Hyla *VOL. 2015., No.1, Str. 28 - 35*
ISSN: 1848-2007

Original Scientific Paper
Kleewein 2015

Investigating temperature tolerance in wild broods of *Trachemys scripta elegans* (Reptilia: Testudines: Emydidae) in Austria

Istraživanje temperaturne tolerance u divljim populacijama *Trachemys scripta elegans* (Reptilia: Testudines: Emydidae) u Austriji

ANDREAS KLEEWEIN

University of Vienna, Department of Integrative Zoology, Althanstraße 14, A-1090 Vienna, Austria, andreas.kleewein@gmx.net

Abstract

Reproduction of the freshwater chelonian, the red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied, 1836), has been documented numerous times in the wild in Europe. The aim of this work was to show the temperature tolerance in wild broods of *T. s. elegans* in Austria. In the Austrian province of Carinthia, this allochthonous subspecies displays a huge tolerance regarding clutch temperature. Clutch temperature was measured in half-hour intervals during the entire incubation period of 118 days. The greatest variation in temperature in a single day ranged from 19.3°C to 37.1°C: a shift of 17.8°C. The maximum temperature reached during the entire incubation period was 38.7°C, the minimum temperature was 11.5°C. Average temperature was 22.5°C. Shell dimensions and weight of hatchlings were low. This indicates a negative influence of the extreme temperatures in clutches that are incubated under natural conditions in Austria. Nevertheless, broods in the wild are to be expected much more frequently in Europe than hitherto assumed.

Key words: Reproduction, clutch temperature, temperature variation, incubation period

Sažetak

Razmnožavanje slatkovodne crvenouhe kornjače, *Trachemys scripta elegans* (Wied-Nuwied, 1836) u divljini u Europi je već zabilježeno nebrojeno puta. Cilj ovog rada je prikazati temperaturnu tolerancu divljih populacija *T. s. elegans* u Austriji. Ta alohtona podvrsta pokazuje veliku tolerancu po pitanju temperature legal u pokrajini Koruškoj u Austriji. Temperatura legla je mjerena u 30-minutnim intervalima kroz cijeli period inkubacije od 118 dana. Najveća razlika temperature u pojedinom danu je bila od 19,3°C do 32,1°C: razlika od 17,8°C. Najviša zabilježena temperature je bila 38,7°C, a najniža 11,5°C. Prosječna temperature je bila 22,5°C. Dimenzije oklopa i masa mladunaca su bile male. To ukazuje na nepovoljan utjecaj temperaturnih ekstrema u leglima u austrijskoj prirodi. Unatoč tome, očekuje se povećanje učestalosti legala u Europi nego što je do sada bilo pretpostavljano.

Ključne riječi: Razmnožavanje, temperatura legla, temperaturna varijacija, trajanje inkubacije

INTRODUCTION

The red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied, 1836), is among the most frequently occurring allochthonous turtle species in the world. The Global Invasive Species Database regards it as one of the "100 of the world's worst invasive alien species" (van Dijk et al. 2013).

This led to an import ban on the trade of this species within the European Union in 1997. Reports of successful reproduction in the wild in various European countries have confirmed that the species is able to reproduce outside of its natural range (Girondot et al. 2012, Vamberger et al. 2012, Kleewein 2014). The majority of sites with released turtles in Austria have been recorded in the province of Carinthia. A comprehensive survey revealed 47 bodies of water in which allochthonous turtles occur (Kleewein 2007). Reproduction in the wild in Carinthia is also already known (Gutleb & Happ 2002, Kleewein 2014).

For many years, the Happ reptile zoo in Klagenfurt has been receiving allochthonous turtles that have largely been found in the wild. The zoo has a dedicated pond for accommodating these animals, providing the turtles with near-natural conditions.

MATERIAL AND METHODS

The outdoor turtle pond of the Happ reptile zoo in Klagenfurt was chosen for data acquisition, because male and female animals are kept here together. Oviposition occurs regularly at an oviposition site made up of an earth-sand mix. The study site is situated in the Klagenfurt basin (E: 14°15'56", N: 46°37'10", WGS84 coordinate system; 441 m a.s.l.), which resembles inner-alpine areas thermally, but with a continental influence. A wire mesh at the surface with 2.5 x 2.5 cm openings provides protection from clutch predators.

