



Examination of successful wintering of turtles

PhD dissertation thesis

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**Gödöllő
2015**

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1. Background and objectives

The importance of trade of the exotic animals and the lust about exotic reptiles is increasing from the last three decades to the present days. In the beginning the incessant demands was satisfied by the export of wild caught animals from their natural habitats, what in point of nature conservation view has caused high damage on native populations. When targeted to stop this process the exporting countries have been established breeding farms and at the end of the '90s also appeared small breeding farms in the importing countries. The red-eared slider (*Trachemys scripta elegans*), which is native in North American continent is one of the species that were especially popular among the hobbyist. In the United States between 1989-1997 a total of 52 millions, mostly fresh hatchlings of red-eared sliders were exported mainly to Europe and Asia. In Europe, the sliders bought as pet animals, in Asia it is typical to eat or use for do religion.

The red-eared slider can be kept simple, decorative pet turtle, which can be easily housing and satisfying its demands in the beginning. The rapid growth is typical in case of this species, which after a few years means is not negligible extra cost for the keeper, because the turtle needs bigger aquaterrarium, more powerful water purification unit, more food and in some case needs veterinary care. During the years the animals become boring and aggressive for the keeper, what means more financial burden, some owner released their turtles into natural waters. This phenomenon has become so common and at the beginning of the 2000s, almost all importing countries observed the species in their natural waters.

The red-eared slider is also published by the IUCN (International Union for Conservation of Nature) in 2000 on the 100 worths invasive species list as well which means the appearance of this species cause

ecological threat. Hungary in the same time of join to the EU banned the red-eared slider imports and the execution of „protection and welfare of animals” law XXVIII in 1998. of implementation of Regulation In 2010, Keeping and marketing of the pets on the implementation of Law 41/2010. (II. 26) of Regulation (hereinafter: Regulation) in Annex I, as the country's native flora and fauna listed ecologically harmful species the red-eared slider with the followings: *Chelydra serpentina*, *Chrysemys picta*, *Macrolemmys temminckii*. It is prohibited to keeping, breeding or offer for sale each mentioned species.

Due to the fact that the observation numbers are increasing on released turtles in the importing countries and in South of France has been reported the successful reproduction in natural habitat I kept carried out a research to investigate the less examined hibernation period in case of the red-eared slider species in question is necessary, which may be the major limiting factor to the colonization of indigenous species.

The objectives of my research were the follows:

- the examination of red-eared sliders survival in winter under natural conditions in Hungary
- the direct and indirect effects of the temperature to their health during the hibernation
- mortality causes during the hibernation
- examination of certain blood parameters after hibernation

Due to the banned red-eared slider trade, in the last period of my investigation I have carried out my study of the hibernation period with an other species also, the Florida Red-bellied turtle (*Pseudemys nelsonii*).

2. Materials and methods

The hibernation examination of the red-eared sliders lasts from 2009. autumn to 2012. spring in three wintering period. Fundamentally I tried to modelling the original wintering strategies from their native distribution in Hungary under natural conditions and in the last period completing the investigation with Florida Red-bellied turtle as well. In the examined wintering periods I have built artificially wintering places. During the research each year modelled mainland wintering strategy with 50 cm and 1 m deep holes. In the first year I investigated the survive of hibernation in a house's attic with wet conditions which was targeted to study the less protected wintering strategy against the weather. In the second winter period (2010. autumn – 2011. spring) I modelled the hibernation in 50 cm, 80 cm and 1 m deep, at least 1 m³ artificially created lake.

The wintering holes were created in a calm garden, which were free of stagnant ground water. When I set up the modelling in each case the turtles were placed at the bottom of the hole and that were uploaded dry, mold-free straw. To prevent of any escape and keep out predators from the holes, I used 40-50 cm high boards around, wich were digged to 8-10 cm deep into the ground and its closed with metal fence. The examined adult turtles were free in the hole, the youngs were in plastic boxes with perforated bottom and sides or use plastic buckets wired on the top.

To investigate of wintering period, when the turtles do not find adequate protection from the direct impact of the weather, I put the turtles in a 90 l plastic bin and placed them in an attic with moderately humid climate and covered them with moldfree straw.

The water-wintering strategy modelled in 50 cm to 1 m deep, artificial, black plastic-lined lake, and a kidney-shaped, 80 cm deep, plastic pond. On the bottom, there were approx. 20 cm deep layer of sludge what I have made by with the excavated soil. The lakes were filled by garden tap water one month before, than the hibernation starts to equalize the temperature between the aire and the water. I filled the ponds water from a garden tap a month before the start of the wintering so that the body of water being close to the ambient temperature and be free from seepage.

