Heliyon 7 (2021) e07607

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Optimal body mass-length ratio during hibernation for *Emys orbicularis* (Linnaeus, 1758) – European Pond Turtle

Ziegler Carina [a], Mărginean George-Ioan [a,*], Vitan Dragoș [b]

[a] *Wilderness Research and Conservation, Bucharest, Romania*
[b] *Military Equipment and Technologies Research Agency, Bucharest, Romania*

ARTICLE INFO

Keywords:
Emys orbicularis
Turtle
Hibernation
Body mass/length ratio
Calculator

ABSTRACT

The aim of this study was to determine optimal body mass/length ratios of *Emys orbicularis* before hibernation. We obtained 213 measurements of wild-caught and captive-bred turtles during 9 years (2011–2019) and determined regression curves that show intervals for optimal or suboptimal body mass/length ratios before winter dormancy. Using this data we designed an online calculator that facilitates the process of establishing if a turtle has an optimal body mass/length ratio before hibernation. We also gathered 45 measurements at the end of the hibernation period which showed an average body mass loss of 5.32%. The present study offers practical means of assessing if an *Emys orbicularis* individual is fit for hibernation.

1. Introduction

Emys orbicularis (Linnaeus, 1758), the European Pond Turtle, is listed on the IUCN Red List as "near threatened" (IUCN 2020). In some regions it is still present in greater numbers (i.e. the Danube Delta, the north-western and south-eastern areas of Romania; Fuhn and Vancea 1961; Cogălniceanu et al., 2013; Sos 2011), but in many parts of its distribution, the populations are declining, especially in regions with a marked anthropic influence, because of multiple factors: direct human disturbance, habitat destruction, pollution of the aquatic environment, poaching, illegal online pet-trade, competition with invasive species (i.e. *Trachemys sp.*) (Fritz 2001; Cordero Rivera and Fernández 2004; Sos 2011; Mărginean et al., 2018). A lot of effort is put in rehabilitation and reintroduction programs (i.e. Germany, Switzerland, Romania, etc.; SwissEmys (2020); Meeske and Poggenburg (2014); Wilderness Research and Conservation (2018)) and this means that the species has to be kept in captivity for various periods of time, may it be quarantine, for medical care, captive reproduction for head-starting programs, rehabilitation after being kept illegally as a pet etc (Mărginean et al., 2018). Therefore natural conditions have to be simulated in a micro-environment as best as possible and this includes their annual cycle.

Emys orbicularis turtles are poikilotherms and because of their range of distribution, their annual cycle includes a period of hibernation (Rogner 2009).

Even in a controlled environment with ideal conditions for hibernation (cellars, fridges, cold rooms etc.), the process of hibernation is a delicate period for the turtles and fatalities are not uncommon (McArthur et al., 2008).

Efforts should be made before any hibernation period to reduce the risk of death to a minimum and this implies basic medical evaluations in order to include only healthy individuals (screening for parasites, injuries, infections, good body condition score, nasal discharge, diarrhea etc.) (Divers 2018).

In an attempt to facilitate the general examination of turtles, lower the risk of fatality during the winter dormancy and therefore reduce losses during rehabilitation and reintroduction programs, by providing body condition baselines, we have collected data about body mass and shell length of wild-caught and captive-bred *Emys orbicularis* individuals, kept in outdoor enclosures, before and after hibernation; any variations from the optimal values should be interpreted from a medical point of view and warrant caution at attempts of hibernation. Similar charts are in use for Mediterranean tortoises (i.e. Jackson's Ratio for *Testudo graeca* and *Testudo hermanni*, McIntyre Ratio for *Testudo horsfieldii*; Jackson (1980); Jepson (2011)), but none exist currently for *Emys orbicularis*. Since body mass and shell length data were collected at the end of the active season for the turtles, at the point at which they are the fittest in preparation for hibernation, this provides an accurate insight into what a normal baseline for a turtle should look at a general examination. Our baselines are different from previous similar studies, where

* Corresponding author.
E-mail address: geomarginean@yahoo.com (M. George-Ioan).

https://doi.org/10.1016/j.heliyon.2021.e07607
Received 28 July 2020; Received in revised form 29 March 2021; Accepted 14 July 2021

measurements were taken either at the start of the active season (Zuffi and Foschi, 2015) or in the middle of it (Jackson 1980) and thus reflecting potential lower values for body mass. Conservation programs focused on *Emys orbicularis* can also make use of the present data to assess body conditions of the turtles and make informed decisions about release, hibernation and potential underlying medical problems that would need further investigation.