Temperature measurements were taken using two Tinytag Talk 2 Temperature Loggers (Type TK-4014). One logger was buried at a depth of 12 cm directly by the clutch, in order to measure the soil temperature. The second logger was positioned 2 m above the ground, measuring outside temperatures. Data recording started on 4 June 2012 after oviposition of a female *T. s. elegans*, and lasted until 29 September 2012, when the eggs hatched. Daily average temperature and daily temperature range were calculated between 00:00 and 24:00 h. The measurement interval was 30 minutes.

Rainfall data was provided by the ZAMG (Austrian central institution for meteorology and geodynamics). The meteorological station from which this data originates is 5 km from the clutch site, also in the Klagenfurt basin (E: 14°19'04", N: 46°38'54", WGS84 coordinate system; 452 m a.s.l.), and therefore it provides authentic data for the study site. Substrate moisture could not be measured and was estimated subjectively.

Clutch temperatures of a wild brood of *Trachemys scripta* (Kleewein 2014) at Tallach, 17 km to the south-west (E: 14°04'13", N: 46°32'20", WGS84 coordinate system; 516 m a.s.l.), are compared and discussed.

RESULTS

On 29 September 2012, a hole measuring 2.5 cm in diameter was discovered at the protected clutch site, indicating hatching. The wire mesh and uppermost layers of earth were removed, revealing a carapace. A live hatchling (hatchling 2) crawled out of the egg chamber and made its way towards the pond. Its yolk sac was already completely absorbed, and a small part of the plastron was not yet closed. The egg tooth was clearly visible. Regarding the carapace scutes, the second vertebral one was divided, meaning a total of six vertebral

scutes were present. There were 12 marginal and five costal scutes on each side of the carapace. The plastron, on the other hand, did not show any meristic abnormalities (Tab. 1). On 1 October 2012, a second hatchling was discovered during maintenance work in the outdoor enclosure – the first-hatched young (hatchling 1). Its yolk sac was not yet fully absorbed, and the animal was altogether smaller, but did not show any meristic abnormalities (Tab. 1). Sex-specific characteristics of both hatchlings, such as the cloaca positioned closer to the end of the tail, indicated that the hatchlings were males. Measurements of both individuals one year after hatching showed that both animals had grown well and had increased in weight. Hatchling 1 nevertheless remained well behind hatchling 2 in both size and weight (Tab. 1).

Three dead individuals in development stages 23, 25 and 26, according to Greenbaum (2002), were also found in relative proximity to the surface. The embryos of all other eggs in deeper parts of the chamber had died in much earlier development stages, and were therefore already completely decomposed and could not be classified by development stage.

The chamber contained a total of 22 eggs. Due to the proximity of the pond and the high soil water level, 17 of the 22 eggs in this Klagenfurt clutch were affected by permanent stagnant moisture. Only the uppermost eggs were surrounded by dry incubation substrate. Therefore, only two eggs were able to develop fully and hatch live young.

Temperature measurement was conducted over 118 days, and the average incubation period can also be assumed to be around that duration, since the first hatchling had already hatched shortly before this time.

The maximum temperature measured in the clutch was 38.7°C (16:30 h; 30 June 2012), the highest daily temperatures always being reached in the afternoons between 15:00 and 18:30 h. The minimum temperature measured in the clutch was 11.5°C (7:30 h; 21 September 2012), the lowest temperatures usually being reached in the mornings between 6:00 and 8:00 h.

The maximum daily temperature range in the clutch was 17.8°C (28 June 2012). In this case, the temperature rose from 19.3°C (7:00 h) to 37.1°C (17:00 h). The smallest daily temperature range was 2.1°C on a relatively cool day with temperatures between 16.3°C (7:30 h) and 18.4°C (16:00 h). The average daily temperature range during the entire incubation period was 10.4°C.

Out of the 118 days of development, 79% of days had a daily average temperature below 25°C (Fig. 1). The first third of the development period was the warmest, with a mean daily temperature of 24.7°C, the second third had a mean daily temperature of 23°C, and the third 19.9°C. The mean daily temperature during the entire incubation period, calculated using the daily average temperatures, was 22.5°C.

