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ABSTRACTS

of the international workshop–conference

“RESEARCH AND CONSERVATION OF EUROPEAN
HERPETOFAUNA AND ITS ENVIRONMENT: *BOMBINA BOMBINA*,
EMYS ORBICULARIS, AND *CORONELLA AUSTRICA*”

www.life-herpetolatvia.biology.lv

Daugavpils University, Institute of Ecology
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The LIFE+ Project LIFE-HerpetoLatvia LIFE09NAT/LV/000239 "**Conservation of rare reptiles and amphibians in Latvia**", which is co-financed by the LIFE Programme of the European Commission, in a scientific cooperation with the: Institute of Ecology of Daugavpils University; Institute of Systematic Biology of Daugavpils University; Latgales Zoo of Daugavpils city council; Latgales Ecological Society, and with support of the: Ministry of Environment of Latvia; Nature conservation agency of Latvia, is organizing the **international workshop–conference** entitled:

RESEARCH AND CONSERVATION OF EUROPEAN HERPETOFAUNA AND ITS ENVIRONMENT: *BOMBINA BOMBINA*, *EMYS ORBICULARIS*, AND *CORONELLA AUSTRIACA*

The international scientific – practical workshop–conference was held in **Daugavpils, Latvia** (Daugavpils University, Parades str.1; 55°52'11.71"N; 26°30'30.80"E) on **8th – 9th October 2012**.

The main topics of the workshop–conference covered different aspects of research, management of populations, and conservation in-situ and ex-situ of three species of herpetofauna: *Bombina bombina*, *Emys orbicularis*, and *Coronella austriaca*:

- Practical results of LIFE, European, national, local and other projects connected with conservation or research on these species;
- Genetic analyses and its role in management of populations;
- Management practices for sustainability of populations;
- General biology and ecology of these species;
- Connections between human impact, water and terrestrial ecosystems and these species;
- Experimental and theoretical approaches to surveys and data processing;
- Distribution, new findings, natural area, climate and habitats;
- Zoocultures of three main species, technologies of keeping, domestication's problems;
- Health conditions, diseases and veterinary;
- Re-introduction programs, population enforcements by releasing in wild and their results or problems.
- Diets and feeding of these species;
- Aquatic and terrestrial invasive species as threats to these species;
- Role of education and public awareness in conservation programmes

Practical part of the workshop–conference was in a form of work visits to:

- sites and territories of Project LIFE-HerpetoLatvia;
- husbandry facilities in Latgales Zoo;
- facilities for *Emys orbicularis* breeding in Reare Amphibian and Reptile Breeding Centre, which is being renovated during LIFE-HerpetoLatvia Project;
- future Natura 2000 territory for *Bombina bombina*;
- sites of *Emys orbicularis* findings in wild;
- territory where largest *Bombina bombina* population in Latvia is found (site called "Demene").

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ECOLOGICAL NETWORK FOR THE EUROPEAN POND TURTLE (*EMYS ORBICULARIS*) IN LITHUANIA

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The population of European pond turtles in Lithuania is estimated to be around 400 individuals, majority of them live in the southern Lithuania. There is an ecological network of five Natura 2000 areas designated for this species, the average rectilinear distance between the closest areas is 8,3 km. Some smaller populations of the pond turtles are more remote from the protected populations. The survival of the northern pond turtles depends on the availability of habitats and ecological connectivity. Therefore a more detailed ecological network for the pond turtles is being created in the south Lithuania. The core areas of the network are in the Natura 2000 areas and the corridors are created as stepping stones, restoring water habitats between the Natura 2000 areas and smaller turtle populations. The stepping stone habitats are protected according to the individual agreements with the landowners. The final goal is ratification of the ecological network by the Ministry of Environment and inclusion of it into the general plans of the municipalities. The network is being created in the frame of LIFE+ Nature project “Development of a Pilot Ecological Network through Nature Frame areas in South Lithuania” 09/NAT/000581.