2. Materials and methods

The present study is a prospective cohort study. We measured the length of the carapace and body mass of 95 *Emys orbicularis* individuals (81 wild-caught and 14 captive-bred individuals from wild-caught parents), and weighed them using household scales accurate to the gram, over a period of 9 years (2011–2019), on the day before introducing them to indoor hibernation setups (cellars). Carapace length was determined by measuring the length of the segment between verticals from the cranial edge of the nuchal scute to the caudal edge of the supracaudal scute (in centimeters) (Figure 1). For practical reasons, values have been adjusted to 0.5 cm increments.

The hibernation period in Romania encompassed the timeframe between late October/early November and late March/early April, depending on local weather conditions.

All turtles were in the process of rehabilitation to be reintroduced into the wild. Individuals ranged from 2 month old hatchlings to fully grown, decades old adults. Unfortunately age cannot be accurately assessed in wild-caught individuals. Estimations using growth rings are inaccurate at best, since juveniles can have more than one growth spurt/year and as such develop more than one growth ring if conditions are good enough and older adults present eroded scute lamellae. These aspects either contribute to over- or underestimations of age of wild turtles (Zuffi and Foschi, 2015; Fritz 2001).

Figure 1. Weighing of an *Emys orbicularis* individual and measuring carapace length.

Every turtle was assessed before hibernation for the presence of open injuries or other symptoms indicating health problems (pathological secretions, respiratory problems, failure to react to stimuli etc.).

Inclusion criteria consisted of turtles that appeared to be healthy during a general medical examination (without paraclinical investigations) and successfully completed the process of hibernation. Exclusion criteria consisted in individuals that deceased during hibernation or that had the process interrupted for various medical reasons (infections, open wounds).

All individuals were hibernated indoors, in controlled environments (cellar with stable temperatures ranging across 4–15 degrees Celsius, depending on the temperatures outside). Some individuals have provided data points for several years, being kept in captivity for various reasons (debilitating injuries – partial limb amputations, cataract, fractures, carapace injuries, hatchlings/juveniles until they reached our desired size for release).

We obtained a chart indicating body mass/length ratios for *Emys orbicularis before hibernation*, in which three regression curves were determined using average (favr), minimum (fmin) and maximum (fmax) values of body mass for the same carapace length. Between these curves there are intervals in which turtles can be considered to have an optimal/average, above average or suboptimal ratio for hibernation.

Using the analytical expression of the regression curves, we defined the function $t:\mathbb{R}^2 \rightarrow \{-1, 0, 1, 2\}$ that determines if a subject has an optimal body mass/length ratio before hibernation:

$$t(1,g) = \begin{cases} 2, g > f_{max}(l) \\ 1, g \in [f_{avr}(l), f_{max}max] \\ 0, g \in [f_{min}(l), f_{avr}(l)] \\ -1g, < f_{min}(l) \end{cases}$$

where "2" represents overweight, "1" above optimal weight, "0" optimal and "-1" underweight turtles.

For a different set of data, one can obtain slightly different functions for the analytical expression of the regression curves, but from a practical point of view these differences are insignificant.

For practical reasons, so that one is able to calculate if a subject has a good hibernation ratio, we provided a simple "software" solution that can be accessed at "https://emyscalc.github.io/". The function on the website has 2 inputs: the length "l" and the body mass "g" of a subject. When the values are introduced, the "calculator" will provide a result stating if a turtle is underweight, of optimal, above optimal weight or overweight. This is done by comparing the end result to the reference intervals between the above mentioned three regression curves.

For 45 data entries we also measured the body mass at the end of the hibernation period to analyze body mass loss.

Statistical analysis was performed in Microsoft Excel (2016) and R (version 3.6.2) using descriptive statistics and Shapiro-Wilk normality test to analyze the distribution of data for our 45 post-hibernation measurements. The test revealed a non-normal distribution of this data and therefore non-parametric statistical tests have been applied to compare our data: Kruskal-Wallis rank and post-hoc tests and Wilcoxon rank sum test. We established statistical significance at $p < 0.05$.

3. Results

We collected data from 95 *Emys orbicularis* individuals, over 9 years (2011–2019) and obtained 213 data points (individuals in captivity for several years provided multiple data points, as pointed out in Table 1), illustrating body mass and length of turtles before hibernation.

The lowest recorded value was 11g for a 3.5cm turtle hibernated in the same year after hatching and the highest 997g for a 19 cm adult female.

Table 1. Body mass/length measurements from individual turtles.