Rainfall from June to September was 593 mm, the most of which (292.5 mm) fell in July, in the second third of the development period. The months of June (84 mm), August (69.9 mm) and September (146.6 mm), on the other hand, were very dry (Fig. 1).

Table 1. Morphometric data of the two *Trachemys scripta elegans* hatched in Klagenfurt (Carinthia, Austria) in 2012, and one year after hatching. Shell measurements in centimetres (cm), body mass in gram (g). HN - Hatchling Number, CL - Carapace length, CB - Carapace breadth, PL - Plastron length, PB - Plastron breadth, SH - Shell Height

Tablica 1. Morfometrijski podatci dvije jedinke *Trachemys scripta elegans* koje su se izlegle u Klagenfurtu (Karintija, Austrija) 2012. godine, i jednu godinu nakon izlijeganja. Mjere na oklopu izražene su u centimetrima (cm) a tjelesna masa u gramima (g). HN – broj mladunca, CL – duljina karapaksa, CB – širina karapaksa, PL – duljina plastrona, PB – širina plastrona, SH – visina oklopa.

	HN	**CL**	**CB**	**PL**	**PB**	**SH**	**Weight**
2012	1	2.8	2.5	2.6	2.2	1.4	5
2013	1	5.5	5.2	4.9	3.9	2.4	31
2012	2	3.1	2.9	2.8	2.5	1.6	6
2013	2	6.8	6.0	5.9	4.7	2.8	52

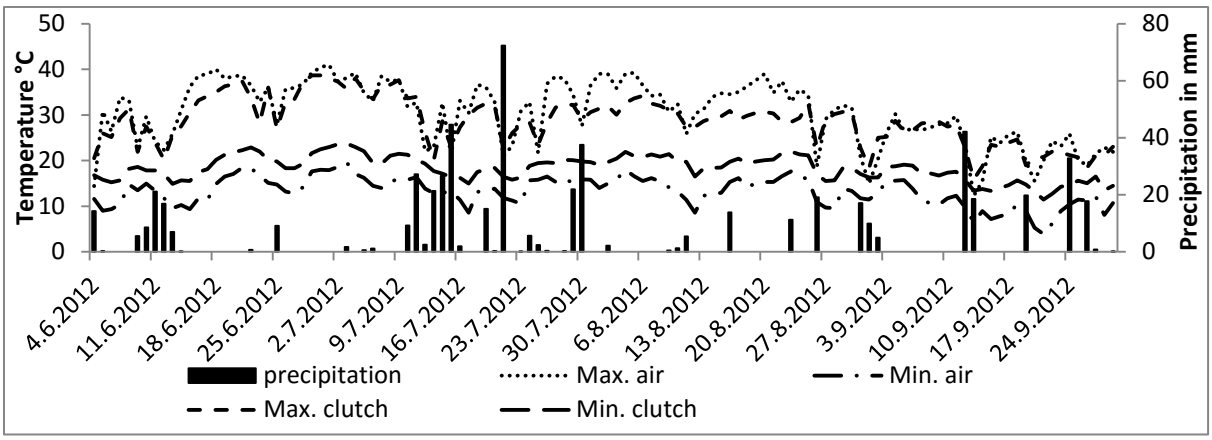

Figure 1. Outside temperature and rainfall data from the clutch site in Klagenfurt. Daily maximum and minimum outdoor temperature were measured 2 m above ground, maximum and minimum ground temperature were measured at a depth of 12 cm. Rainfall data refers to the period from the beginning of June to the end of September.

Slika 1. Podatci o vanjskim temperaturama i padalinama zbilježenim za leglo u Klagenfurtu. Dnevni maksimum i minimum su mjereni na 2 m iznad zemlje a dnevni maksimum i minimum temperature tla su mjereni na dubini od 12 cm. Podatci o padalinama se odnose na period od početka lipnja do kraja rujna.

