THE FIRST EXPERIENCE IN NEW TECHNOLOGIES OF BREEDING AND SEMI-NATURAL EGGS INCUBATION OF NORTHERN *EMYS ORBICULARIS* IN GLASS-HOUSE AQUACULTURE IN LATVIA

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Autochthonous *Emys orbicularis* are kept in herpetoculture in Latvian Rare Amphibian and Reptile Centre with the aim of their further releasing in the wild since 1984. In 2014-2015 we preliminary researched possibility of breeding and semi-natural eggs incubation of northern *E.orbicularis* in unheated glass-house aquaculture in Latvia. In a result of the research we found that adult males, semi-adults females and adult females begin spring sun-basking consequently in the experiment. Adult females for its first spring sun-basking need for higher temperature of shore than males. When adult *E.orbicularis* are ready for breeding, mating behaviour realises also if temperature of shore is low, temperature of water is more important. Eggs can be successfully semi-naturally incubated in unheated glass-house in Latvian climate. Glass-house herpetoculture can be efficiently used for breeding of northern *E.orbicularis*, allowing prolongation of active season and lowering the dangers of wintering for turtles.

Key words: *Emys orbicularis*, wintering, eggs incubation, aquaculture technologies, glass-house basin, nature conservation, Latvia.

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INTRODUCTION

Breeding of rare and protected species in the regulated conditions of zooculture with the aim of their releasing in the wild is widely popular method of nature conservation around the world and needs to more effective technologies development (Beck et al. 1994, Balmford et al. 1996, Snyder et al. 1996, Mathews et al. 2005).

Especially relevant are zoocultures of reptile species, populations of which are small in number and fragmented, and abilities for distribution and reproduction are limited (small number of offspring, long time for reaching of sexual maturity, high mortality on the initial stages of development caused by predators etc.). One of such species in Latvia is European pond turtle *Emys orbicularis* (Linnaeus 1758). Inhabiting and breeding in Latvia on the most northern border of its European range (Siliņš & Lamsters 1934, Berdnikovs 1999, Meeske 2006, Meeske et al. 2006, Pupins & Pupina 2008a,b), *E.orbicularis* is influenced here by many limiting factors, such as cold climate, retarding the development of turtles and making egg development impossible in cold years, and natural predators, competitors, and parasites (Fritz 2003, Meeske & Pupins 2009).

Among negative factors of anthropogenic origin there is predatoriness, parasitism and its new vectors, and competition from invasive species introduced by human: Red-eared slider *Trachemys scripta* and other exotic turtles species (Cadi & Joly 2003, 2004, Pupins 2007, Pupins & Pupina 2011), American mink *Neovison vison*, Racoondog *Nyctereutes procyonoides*, Ondatra *Ondatra zibeticus*, Chinese sleeper *Perccottus glenii (*Reshetnikov 2005, Pupins & Pupina 2012, Luca & Ghiorghița 2014), capturing of turtles by human and transformation of habitats (drainage, amelioration of ponds, roads etc.) (Pupins & Pupina 2007).

Autochthonous *E.orbicularis* are kept in the herpetoculture in the Latvian Rare Amphibian and Reptile Centre (former Centre of conservation of *E.orbicularis*) with the aim of their further releasing in the wild since 1984 (Pupins & Pupina 2014a,b). During this time, various methods of their keeping in indoor terrarium and in outdoor basin (in aquaculture) were tried, positive and negative sides in the trialled methods were found. Keeping in outdoor basin allowed keeping turtles in the natural climate conditions in Latvia and in semi-natural habitat. However, in the conditions of such keeping, *E.orbicularis* sun-basked naturally (Pupins & Pupina 2009) but is exposed to the influence of relative low temperatures which retards their development and exposes to high risk during hard under-water overwintering (the artificial basin isn't the same as a natural pond and has not so wide spectrum of conditions for wintering), on the whole decreasing the safety and efficiency of the *E.orbicularis* aquaculture.

An attempt to decrease this dangerous impact of cold climate in small basin but to preserve natural periods in temperature and lightening change can be keeping and breeding in unheated glass-house with a basin. The necessity to increase efficiency and safety of this aquaculture of northern *E.orbicularis* made the research of possibilities for breeding and semi-natural egg incubation in glass-house aquaculture in Latvia topical.

