & Pasanen, 1974; Hagström, 1982; Pasanen & Sorjonen, 1994), we show that in an alpine population aquatic hibernation prevails and that frogs prefer streams over bodies of standing water. These results agree with the findings of Elmberg (1990), who observed common frogs in Sweden hibernating in rivers and migrating to breeding ponds in spring. Therefore, ambient constraints for hibernation in northern latitudes and high altitudes seem to be similar. There might be several factors favouring aquatic hibernation in common frogs. Since *R. temporaria* has only limited abilities to tolerate temperatures below zero (Voituron et al., 2009), they need to hibernate in frost- free sites. However, in terrestrial habitats without distinct layers of leaves and woody debris, such as in our study area, it might be difficult to find safe hibernacula. A constant low water temperature allows frogs to keep their metabolic rate at a consistent reduced level, which might be crucial for their overall energy balance (Tattersall & Ultsch, 2008; Jackson & Ultsch, 2010).

Frogs may prefer lotic over lentic water for hibernation because hibernating in lentic water bares the risk of low oxygen concentration or complete oxygen depletion during winter. Although common frogs show metabolic adaptations to deal with low oxygen levels, it is unknown if and to which extent hypoxic conditions influence their metabolic activity and overwinter survival (Tattersall & Ultsch, 2008; Jackson & Ultsch, 2010). Smaller ponds bear the risk of completely freezing, whereas streams exhibit permanent normoxic conditions. However, to estimate the impact of environmental factors on hibernation site selection, hibernation behaviour needs to be observed over several years and in several populations at sites with differential habitat characteristics.

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