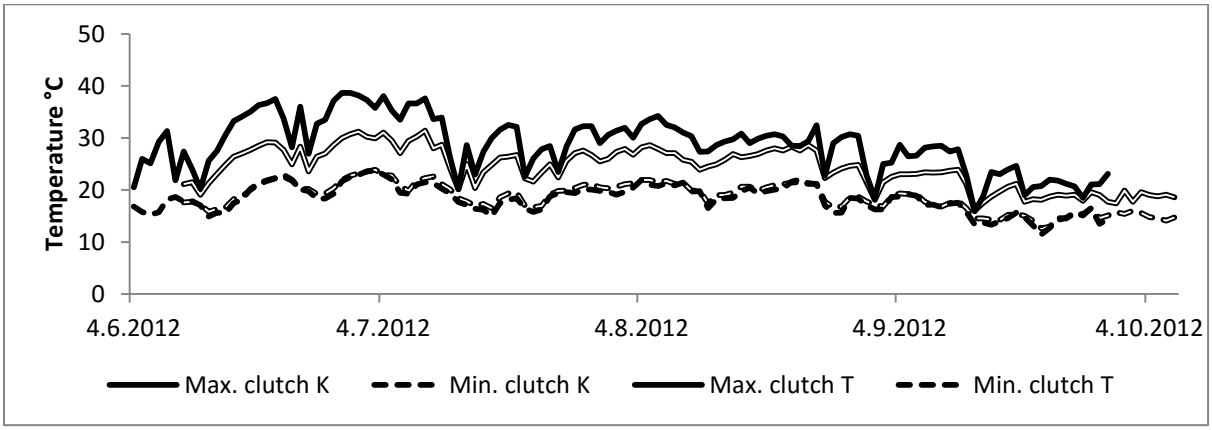

Figure 2. Comparison of maximum and minimum ground temperatures between the clutches in Klagenfurt (K) and Tallach (T). Temperatures in Klagenfurt were measured at a depth of 12 cm, in Tallach at 10 cm. Minimum temperatures of both clutches are almost identical, the maximum temperatures in Klagenfurt are much higher.

Slika 2. Usporedba maksimuma i minimuma temperature tla između legal u Klagenfurtu (K) i Tallachu (T). Temperature u Klagenfurtu su mjerene na dubini od 12 cm, a u Tallachu na dubini od 10 cm. Minimalne temperature oba legla su skoro identične, dok su maksimalne temperature u Klagenfurtu značajno veće.

DISCUSSION

Survival rate and development of embryos in eggs with flexible, porous shells – as is the case in *T. s. elegans* – are strongly influenced by the moisture of the incubation substrate, due to the increased exchange between the egg and its surroundings (Congdon & Gibbons 1990). In dryer substrates, hatchlings are generally smaller and lighter (Congdon & Gibbons 1990). This was also evident in the two uppermost eggs from the Klagenfurt clutch which hatched live. These two hatchlings, which had a body mass of 5 and 6 g respectively, thereby lying at the lower end of the range of the body mass of wild hatchlings in North America (4.4-10.3 g; mean 8.2 g; n=151) (Tucker 2000). The Tallach hatchlings had only between 5 and 6 g and the incubation substrate was dry (Kleewein 2014).

On the one hand, moist substrate promotes an increase in embryonic mass; on the other hand, eggs in moist substrate require a longer incubation period than eggs in drier substrate (Tucker & Paukstis 1999). If moisture levels are too high during incubation, this can kill the embryos (Tucker et al. 1997). This was also the case at the deeper part of the clutch at the Klagenfurt site. The combination of the high groundwater level and waterlogged soil caused the embryos in deeper parts of the chamber to die.

Average incubation period is dependent on temperature and lasts 112.5 days under laboratory conditions at an average temperature below 25°C, according to Ernst & Lovich (2009). The development period of 118 days and the low incubation temperature measured in Klagenfurt match these laboratory results. Under natural conditions in their native habitat, the young hatch after 60-80 days (Ewert 1979). The Tallach clutch also had a long incubation period of 119 days (Kleewein 2014) (Fig. 2). This shows that incubation periods under natural conditions in Austria are longer by one half to one third than incubation periods in the species' native North American habitat. Furthermore, the possibility of successful reproduction despite low incubation temperatures and huge temperature variation could

be proven. Ewert & Nelson (1991) describe the development of males at 22.5, 25 or 27°C, and the development of females at 30°C. The transitional range of temperatures under stable laboratory conditions lies between 29.0 and 29.5°C (Bull et al. 1982, Paukstis & Janzen 1990). Cadi et al. (2004) state a transitional range of temperatures between 28.30°C and 30.61°C, at which both sexes can develop equally. The most decisive phase for the maturing *T. scripta* embryo is the middle third of the incubation period. It is only after this time that the sexual organs begin to differentiate, lasting until the onset of sex-specific gonadal morphogenesis (Wibbels et al. 1991). In the Klagenfurt case, clutch temperature dropped constantly from oviposition to hatching, the mean daily temperature already being below 25°C in the first third of development. In Tallach, at an overall average temperature of 21.4°C, it was also only males that hatched. Due to the low recorded average incubation temperatures, it is currently not possible for females to develop in Austrian clutches in the wild.