MATERIAL AND METHODS

Preliminary experiment was conducted in 2014-2015. Its goal was to assess the possibilities for wintering, keeping, breeding and egg incubation in the conditions of glass-house in the north of *E.orbicularis* range with the aim to increase the efficiency of aquaculture of the species. A breeding group of 20 adults and 8 semi-adults *E.orbicularis* from the most northern border of its range in Europe was used for the research. The experiment was conducted in Rare Amphibian and Reptile Centre in the south-east of Latvia, 55°50'5.35"N; 26°29'6.64"E.

The unheated plastic glass-house had an area of 104 m^2. and transparent polycarbonate roof and walls (Fig. 1). For the experiment parts of a common basin were used, fenced off with plastic mesh bulkhead, with the areas of 35 m^2 (for adult

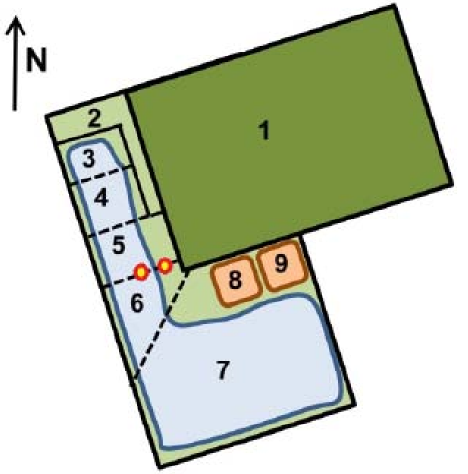

Fig. 1. Scheme of the construction of the experimental glass-house: 1 – building of the Centre; 2 – filter and air compressor part; 3, 4, 5 – unused in the experiment parts of the basin; 6, 7 – experimental parts of the basin; 8, 9 – places for eggs-laying; two small red-yellow circles – A and B points of measurement of temperature on concrete shore and in water.

turtles) and 8 m^2 (for semi-adults turtles). The average depth of the basin was 0.7 m; maximum – 1.2 m.

The basin had an Emys-friendly special design: easy-to-toe claws concrete bottom, painted in natural green-yellow tones, with gently sloping sides. It was filled with water taken from natural pond and equipped with an air compressor in each of the fenced parts and joint filtration. There were plastic containers with water plants (*Acorus calamus*) on the bottom, snags and planks in the water and on the watersides (Fig. 2). On the gently sloping side in the outdoor cage for adults there were two plastic boxes for egg laying, which had an area of 2 m^2 and depth of 0.2 m each, filled with fine semi-humid sand, with drainage. *E.orbicularis* were being fed daily in warm weather with raw cut *Clupea harengus membras* to satiety, feeding was stopped in autumn, when turtles systematically refused to feed.

For the research only available in Latvia and collected since 1984 according permissions of Nature Conservation Agency extremely small in number breeding group was used, but its main purpose was the reproduction of *E.orbicularis*. Therefore, any invasive intervention in keeping of the breeding group during the experiment was minimized. In the glass-house we noted time and temperature of the first sun-basking in spring for males, females and semi-adults, first time of egg lying and appearance of the first hatchlings.

Fig. 2. Emys-friendly design of the experimental glass-house basin in summer.

Temperature measurements were taken since 2014.12.26 in the afternoon (14:00 – 15:00), when the glass-house was warmed up in the sun. We took measurements in the middle part of glass-house of water at depth of 0.3 m in the point A (temperature of water) and the temperature of horizontal surface of sunlit concrete side in the point B (temperature of shore), as a model points of potential sites for wintering and sun-basking (Fig. 1). The points A and B were in distance of 0.7 m between the points.

As a control group we also registered the first sun-basking *E.orbicularis* in spring after wintering in the nearby outside fenced artificial concrete basin. The design of the outside basin was Emys-friendly also and all the conditions (water from natural pond, the deep, sloping sides of natural colours, water plants, snags and planks in water and on watersides) were similar in both basins.

Laid eggs were incubated semi-naturally in the eggs-laying places. We kept the sand being semi-humid. After finding of the hatchlings inside of the sand these were captured and measured. We measured its carapace length (CL) and carapace width (CW) by electronic callipers with accuracy 0.01 mm and naturally wet body weight (BW) by electronic weighing-machine with accuracy 0.01 g. Hatchlings found out-side of sand and in the basin were not measured.

The obtained results were processed using methods of descriptive statistics and ANOVA.