OCCURRENCE OF THE FIRE-BELLIED TOAD *BOMBINA BOMBINA* IN LARGE POLISH CITIES

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The fire-bellied toad *Bombina bombina* is one of 18 species of Polish batrachofauna. The species is widespread throughout the country with the exception of the Carpathians and the Sudetes (Szymura 2003). In Krakow city, it is observed since 1922 (Juszczuk 1989).

The aim of the study was to determine the current distribution of the fire-bellied toad in Krakow and in two other large Polish cities (Wroclaw, Warsaw) on the basis of literature data.

Krakow is located in southern Poland, in the central-northern part of the Lesser Poland Voivodeship. The valley of Vistula river makes extended latitudinal axis of the city. In the period April - June 2011 and 2012 inventory studies on 32 water bodies in 12 districts of the Krakow were performed. The presence of amphibians was determined by direct observation of adults, juveniles, tadpoles and by males mating calls.

The occurrence of the fire-bellied toad was found in 9 sites, located in the western and eastern parts of Krakow. 7 sites of the species are situated in two the largest districts, where the population density is relatively low. The proportion of occupied habitat by the *Bombina bombina* is 0,28. In Warsaw the species is very rare - the proportion of the habitat is 0,09 (Mazgajska 1998), whereas in Wroclaw it is the third common amphibian of the city, and the proportion of the habitat is 0,45 (Ogielska & Kierzkowski 2010).

Conclusions:

- Proportion of habitat occupied by the fire-bellied toad varies in different Polish cities.
- The *Bombina bombina* occurs most numerous in Wroclaw.
- Different number of the fire-bellied toad habitats may reflect the presence of preferred by the species warm, shallow natural water bodies within the cities.

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DISTRIBUTION OF THE FIRE-BELLIED TOAD *BOMBINA BOMBINA* AT THE RAILWAY LINE WITHIN THE HYBRID ZONE IN POLAND

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Occurring in Poland the fire-bellied toad *Bombina bombina* and the yellow-bellied toad *Bombina variegata* are parapatric distributed and interbreed in a narrow, approximately 20-kilometers long hybrid zone of their ranges on the threshold of the Carpathian Foothills (Szymura 1993). Although hybridization in the contact zone, both species maintain their separateness (Szymura & Barton 1991). The main goal of the study was to determine the current distribution of the fire-bellied toad in areas in the vicinity of the railway line (Podłęże - Biadoliny). Additionally, the aim was to check whether the study area is situated in the hybrid zone of the *Bombina*.

Area of the study concerned part of the railway line on the section Krakow - Tarnow, in the south-eastern Poland. The research was conducted during two breeding seasons of amphibians over a distance of 45 km Podłęże - Biadoliny railway line (2011) and 22 km Bochnia - Biadoliny railway line (2012). Inventory of amphibians concerned areas located on both sides of the track (about 150 m) and the same track. The presence of amphibians was determined on the basis of direct observation of adults, juveniles, spawn, tadpoles and males mating calls. The main criterion differentiating the *Bombina bombina* from the hybrid was spots pattern on the ventral side of the body.

At the foot of the railway embankment, there are numerous marshes and ditches which are habitat, inter alia, for the fire-bellied toad. The *Bombina bombina* was observed most frequently and the most numerously in very humid areas in Podłęże, Kłaj and Sterkowiec. In the latter place its habitat often overlap with agile frog reproductive habitats. The individuals of hybrid phenotype was observed in Kłaj and Sterkowiec. There was no fire-bellied toads on the overhead trolley, while on the road under the viaduct one dead individual was reported.

Conclusions:

- Wetlands beside the railway lines are habitat for the fire-bellied toad.
- The study area is a part of the hybrid zone of the *Bombina*.
- The track is probably not-to-cross barrier for the *Bombina*.