The maximum daily range of temperature in a *Trachemys scripta troostii* (Holbrook, 1836) clutch in Tennessee was 8°C (Cagle 1937). For comparison's sake, the average daily range of temperature in a 10.7 cm deep clutch of *Chrysemys picta* (Schneider, 1783) was 7°C (Refsnider et al. 2013). With 10.4°C at a depth of 12 cm, the measurements in Klagenfurt revealed only a slightly higher average daily range of temperature; but they indicated a much greater tolerance regarding the maximum daily range of temperature, with 17.8°C. Since substrate moisture and clutch temperature are decisive for development of the embryos, and as these values were considerably lower in Carinthia than in the species' native habitat, this shows that the species possesses considerable tolerance regarding temperature in central European climates.

Generally, there would be enough habitats available around the eastern Alps that could allow the development of hatchlings.

T. scripta can reproduce for the first time around the age of eight years on average (Gibbons et al. 1981), and at this age their body mass is still smaller than that of adults. Compared to adult animals, however, the young are subject to much greater predation pressure, especially from raptors and corvids, as well as from carnivorous mammals such as foxes. What is more, even in South Carolina, only 10% of a population reach the age of ten years, and only 1% of turtles reach 20 years (Gibbons 1987). For these reasons, only a small number of hatchlings could be expected to reach maturity in Austria.

Long, harsh frosts reduce the hatching rate, body temperatures below 0°C down to -4°C are tolerated only for a few hours (Churchill & Storey 1992). Slider hatchlings of the northern North American populations generally overwinter in the nest, and react sensitively to temperatures around -0.6°C-4.0°C (Packard et al. 1997, Tucker & Packard 1998, Costanzo et al. 2008). An analysis of soil temperatures during the course of one year, measured at the hatching site in Klagenfurt from the winter of 2011 to the winter of 2012, showed minimum temperatures reaching -3.5°C, which would have had a detrimental effect on survival rate in any overwintering clutch.

One year, or a few years, with low reproductive success will not lead to the extinction of populations. What is more decisive for the geographic range and persistence of a population is the long-term average temperature (Rödder et al. 2009). Due to the low clutch temperatures measured in Carinthia, only males are able to develop at the moment. This can, however, provide the basis for future reproductions in certain bodies

of water that already contain released, sexually mature females.

REFERENCES

Bull, J.J., Vogt, R.C. & McCoy, C.J. (1982): Sex determining temperatures in turtles: a geographic comparison. Evolution 36: 326-332.

Cadi, A., Delmas, V., Prévot-Julliard, A.-C., Joly, P., Pieau, C. & Girondot, M. (2004): Successful reproduction of the introduced slider turtle (*Trachemys scripta elegans*) in the South of France. Aquatic Conservation: Marine and Freshwater Ecosystems 14: 237-246.

Cagle, F.R. (1937): Egg laying habits of the slider turtle (*Pseudemys troostii*), the painted turtle (*Chrysemys picta*), and the musk turtle (*Sternotherus odoratus*). Journal of the Tennessee Academy of Science 12: 87-95.

Churchill, T.A. & Storey, K.B. (1992): Responses of freezing exposure of hatchling turtles *Trachemys scripta elegans*: factors influencing the development of freeze tolerance by reptiles. Journal of Experimental Biology 167: 221-233.

Congdon, J.D. & Gibbons, J.W. (1990): Turtle Eggs: Their Ecology and Evolution. pp. 109-123. In Gibbons, J.W. (ed.) Life History and Ecology of the Slider Turtle. Smithsonian Institution Press.

Costanzo, J.P., Lee Jr., R.E. & Ultsch, G.R. (2008): Physiological ecology of overwintering in hatchling turtles. Journal of Experimental Zoology 309A: 297-379.

van Dijk, P.P., Harding, J. & Hammerson, G.A. (2013): *Trachemys scripta*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 22 May 2015. http://www.iucnredlist.org/details/22028/0.