Table 1. First observations of *E.orbicularis* spring sun-basking behaviour in the experiment

Observed behaviour in the glass-house	Date	Time	Temperature of shore	Temperature of water
First sun-basked adult male	2015.03.05	15:07	+11.9°C	+7.4°C
First sun-basked semi-adult female	2015.03.13	14:48	+25.4°C	+9.0°C
First sun-basked adult female	2015.03.14	14:15	+30.6°C	+12.5°C
Mass sun-basking: adult and semi-adult females and males	2015.03.15	15:29	+30.6°C	+13.7°C

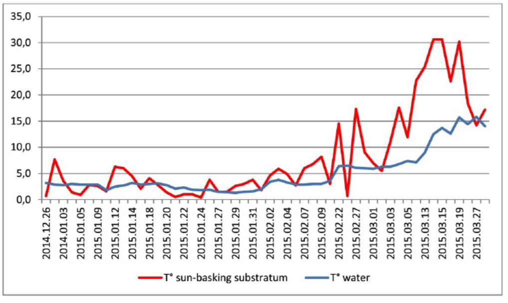

Fig. 3. Dynamics of measured temperatures of shore and water in the experimental glass-house.

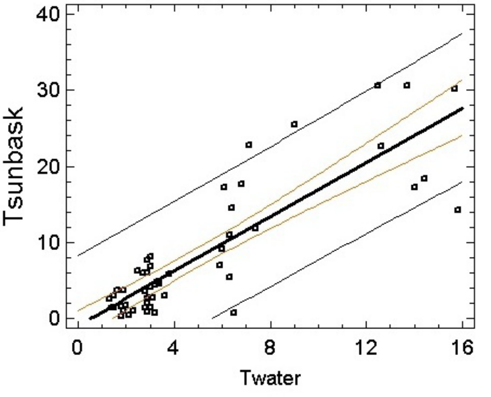

Fig. 4. Plot of fitted model for temperature of sun-basking place (Tsunbask) and temperature of water in the basin (Twater) of the glass-house herpetoculture of *E.orbicularis*.

RESULTS

For the experiment the *E.orbicularis* were placed in the glass-house on 2014.06.28. That winter was unusually very warm for Latvia. Throughout the overwintering the basin was periodically covered with tin ice, 20-30% of surface, but it did not freeze completely. Measured temperature of shore was ≥+0.4°C, temperature of water was ≥+1.3°C for all the time of the experiment (Fig. 3).

To describe the relationship between temperature of sun-basking place (Tsunbask) and temperature of water in the basin (Twater) we estimate the results of fitting a linear model. The equation of the fitted model is Tsunbask = -0.891541 + 1.78394*Twater (Fig. 4). Since the P-value for the ANOVA is less than 0.05, there is a statistically significant relationship between temperature of sun-basking place and temperature of water in the basin at the 95% confidence level.

During the winter some *E.orbicularis* kept their weak motions, periodically slowly changing their location sites in water. After the wintering the first sun-basking male was observed in 2015.03.05. In the outside control basin the first sun-basking male was registered on 2015.03.14 at 14:22 – for nine days later.

Eight days after the first sun-basked male, we observed in the experimental glass-house the first sun-basking semi-adult female, on the next day – first sun-basking adult female, afterwards sun-basking became mass for all *E.orbicularis*. The observations of the sun-basking actions, dates and temperatures of sunlit concrete surface and of the water are shown in the table (Table 1).

It was also observed the case, that the same first sun-basked female stayed on the sun-basking site, without leaving it, since 2015.03.16 (t° of shore = +22.6°C; t° of water = +12.6°C) until 2015.03.19 (t° of shore = +30.2°C; t° of water = +15.7°C) when mowed to water.

First breeding behaviour, when males were on female carapaces, was observed on 2015.04.23 in two pairs, one of them was sun-basked for 2 hours ashore (Fig. 5) (t° concrete surface = +17.2°C; t° water = +14.0°C).

The temperatures of shore and water were important for beginning of sun-basking in all three groups: adult males, semi-adults females and adult females, which begin spring sun-basking consequently. First sun-basking of adult females was an index of beginning of mass sun-basking of *E.orbicularis* of all ages and sizes. When adult *E.orbicularis* are ready for breeding, this behaviour can realise also, if temperature of shore is low, probable, the temperature of water is more important (Fig. 6).

Fig. 5. First breeding-sun-basking behaviour observed in the experiment.