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- Szymura J.M. 1993. Analysis of hybrid zones with *Bombina*. In: Harrison R, ed. Hybrid zones and the evolutionary process. New York, NY: Oxford University Press, 261-289.
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IS DETECTABILITY A PROBLEM IN SMOOTH SNAKE RESEARCH AND CONSERVATION? – REVIEW

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Introduction

Smooth Snake is commonly defined as a species difficult to study. Mainly because of its rarity, even most publications from XX century described this species as a very rare and endangered in Poland. For example Juszcyk (1974) wrote: “Although Smooth Snake occurs in whole country, but it is rare snake, without known large populations”. Also Najbar (1997) treat Smooth Snake as a rare species, even “in danger of extinction”.

However it appears that Smooth Snake maybe not so rare in Poland, as it is commonly said. Review of Polish literature shows non-compliance on the status of Smooth Snake.

Situation in “Ojcowski National Park”

For a long time Smooth Snake was considered as a vulnerable species in Ojcowski NP:

- Antoszevska-Bugno, Młynarski 1977: “rare and low-numbered”
- Młynarski 1990: “almost extinct”
- Szynclar 1995: describes only one observation

In publication from 2007 Wiśniowski and Rozwałka described observations of 9 Smooth Snakes in Ojcowski NP. Those observations were done accidentally, during arachnological research.

As we can see, previous statements were misjudged.

Wiśniowski and Rozwałka (2007) observed that common opinion about Smooth Snake activity, which said that this species is the easiest to find during hot, sunny weather is incorrect. That statement was confirmed by Bury (2009).

Situation in Bieszczady Mountains

According to Młynarski (1971) Smooth Snake is common species in Bieszczady Mountains. However review of literature (Bury 2009) shows that it is not true and *Coronella austriaca* is known only from few localities in Bieszczady Mountains.

New localities of Smooth Snake after 2000.

Most of new localities of Smooth Snake are published in polish journal “Let’s protect our nature” (“Chrońmy przyrodę ojczystą”). Since 2000 more than 30 new localities were described (f.e. Żmihorski et al. 2011, Dembicka et al. 2006, Zieliński et al. 2002) in this journal. Still new are found. In most cases those findings describe small populations or just few specimens. Taking into account above examples (Ojcowski NP, Bieszczady) it is difficult to say whether it is result of actual low number of snakes whether of detection only small number of snakes. However it is well shown that first publications were mistaken as to the status of *Coronella austriaca* in Poland.

Monitoring of Smooth Snake in Poland – what is the real status of species?

In years 2009-2010 professional project of monitoring of Smooth Snake in Poland was carried out (“Monitoring of species and natural habitats” led by Institute for Nature Conservation PAS). This action shows that Smooth Snake is not common species indeed and should be protected, in many case through active conservation, but also population status not critical.

Conclusions

- Despite doubts connected with difficulties in estimation of real occurrence and density because of low detectability, Smooth Snake still should be defined as a very rare species in Poland (according to results of Polish wide monitoring).
- Detectability is anyway big problem that can lead us to faulty planning of research, conservation and management of the species.

PROBLEMS WITH LAW PROTECTION OF SMOOTH SNAKE IN POLAND – CASE STUDIES

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Introduction

Smooth Snake is very rare species of snake in Poland. It is found in whole country, but there are only few localities where exist large, stable populations. In Polish Red Data Book of Animals smooth snake is categorized as a vulnerable.

According to polish law this species is under total protection. Killing, capturing, damaging, startling etc. is forbidden. Also habitats of *Coronella austriaca* are protected (within 500 m radius between 1 march and 31 august, and 100 m radius the whole year). In such protected area staying of all people (except of the owner of the area) and any activities that could change the habitat are forbidden. Felling of trees and shrubs, and a change in water are possible only with legal authorization, when they are necessary to protect the habitat.

Unfortunately some restrictions can impede conservation of Smooth Snakes.

Case 1 – Smooth Snakes in Bieszczady Mountains (locality Bóbrka)

In 2005 in stone-pit in Bóbrka smooth snakes were found. In 2007 locality was reported to Regional Directorate of Environment Conservation. RDEC attempted to create protected area (see above). In such situation agreement of the owner is not necessary to create protected area. However, by RDEC, it is necessary to inform the owner. In this case attempts to contact with the owner failed. Local authorities were not able to find the owner. Case was consider completed.