Ernst, C.H. & Lovich, J.E. (2009): Turtles of the United States and Canada. Second Edition. John Hopkins University Press, Baltimore.

Ewert, M.A. (1979): The embryo and its egg: Development and natural history. pp. 333-413. In Harless, M., Morlock, H. (eds.) Turtles: Perspectives and research. John Wiley and Sons.

Ewert, M.A. & Nelson, C.E. (1991): Sex determination in turtles: Diverse patterns and some possible adaptive values. Copeia 1991: 50-69.

Gibbons, J.W. (1987): Why do turtles live so long? Bioscience 37: 262-269.

Gibbons, J.W., Semlitsch, R.D., Greene, J.L. & Schubauer, J.P. (1981): Variation in age and size at maturity of the Slider turtle (*Pseudemys scripta*). The American Naturalist 117: 841-845.

Girondot, M., Delmas, V. & Prévot-Julliard, A.-C. (2012): Nouveles données sur la ponte de la tortue de Floride (*Trachemys scripta elegans*) en Île-de-France. Bulletin de la Société Herpétologique de France 142-143: 71-78.

Greenbaum, E. (2002): A standardized series of embryonic stages for the emydid turtle *Trachemys scripta*. Canadian Journal of Zoology 80: 1350-1370.

Gutleb, B. & Happ, H. (2002): Schildkröten in Kärnten. Carinthia II 192/112: 155-160.

Kleewein, A. (2007): Verbreitung der Rotwangen-Schmuckschildkröte (*Trachemys scripta elegans*) in Kärnten. Carinthia II 197/117: 53-58.

Kleewein, A. (2014): First record of reproduction in open land of *Trachemys scripta troostii* (Holbrook, 1836) with the nominate species *Trachemys scripta* (Schoepff, 1792) in Austria. Herpetozoa 26: 183-185.

Packard, G.C., Tucker, J.K., Nicholson, D. & Packard, M.J. (1997): Cold tolerance in hatchling Slider turtles (*Trachemys scripta*). Copeia 1997: 339-345.

Paukstis, G.L. & Janzen, F.J. (1990): Sex determination in reptiles: summary of effects of constant temperatures of incubation on sex ratios of offspring. Smithsonian Herpetological Information Service 83: 1-28.

Refsnider, J.M., Bodensteiner, B.L., Reneker, J.L. & Janzen, F.J. (2013): Nest depth may not compensate for sex ratio skews caused by climate change in turtles. Animal Conservation 16: 481-490.

Rödder, D., Kwet, A. & Lötters, S. (2009): Translating natural history into geographic space: a macroecological perspective on the North American Slider, *Trachemys scripta* (Reptilia, Cryptodira, Emydidae). Journal of Natural History 43: 2525-2536.

Tucker, J.K. (2000): Annual variation in hatchling size in the red-eared slider turtle (*Trachemys scripta elegans*). Herpetologica 56: 8-13.

Tucker, J.K., Janzen, F.J. & Paukstis, G.L. (1997): Response of embryos of the red-eared turtle (*Trachemys scripta elegans*) to experimental exposure to water-saturated substances. Chelonian Conservation and Biology 2: 345-351.

Tucker, J.K. & Packard, G.C. (1998): Overwinter survival by hatchling sliders (*Trachemys scripta*) in West-Central Illinois. Journal of Herpetology 32: 431-434.

Tucker, J.K. & Paukstis, G.L. (1999): Post-hatching substrate moisture and overwintering hatchling turtles. Journal of Herpetology 33: 608-615.

Vamberger, M., Lipovšek & G., Gregorič, M. (2012): First reproduction record of *Trachemys scripta* (Schoepf, 1792), in Slovenia. Herpetozoa 25: 76-79.

Wibbels, T., Bull, J.J. & Crews, D. (1991): Chronology and morphology of temperature-dependent sex determination. Journal of Experimental Zoology 260: 371-381.

ACKNOWLEDGEMENTS

I would like to thank Helga Happ, of the Happ reptile zoo, for the possibility to conduct this study in her zoo. Thanks are also due to the Department for Integrative Zoology, especially to Walter Hödl, for material and professional support.