Number of data points/turtle		Number of turtles
9		1
8		1
7		1
6		1
4		11
3		10
2		39
1		31
Total	**213**	**95**

Based on the 213 measurements, *Emys orbicularis* body mass/length ratios before hibernation with regression curves of optimal, above optimal or suboptimal ratios for hibernation, are illustrated in Chart 1.

The analytical expression of these regression curves is represented by the following three real functions:

$f_{max}(x) = 0.3354 \times {}^{2.7976}$;

$f_{avr}(x) = 0.3424 \times {}^{2.7315}$;

$f_{min}(x) = 0.3625 \times {}^{2.6425}$;

in which the pairs {x, f(x)} represent the {length, body mass} of a subject.

Our 45 measurements after hibernation showed an average body mass loss of 5.32%, with a minimum of 0.22% and a maximum of 18.87% of the total body mass.

Shapiro-Wilk normality test of these 45 measurements after hibernation showed that the distribution of our data is statistically different from the normal one (W = 0.83273, p-value = 1.355e-05). As a consequence we used a Kruskall-Wallis test that revealed no statistically significant difference in body mass loss percentages between males, females and juveniles (chi-squared = 0.85098, df = 2, p-value = 0.6535). We also analyzed if there is a difference in body mass loss between adults and juveniles and chose a cutoff size of 11cm carapace length. Wilcoxon rank sum test showed no statistical significant difference in body mass loss percentages between turtles above or under 11cm carapace length (W = 257, p-value = 0.7569).

4. Discussions

Rehabilitation of wildlife is always a challenge and often a long and difficult process. For species of turtles in temperate climates, like *Emys orbicularis*, conservationists also need to provide the optimal conditions for the whole annual cycle during the process of rehabilitation and this includes recreating a controlled environment for hibernation.

Hibernation is a delicate process during the cold season in which turtles lower their metabolism by as much as 95–99% (Jackson and Ultsch, 2010) and even in controlled environments like cellars and fridges it can be a fatal process. Death can occur for various reasons (infection, lack of sufficient resources – underweight turtles -, previously undiagnosed or idiopathic conditions, predation, freezing, dehydration etc.) (McArthur et al., 2008; Boyer and Boyer, 1994).

Often, just by measuring and weighing a turtle, one can get a general idea regarding the health of that individual, as a body condition score can be the reflection of various underlying medical problems.

This study aimed to provide reference intervals for assessing *Emys orbicularis* turtles before hibernation, to determine if the turtles are of optimal body mass and therefore have a reduced risk of mortality, especially from lack of sufficient energy storages (i.e. underweight turtles) during this delicate time of their annual cycle.

By conducting a study on Romanian wild caught *Emys orbicularis* individuals or hatchlings from wild caught individuals that were kept in semi-wild, outdoor enclosures, with minimal human contact only during feeding times, our measurements might offer a closer insight into optimal body mass/length ratios of hibernating individuals in the wild and also provide novel biometric data in a local ecological context for this particular turtle species.

Having reference intervals and the means to assess optimal body mass/length ratios before hibernation, can represent a useful tool to reduce the risk of mortality during this period and to exclude and treat potential underweight or overweight individuals. After all, potentially unhealthy individuals cannot be safely released back into the wild and need further investigations and treatments. However errors can occur in specific cases, where body condition scores are artificially elevated due to the presence of intestinal gravel, uroliths, coelomic exudates or even eggs (Jacobson et al., 1999). Therefore, a body condition score must always be accompanied by complementary examinations to properly interpret the values, such as a general medical examination, with or without paraclinical investigations, including visual inspection, auscultation and palpation to further exclude potential medical risks.

Our devised online calculator for comparing body condition scores in *Emys orbicularis*, accessible at https://emyscalc.github.io/, is a very simple and practical tool that can be used by anyone, be it veterinarians, rehabilitators, conservationists, or even pet owners to provide an initial insight into the condition of their turtles, without the need of a scientific background to analyze the data. Because the calculator also shows the baseline limits of the interval for specific values, one can also establish if the body condition score of a turtle is closer or farther away from the minimal threshold and if that individual requires closer monitoring during hibernation or a shorter time in this state.

Because our measurements were obtained at the end of the active season, the obtained numbers could be considered to be from turtles at their peak condition, after a full season of activity, eliminating potential biases of calculated body condition scores during different periods of the year (i.e. mating season during springtime when turtles are not at their peak condition and have only recently emerged from hibernation). We have therefore also avoided biases like the presence of eggs (Zuffi and Foschi, 2015) in our calculations, which can alter body condition scores (Jacobson et al., 1999).