Eventually in 2010 protected area was created, despite problems in contact with owner. This time application to create that area was filled by Society for Amphibians and Reptiles Conservation “Tryton” (Mazgajska J. – personal communication).

Case 2 – Smooth Snakes In Cracow (Zakrzówek)

In 2010-2011 more than 20 smooth snakes were observed in the city of Cracow.

Therefore it may be one of the biggest populations of *Coronella austriaca* known from Poland. Because of high number of individuals it should be protected through making protected areas. Unfortunately smooth snakes localities are very often visited by people (recreation activities). According to the law presence of people in such areas is forbidden, without any exceptions. However in Zakrzówek presence of people is not harmful for snakes. It is even beneficial, because it helps in sustaining xerothermic meadows.

This is the biggest problem of this case. In 2010 “Skołczanka” Nature Reserve was set on fire, probably because of local aversion to nature conservation. In smooth snake case such situation is possible too. There is high possibility that forbidding entry to Zakrzówek would be badly received by local society.

Still, official protection of those localities is required, because of pressure from the developers.

Problem of smooth snake conservation in Cracow is not solved yet.

Conclusions

- Way to create protected area should require owner agreement to minimize possible conflict with the owner, by taking from the owner possibility to make a decision about his property.
- There should be legal possibilities to derogation from restrictions, such as presence of people in protected area, when it is not harmful.

OCCURENCE OF THE SMOOTH SNAKE *CORONELLA AUSTRIACA* (LAURENTI 1768) IN URBAN AREA (CRACOW, SOUTHERN POLAND)

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Introduction

Smooth snake (*Coronella austriaca*) is a snake found in central and northern Europe. In most areas of distribution Smooth Snake is rare and protected species.

It is one of the rarest species of reptiles in Poland. According to the Polish Red Data Book of Animals smooth snake is categorized as a vulnerable.

The aim of this study was to determine the size and structure of the population of Smooth Snakes in the urban area of Cracow.

Methods and study area

The study was carried out in 2011 between April and September (whole season of Smooth Snakes activity) and in August 2010. Typical habitats of Smooth Snakes, such as xerothermic meadows, rocky places, forest edges, were penetrated in favorable weather conditions mostly in morning and afternoon hours.

The study was conducted in Kraków. Kraków is the second largest city of Poland

Results

Tab. 1. In both areas 29 Smooth Snakes were found:

Specimens Locality	Adult, males	Adult, females	Juveniles, subadults	Adult, sex not known
Tyniec	8	4	5	0
Zakrzówek	5	4	2	1

Additional observations:

- In both areas 2 pregnant females were found.
- One dead specimen (killed by car) was found in Tyniec.
- 4 harmed specimens (3 in Tyniec, 1 in Zakrzówek) were found.

Discussion

My data shows the presence of vulnerable species in urban area, in big city, which is rare situation. Despite the activity of human population of Smooth Snake still persists.

In the studied areas exist probably quite large and stable population of Smooth Snakes. However according to quite short time of research total, reliable number of snakes is impossible to estimate.

The important information is observation of pregnant females and young snakes. This shows a certain, but difficult to precise reproductive potential of the population.