In my research I considered particularly important the temperature measurement in wintering places which have the direct and indirect impact on the health of turtles and can answer many questions of examining the mortality causes during hibernation. In each hole there was a digital sensor placed among the turtles (VOLTCRAFT DL-120 TH), what can record downloadable temperature datas. The devices have started at the hibernation period and its recorded the current temperature datas in every 60 minutes until the end of the wintering. At the attic of the house and also at created the artificial ponds I have measured the effect of temperature exposure such as 50 cm from the ground surface with outdoor thermometer and recorded three times a day (morning, noon, evening).

In this study all of the investigated turtles were from external keeping mode. The turtles had been starving before the wintering period to prepare them for the hibernation.

Regarding the animal welfare law each turtles have been painlessly marked. In case of older turtles the marginal shield edges of the carapace were rasped by iron saw. The hatchlings had softer carapace, so they were marked by a sharp scissors to cut small nick on the marginal edge. These markings on the animals were based on a predetermined code, which helped

to identify the animals correctly. Additionally I have made high-resolution pictures to make sure about the identification in the future. Before the hibernation period have started the animals were subjected to thorough clinical examination and the data about body weight, plastron and the carapace length was recorded.

When I have found a dead turtle in a wintering place, I have done post-mortem examination to looking for the cause of the death every time at the laboratory of Szent István University Faculty of Veterinary Science Department of Pathology and Forensic Veterinary Medicine. My supervisor Dr. Janos Gal made me guidelines to do the dissection.

I had license to investigate the red-eared sliders in the Margaret-Islands lake. In case of red-eared sliders from Margaret-Island Lake I have collected cloacal swab from adult turtles to investigate the extent of infection by ciliated and flagellated protozoan. After the sample collection I examined the adhered mucus from the cloacal swab by microscope and evaluated the severity of the infection based on a number of briskly moving protozoans per field of vision by the microscope.

The posthibernation blood parameters investigation was made in March of 2011, when I had opportunity to get bloods from adult red-eared sliders, which were sampled general animal health purposes originally. The turtles were living in the Budapest Zoo and Botanical Garden Great Lake and they just awaking from the hibernation. The blood sample was taken from the generally accepted vein subcarapacialis. After clotting the blood the serum was pipetted into Eppendorf tubes with sterile pipette and then sent to SYNLAB Hungary Kft. Animal Health and Diagnostic Department for determine the volume of glucose, uric acid, total calcium, total protein, albumin, glutamic-oxaloacetic-transaminase (GOT), glutamate-pyruvate-

transaminase (GPT), lactate dehydrogenase (LDH), creatine kinase and lipase from the serum.

3. Results

The results of three investigated wintering periods showed, that the success of hibernation is strongly influenced by the effect of temperature during wintering at domestic natural environment. Based on literatural data the optimal temperature for the red-eared slider and the Florida Red-bellied turtle in wintering place is optimal at 2-4 °C .

The red-eared sliders, which wintering in the attic of the house get reached the highest temperature fluctuation, what was much more than the optimum values and temperatures were recorded below the freezing point also. During hibernation the reptiles life processes slowed down and reduced the overall resistance as well. In addition in case of non-optimal wintering temperature within a short time can causes immunosuppressive condition and can promotes to multiply of the facultative pathogens in the reptile which can give rise the chance for pathological processes. In this case from the eight adult red-eared slider (four males and four females) seven dead, except one female have survived and suffered a minimal (0,8%) decrease of body weight. It is not negligible, that the males died firstly in the first two months, and after followed by the females.

The success of the wintering strategy on ground was highly dependent by the temperature and its durability at different depths in case of the red-eared sliders and Florida Red-bellied turtle. The wintering animals in 50 cm deep holes in the first (2009/2010) and the last (2011/2012) year the turtles died because of the temperature and its durability below freezing point (310 hours, -10,8 °C -; 1022 hours, -2,7 °C). Note that in this depths the animals also had a negative effect by the temperature fluctuation until the freezing impact. In the first year at 1 m deep wintering place the lowest temperature was -0,5 °C and was below 0 °C during 65 hours and it caused 20% mortality.

In the second year (2010/2011) of all wintering animals (50 cm deep also) has survived successfully the hibernation period in the holes, and in the third year only the 1 m deep hole was suitable for hibernation. Note that in the second winter (2010/2011) at 50 cm deep the animals suffered lower weight loss, than the warmer conditions in 1 m deep because the mild winter.