Our average body mass loss of 5.32% during hibernation can also serve as a reference for assessing individuals after the cold season. Considering that the hibernation period was 5–6 months each year, this seems to be in accordance with previously recorded data in other turtle species with body mass loss of 1%/month spent in hibernation (McArthur et al., 2008). Body mass loss during hibernation does not seem to fluctuate statistically significant with size or sex. Increased body mass loss during winter dormancy might indicate an underlying health problem or improper hibernation conditions (i.e. higher temperatures can raise the metabolism of hibernating turtles and caused increased consumption of glycogen storages) (Jackson 2011) and should exercise caution in continuing the hibernation process.

5. Conclusions

Our study provides reference intervals and practical means of calculating if an *Emys orbicularis* individual is fit for hibernation.

Declarations

Author contribution statement

Ziegler Carina, Mărginean George-Ioan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Vitan Dragoș: Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2021.e07607.

Acknowledgements

We are grateful to Niculae Alexandru tefan for his support and help in analyzing our measurements. We also would like to express our gratitude to Gherman Elena and Cujerean Cristian for contributing to our data and to Doboș Ionuț-Daniel for providing invaluable assistance in creating the online calculator.

References

Boyer, D.M., Boyer, T.H., 1994. Tortoise care. Bulletin of the Association of Reptilian and Amphibian Veterinarians 4 (1), 16–28.

Cogălniceanu, D., Rozylowicz, L., Székely, P., Samoilă, C., Stănescu, F., Tudor, M., Székely, D., Iosif, R., 2013. Diversity and distribution of reptiles in Romania. ZooKeys 341, 49–76.

Cordero Rivera, A., Ayres Fernández, C., 2004. A management plan for the European pond turtle (*Emys orbicularis*) populations of the Louro river basin (Northwest Spain). Biologia 59 (Suppl. 14), 161–171.

Divers, S.J., Stahl, S.J. (Eds.), 2018. Mader's Reptile and Amphibian Medicine and Surgery-E-Book. Elsevier Health Sciences.

Fritz, U., 2001. *Emys orbicularis* (Linnaeus, 1758) – Europäische Sumpfschildkröte. In: Fritz, U. (Ed.), Handbuch der Reptilien und Amphibien Europas. Schildkröten I. Aula-Verlag.

Fuhn, I.E., Vancea, ., 1961. Fauna Republicii Populare Române – Reptilia. Editura Academiei R.P.R., București.

IUCN, 2020. The IUCN Red List of Threatened Species. Version 2020-1. http://www.iucnredlist.org. accessed at: 2020.04.02.

Jackson, D.C., 2011. Life in a Shell. Harvard University Press.

Jackson, D.C., Ultsch, G.R., 2010. Physiology of hibernation under the ice by turtles and frogs. J. Exp. Zool. Part A: Ecological Genetics and Physiology 313 (6), 311–327.

Jackson, O.F., 1980. Weight and measurement data on tortoises (*Testudo graeca* and *Testudo hermanni*) and their relationship to health. J. Small Anim. Pract. 21 (7), 409–416.

Jacobson, E.R., Behler, J.L., Jarchow, J.L., 1999. Health assessment of chelonians and release into the wild. Zoo and wild animal medicine. Curr. Ther. (Phila.) 4, 232–242.

Jepson, L., 2011. Tortoise (Pet Expert). Edition Magnet&Steel.

Mărginean, G.I., Gherman, E., Sos, T., 2018. The illegal internet based trade in European pond *turtle Emys orbicularis* (Linnaeus, 1758) in Romania: a threat factor for conservation. N. West. J. Zool. 14 (1).

McArthur, S., Wilkinson, R., Meyer, J. (Eds.), 2008. Medicine and Surgery of Tortoises and Turtles. John Wiley & Sons.

Meeske, A.C.M., Poggenburg, C., 2014. Reintroduction of the European pond turtle (*Emys orbicularis*) in northwest-Germany – first results after first release. Herpetological Facts 1, 46–60.

Rogner, M., 2009. European Pond Turtle: *Emys orbicularis*. Edition Chimaira.

Sos, T., 2011. În obiectiv: Țestoasa de apă europeană, *Emys orbicularis*. Asociaţia Ecouri Verzi, Cluj-Napoca, Romania.

SwissEmys, 2020. http://emys.ch. accessed at: 2020.04.02.

Wilderness Research and Conservation, 2018. https://www.facebook.com/WildernessResearchandConservation/. accessed at 2020.04.02.

Zuffi, M.A., Foschi, E., 2015. Reproductive patterns of European pond turtles differ between sites: a small scale scenario. Amphibia-Reptilia 36 (4), 339–349.