CONSERVATION OF THE SMOOTH SNAKE (*CORONELLA AUSTRICA*) IN LATVIA

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Smooth Snake (*Coronella austriaca*) is very rare species in Latvia, located on the northern border of its species range, with confirmed records mainly in the coastal area west and south from the Riga Gulf. It has specific habitat requirements and feeding objects, slow reproduction rates and poor dispersion ability. Studies are bothered by its secretive habits. The Smooth Snake is one of the LIFE-HerpetoLatvia Project target species in Natura 2000 area – Ķemeri National Park. Here, best-known and probably largest population is located in the Slokas Bog, where habitats have been subjected to the various degree of degradation due to the drainage and peat mining activities. The snake inhabits clearings created by old burns, periphery of raised bogs, and open roadside vegetation belts. Size of population, estimated from mark-recapture study, is ~50 adult snakes. In some locations, there have been significant changes due to vegetation succession in last decades resulting in the decreasing of reptile population. Survey in 2011-2012 has showed poor connectivity between different sub-populations of the Smooth Snake in the Slokas Bog. Proposed population management here is the habitat improvement and creation of the corridors for exchange of the individuals from different sub-populations. These are clearing of sides of minor roads on a dam (2,3 km; 2.3 ha) for population connectivity improvement and enforcement of the lizard population (snake's feeding object), and forest improvement cutting (area of 3,5 ha) for existing snake habitat improvement, creating of new habitats and population connectivity corridors. The habitat succession in these sites latter should be monitored. In the future populations in other parts of Latvia also should be surveyed for necessary management actions, and their connection to the population in the Ķemeri National Park should be enhanced.

CAPTIVE MANAGEMENT OF EUROPEAN FIRE-BELLIED TOAD, *BOMBINA BOMBINA*, IN RIGA ZOO

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The zoos' role in species conservation is well known. Zoo setting provides the advantages for creating and maintaining captive populations of threatened species, and those can serve as a source for reintroduction and wild population supplementation.

The Amphibian department of Riga Zoo (former Laboratory of Ecology) has kept *B.bombina* since 1987. The first breeding cases of this species were achieved with hormonal stimulation. Since 1998 *B.bombina* bred both in display and laboratory naturally, without hormonal stimulation.

During years, a good experience of keeping, breeding, tadpole rising and hibernating of species is accumulated in Amphibian department. Tadpole survival rate is up to 86% during last years, as well as the losses of animals during hibernation are reduced noticeably.

Nevertheless, there are still unanswered question regarding captive management of this species, i.e., the influence of inbreeding to long term captive population.

ANTHROPOGENIC IMPACT ON *BOMBINA BOMBINA* (L.) POPULATED HABITATS IN THE SOUTH PART OF THE EGLAINE PARISH (ILUKSTE DISTRICT, LATVIA)

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Introduction. *Bombina bombina* is a very rare amphibian in Latvia that is found only on the very south of the country. A Plan on conservation of *Bombina bombina* in Latvia (Pupiņš, Pupiņa 2006) recommends conservation and restoration of biotopes for *Bombina bombina*. Anthropogenic influence on biotopes of *Bombina bombina* (amelioration, use of water, breeding of fish, etc.) might influence negatively on the quantity of populations of *Bombina bombina*. Roads might be dangerous for *Bombina bombina* during their migration and settling. In addition to that, *Bombina bombina* in Latvia inhabit many biotopes that, to different degrees qualitatively and quantitatively, undergo influence of economical activities of people. This makes the study of anthropogenic influence on the biotopes of existing populations of *Bombina bombina* in Latvia urgent.

Material and methods. The study was carried out in 2011-2012 in Eglaine parish (Ilukste district), Latvia. Water biotopes, inhabited by *Bombina bombina*, were detected by auditive calculation of vocalizing males. Distance to human settlements and roads was defined by range finder or with the help of service Google Earth. Use of reservoir by people was defined by interviewing.

Results. In the result of the study in 2012 were registered 19 reservoirs inhabited by *Bombina bombina*. An average distance from a reservoir to a soil rarely used road comprised 195 m (St.dev.± 234 m); to a rubble road with rare traffic comprised 306 m (St.dev.± 299 m); to an asphalt road with busy traffic – 2396 m (St.dev.± 662 m). Distance from the reservoir of *Bombina bombina* to the nearest house comprised in average 279 m (St.dev.± 368 m). The biggest part of reservoirs (57 %) is used for giving water to cattle, only two reservoirs are used for watering (14%), 29% are not used by people in economical activities.