The strategy of wintering in water was modelled with few months old and subadult and adult red-eared sliders in the second year. The examined turtles hibernated in the artificial lakes in three depths (50 cm, 80 cm and 1 m) at the bottom of the lake, bathed in sludge and successfully survived the hibernation without any exception. The turtles collected in Marc at awakening and all the animals appeared to be healthy. The suffered weight loss during wintering was not exceeded the 6 %, which is considered for ground tortioses. The best weight loss showed by the adult turtles in the pond with 80 cm deep, where the average value was 0,8%. The subadult sliders in this lake suffered more average weight loss (8,4%). In case of few months old hatchlings at 50 cm deep I observed 3,3 % average body weight loss, but at 1 m this showed 2,8 % decrease. Overall, the 30 wintering red-eared sliders weight loss was 3,57 % (SD = 3,06) in ponds.

If the condition of carcasses were enable all of the died animals dissected. To start the post mortem process I cut the carapace at the junction of the plastron and the carapax with an iron saw on both sides and lifted the plastron to view the ventricle.

I found same results in case of turtles which wintering at the attic of the house (2009/2010). All of the carcass, plastron and carapax proved to be intact. The place of organs in the body was normal. After opening the digestive system there was a small amount of yellowish-white mucous content. On the mucosa bright red pleats pin-point bleeding were observed.

The intestine contained little content. The other organs were free from any lesions. The scrapings from the gastric mucosa examined by microscope, when I observed a large number of moving briskly ciliated and flagellated protozoa. From these turtles digestive tube identified successfully grown primarily *Aeromonas* sp., *Pseudomonas* sp. and in some cases *Escherichia coli* bacteria.

In this season, each wintering turtles died in 50 cm deep holes. The recorded temperature data showed that the temperature was 7-15 °C among the animals at the beginning of the hibernation for a month, after the wintering it continued with a bit higher than the optimal temperature values and finally the freezing supervened. After the wintering period four cadavers from ten were autolyzed such a way that those substantive examination could not be carried out. It is assumed that these four animals had died in the early stage of the hibernation. At the post mortem examination of the remaining six in case of four turtles observed signs of gastritis what caused by ciliated and flagellated protozoans. The other two turtles had bright red coloration on their plastron. The body cavity of both animals had large amounts of bright red, clear serous liquid. The animals showed the signs of septicaemia and *Aeromonas hydrophila* bacteria were isolated from its livers and kidneys. In this year two turtles died also at 1 m deep during wintering, when diagnosed the above described septicaemia. In this case during the in vitro bacteriological examination *Aeromonas hydrophila* was isolated.

In the third wintering season (2011/2012) all of the seven young red-eared sliders died at the 50 cm deep holes. During the pathological examination the plastron of each carcasses flushed and in some cases the eyes bulged and the skin become redness. After opening the body cavities large amounts of bleary content was visible. Above the serous membranes

sometimes bleeding and in the liver inflammatory necrotic lesions was observed. In any case of the young turtles lungs showed signs of plethora. In each turtles *Aeromonas hydrophila* and from two animals *Pseudomonas* sp. was isolated in same time.

That same year, in the 50 cm deep wintering holes all of the five subadult and adult Florida Red-bellied turtle died. On one of the turtle's skin there was various sized and shaped, blackish-brown necrotic lesions observed that there were deep in varying values. After the opening of the body the organs showed no abnormalities. The size and shape of the liver was normal, but smaller or larger stock nodules were observed on it. The result of bacteriological test identified *Pseudomonas* sp. from here. In three turtles at the post-mortem examination the lesions were referring to septicaemia. From the turtles' liver in this case could be isolated *Aeromonas hydrophila* bacterias. The remaining one turtle before the start of wintering have discontinuities on its plastron in various sizes and shapes, which had different depth. This animal also dead to the end of the hibernation also. On the liver there were yellowish-white necrotic nodules observed, and in the in vitro examination *Flavobacter* sp. was culturable.

In September of 2010 I justified with cloacal swab examination in case of caught red-eared sliders with appropriate resistance from an artificial lake at Margaret Island, that the ciliated and flagellated protozoans parasites may be exist in turtles at active period without any health damage between optimal environmental conditions. The tested ten (two males and eight females) adult turtles shown healthy clinical symptoms during the sampling, but I have found the belows for infection of ciliated and flagellated protozoans (Table 1).