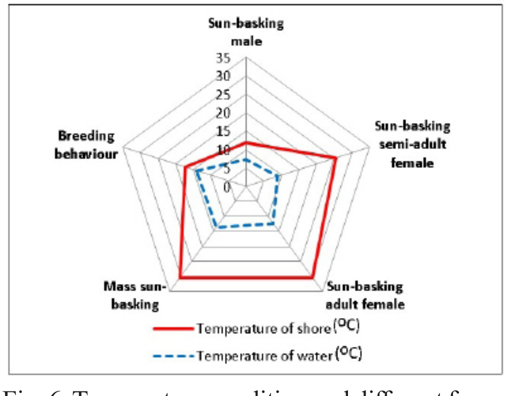

Fig. 6. Temperature condition and different forms of behaviour, observed in the experiment for the first time.

First eggs-laying was observed on 2015.05.22 and continued from 19:57 to 21:22 (time=85 min). Last eggs-laying was observed on 2015.06.11 at 21:22. All laid eggs and its places were not disturbed in any way and the eggs were left in laying sites for the semi-natural incubation.

First *E.orbicularis* hatchlings, which appeared independently out of sand, were found in water, in the basin on 2015.08.26. They developed from eggs laid on 2015.05.22 and their development took 97 days. Subsequently, two new groups of hatchlings were found in the basin and ashore on 2015.08.28 and on 2015.09.10.

As other hatchlings did not come out from the ground preparing for wintering and it can be high risk for the hatchlings, 2015.10.13 we dug out all sites for egg laying and collected 37 hatchlings (Fig. 7).

Its carapace length (CL), carapace width (CW), and body weight (BW) were measured. The values of these parameters are cited in the table (Table 2).

Fig. 7. One of *E.orbicularis* hatchlings found in sand.

Table 2. Summary statistics of *E.orbicularis* hatchlings' CL, CB, and BW (n=37)

	CL	**CB**	**BW**
Average	26.7195	24.7154	5.10919
Standard deviation	1.61313	1.46353	0.820008
Coefficient of variation	6.03729%	5.92151%	16.0497%
Minimum	22.38	20.0	3.38
Maximum	29.3	27.04	6.26
Range	6.92	7.04	2.88

Table 3. Correlations between each pair of variables: CL and BW, CL and CB, BW and CB. P-Value typed in bold

	CL	BW	CW
CL		0.8771	0.5673
		0.0000	**0.0003**
BW	0.8771		0.5682
	0.0000		**0.0002**
CW	0. 5673	0.5682	
	0.0003	**0.0002**	

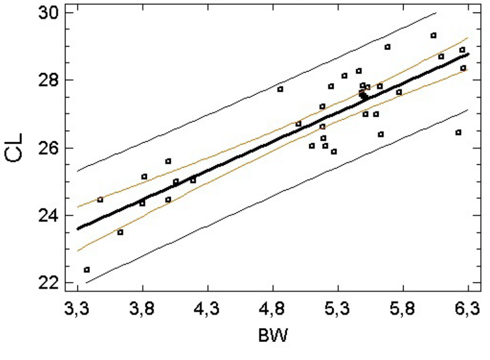

Fig. 8. Plot of fitted model for CL and BW (n=37).

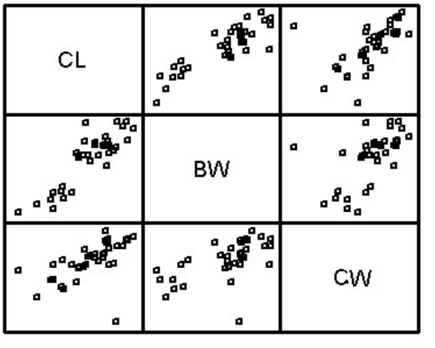

Fig. 9. Scatterplot matrix for each pair of variables: CL and BW, CL and CB, BW and CB.

For description of the relationship between CL and BW, which can be most important values for hatchling's successful wintering fitting a linear model was used. The equation of the fitted model is CL = 17.9034 + 1.72554*BW (Fig. 8).

Correlations exist between all three pairs of the variables (Fig. 9). The table (Table 3) shows correlations between each pair of variables: CL and BW, CL and CW, BW and CW. The correlation between CL and BW is stronger, that between CL and CW, BW and CW. The third number (in bold type) in each location of the table is a P-value. All correlations have P-values below 0.01.

In total, in 2015 there were 56 hatchlings obtained, which was a record number in our *E.orbicularis* breeding practices.

DISCUSSION

Based on our many-years' experience, exactly overwintering is the most threatening period for *E.orbicularis* in Latvian herpetoculture. Conducted research showed that northern *E.orbicularis* can be successfully kept all year round in the conditions, described above, of glass-house herpetoculture in Latvian climate. All kept *E.orbicularis* overwintered successfully in the glass-house. At the same time, the winter of 2014-2015 was extraordinary warm for Latvia, thus, it cannot be claimed that such keeping will be equally successful in common for Latvia cold winters with usual temperatures below -25°C.