Table 1: Ciliated and flagellated protozoans infection in case of caught adult red-eared sliders from a Lake at Margaret Island

Sex	n	-	+	++	+++
		Negative	Slight infection (1-3)	Medium infection (4-8)	Intense infection (9 or more)
male	2	1	1	0	0
female	8	4	2	1	1

The blood test showed, that the posthibernation blood parameters significantly vary from the measurable values in active period. The blood values differences caused by the physiological processes in the awakening period. The glucose level was higher than the active period and the uric acid (this is four times higher than normal values), the values of GOT (AST), and GPT (ALT) as well. The amount of total protein and albumin did not show significant difference when compared two periods. The levels of LDH was higher in active period, than the measurable values in posthibernation. It is justified by the physiological processes during the hibernation and posthibernation period, when the roughage is not fully excreted from the body. The conditions of evacuation and life process initiation is become to be more favorable environmental conditions.

New scientific results

- The red-eared slider (*Trachemys scripta elegans*) and the Florida red-bellied turtle (*Pseudemys nelsonii*) surviving at natural conditions in Hungary is influenced by the environmental conditions during hibernation, when the temperature is the primary limiting factor in each year.

- The wintering is mainly successful if the temperature do not drops below freezing point for durable time, and it is not exceed 8-10 ° C for long term.

- Below the freezing point during the winterig the red-eared slider (*Trachemys scripta elegans*) and the Florida red-bellied turtle (*Pseudemys nelsonii*) suffered serious health damage which is fatal.

- If the temperature is hihger than the optimal during wintering in the body of the red-eared slider (*Trachemys scripta elegans*) and Florida red-bellied turtle (*Pseudemys nelsonii*) can multiply facultative pathogens to cause of turtle mortality.

- Body weight loss during the hibernation in case of the red-eared slider (*Trachemys scripta elegans*) is lower in case of the water wintering than in grounds or on the surface.

- The male red-eared sliders (*Trachemys scripta elegans*) can tolerate lower body weight loss than females.

- In case of the red-eared slider (*Trachemys scripta elegans*) I established a physiologically acceptable posthibernations blood parameters that would later be utilized for patient care activity practice for exotic animals.

4. Conclusions and recommendations

Based on the results obtained in the research it can be assumed that the red-eared sliders males less likely to survive the winter when the hibernation temperature is higher than optimal, which may strongly influence the possibility of making population. In my opinion, the chance of population creation process continue to reduce by laws, which not allow to keep red-eared slider legally from 1st of July 2010 (except the announced individuals during the grace period of entry into force), thus reducing ongoing supply by irresponsible owners into the natural waters. It should be noted that most publications describe observations from prevalence of females in water. I can only confirm this thesis by my observing turtles at the Lake of Margaret-Island and the Budapest Zoo and Botanical Garden Great Lake. The explanation of this fact is influenced by sexual dimorphism because the adult females have 30 cm length carapax or more, the adult males only 14-19 cm. The bigger females means more additional costs, than smaller males, so the females have greater chance realise them by owners.

The possibility of befall mild winters suggest the reproductive examination of red-eared sliders under natural conditions in Hungary and do investigations on free choosen wintering strategy by hatchlings and older turtles (in water, holes and on ground among stones etc.). It is highly recommend to implant into the animals temperature and hearth beating recording sensors which would give further scientific informations for understand the hibernation period individually. Knowing the hibernation success in Hungary and the increasing number of observations in the wild it can be considered to place floating traps to collect red-eared sliders from the areas where it is observed.

Because of the ban on breeding and keeping the red-eared slider it is expected the appearance of other species in pet markets in the coming years such as a „substitute” turtle. The yellow-eared slider (*Trachemys scripta scripta*) has a good chance to be the most common turtle among the pet owners. This species needs is very similar to red-eared sliders, and the breeding in large quantities can be solved. Due to the above mentioned, the revision for possible substitute species is particularly important because of the nature conservation and should do preventive steps by new legislations.

During the research have repeatedly founded ciliated and flagellated infections in different value. I consider it's appropriate to investigate the volume of infection at artificial habitat individually in every two weeks at least for a year regarding with the environmental conditions.

It is recommended to examine and specify the blood parameters of the red-eared sliders at least two times during wintering, additionally in the pre- and posthibernation periodically until they reach the activity. To get the adequate amount of blood necessary for regular and safe removal without any damage of animals they have to be at least 1500 g.

Publications in the topic

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