The same also concerns semi-natural egg incubation. For the first time in our practices, we did not incubate eggs in an incubator, but left them untouched to develop in the egg laying site. The warm summer of 2015 facilitated relatively successful development of eggs, though cold and rainy summers can interfere with their development.

In the experiment, in glass-house males of *E.orbicularis* were the first to come out for sun-basking, then semi-adults, and on the next day – adult female. This matches the data on natural conditions from other authors (Lukashevich V., Bakharev V. pers.com.) and our experience from our field research in Belarus. It should be noted that, the first sun-basking male in the control group (outdoor basin) was being observed simultaneously with the first sun-basking female in the glass-house (2015.03.14).

Thus, higher spring temperatures in the glass-house allowed females of *E.orbicularis* to start sun-basking much earlier (as males in natural temperature conditions), as a result, allowing to start preparation for reproduction earlier.

We took the temperature measurements of prevailing in the glass-house substratum for sun-basking-concrete and in one model point of glass-house on horizontal surface. At the same time, in the experiment, *E.orbicularis* actively used for sun-basking gently sloping sides and other substratum, available in the glass-house: snags, tree branches, planks. This substratum was different from the painted concrete in colour, chemical composition, texture, thermal capacity, thermal conductivity, humidity etc. On this basis, the temperature of other kinds of substratum can differ from the surface temperature of model concrete site measured during the experiment. Therefore, presented data on temperature of surface for sun-basking have comparative value.

During the experiment, *E.orbicularis* males mostly did not leave females while their coming out ashore for sun-basking, remaining on female carapace. It allowed males not only to protect chosen female from other competing males (Poschadel et al. 2006), but also to get essential in this period spring sun-basking. Thus, the other individual of *E.orbicularis*, female of their own species, became the substratum for sun-basking male. Such "substratum" was active, that is, female moved independently and chose sites heated by sunlight and maintained optimal temperature, thus saved males' energy which they had spent for maintenance of the optimal temperature of their body. At the same time, female own sun-basking became less effective.

As a possible benefit for females and for survival of the population on the whole, because usually there are more males in the population than females, we can assume possible better protection with male's body against predators, which (in our case experimenter) attack on top, and it can be especially relevant during spring sun-basking, when females have not yet warmed up and are relatively slow.

Some of the possible issues for sun-basking of *E.orbicularis* in glass-house herpetoculture are possible decrease of received ultraviolet (UV) lightening due to polycarbonate roof and walls. It is possible that vitamin D_3 is necessary to be additionally included in *E.orbicularis* diet. Based on importance of ultraviolet for *E.orbicularis* sun-basking in the north of its range in Latvia (Pupins, Pupina 2009), it might be a threat for glass-house herpetoculture *E.orbicularis*.

Eggs of northern *E.orbicularis* can be successfully semi-naturally incubated in the conditions of unheated glass-house herpetoculture in Latvian climate. In the experiment the Mean (± SD) carapace length (mm) of hatchlings was 26.71 (± 1.61) and the Mean (± SD) body weight (g) of hatchlings was 5.11 (± 0.82), that is more than in more southern *E.orbicularis* hatchlings in Doñana National Park: (Mean (± SD) carapace length of hatchlings (mm) 26.15 (± 0.73); Mean (± SD) body mass of hatchlings (g) 4.75 (± 0.44)) (Carmen et al. 2014). The hatchlings in our experiment had also longer CL and more BW than hatchlings from Poland (Najbar,. Mitrus 2013). The difference can be connected with more optimal temperature conditions in the glass-house or with most northern hatchling's evolutional adaptation for harder and longer wintering on northern part of *E.orbicularis* distribution in Latvia.

Taking into consideration progressing warming of Latvia's climate, experiments with northern *E.orbicularis* in warmer conditions of glass-house can be a preliminary experiment for further ecological research of adaptation possibilities for northern *E.orbicularis* to the warming conditions of global climate change.

CONCLUSIONS

Adult *E.orbicularis* males, semi-adults females and adult females begin spring sun-basking in north of its range of distribution consequently. Adult females for its first spring sun-basking need for higher temperature of shore than males.

Glass-house herpetoculture *E.orbicularis* can be efficiently used for breeding of northern *E.orbicularis*, allowing prolongation of active season and lowering the dangers of wintering for turtles.

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