Discussion. An active economical activity of people, undoubtedly, influences the condition of biotopes and, correspondingly, populations of *Bombina bombina*. Thus, on the results of the study, it was noted that minimal distance from inhabited biotope to the asphalt road with busy traffic comprised 1568 m. The data of the study might be used at planning of development of Eglaine parish and other regions of Latvia, in which *Bombina bombina* is distributed, as well as the data will be used at the inspection of the Plan on protection of *Bombina bombina* in Latvia.

Acknowledgements. The research has been executed owing to support of Daugavpils University, Daugavpils Dome, Latgales Zoo, part of information was received in a cooperation with LIFE+ Project LIFE-HerpetoLatvia LIFE09NAT/LV/000239.

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EMYS ORBICULARIS, BOMBINA BOMBINA, CORONELLA AUSTRIACA IN BALTIC REGION ZOOCOLLECTIONS

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Introduction. There are various public zoological collections located in Europe and around the world such like National zoological gardens, safari and other kinds of animal collections that have an important role in society. First of all, zoos serve as extensive centers of information presenting its visitors with the data concerning specific animal as well as providing info on certain animal habitats, their characteristic features, alimentary features and important information regarding the protection the selected species need and their role in national biological diversity. One of the main aims of the National Program of Biodiversity Preservation is *ex-situ* species protection and preservation measures outside their natural environment. Zoos take a part in educated society in many questions what is connection with nature. First of all at zoos made informational systems what describes people role in sustainable development, and people's relations with the wild nature [1,2]. A lot of zoos and aquariums association (EAZA, ERAZA, WAZA) accents that zoo aim is motivate people understanding decrease of biological diversity problem and motivate people to take act for it saving [3].

Material and methods. For research object was chosen nine Baltic Sea region zoos: Riga national zoological garden (Latvia), Latgale zoo (Latvia), Kaunas zoological garden (Lithuania), Tallinn zoological garden (Estonia), Helsinki zoological garden (Finland), Warsaw zoological garden (Poland), Plock zoological garden (Poland), Minsk zoo (Belorussia), Kiev and Odessa zoos (Ukraine), totality eleven visits. The given researches were carried out during the 2010 September to 2012 August. During this period were studied zoo information environment, what is about endangered animals and animal collection. For research was made a protocol, what describe zoos informational and expository environment. During the study attention was taken on zoo environmental, kind of information, and zoo breeding or reintroduction program.

Results. In total numbers of visitors per year in chosen zoos is near 2 million people. In average number about amphibian's species what is showed in zoo collection is 19, and reptiles species near 48. It means that reptiles in zoos are very popular. Two species what was chosen: *Bombina bombina*, *Coronella austriaca* isn't popular at zoos collection, *Coronella austriaca* was shown only in Riga national garden, *Bombina bombina* in Riga National garden and Latgale zoo. *Emys orbicularis* is more popular it shown in Riga national zoo, Latgale zoo, Minsk zoo, Warsaw and Plock, Helsinki zoos.

Conclusions. Baltic region zoo more show tropical amphibians and reptiles they are more colored, end easy in maintenance, they do need in artificially conditions winter time, lower temperature. *Coronella austriaca*, *Bombina bombina* is not popular, *Emys orbicularis* is most popular. Baltic region zoos are popular in society, for what testify multitude visitors.

Acknowledgements. Part of information was received in cooperation with LIFE+ Project LIFE-HerpetoLatvia LIFE09NAT/LV/000239, Lathales Zoo, Daugavpils University.

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RESEARCH OF RIVER DZEGUZE (KRASLAVAS AND DAGDAS NOVADS, LATVIA) AS A POSSIBLE PLACE FOR RELEASING OF *EMYS ORBICULARIS*

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Introduction. *Emys orbicularis* is a very rare species of Reptile in Latvia (Ministru kabinets 2000). According to the Plan on conservation of *Emys orbicularis* in Latvia (Pupiņš, Pupina 2007) for conservation and renewal of previously registered populations is very important for conservation of biotopes of the species. The Plan also recommends releasing reared *Emys orbicularis* into places, in which they were previously registered. In 2010-2014 activities are supported by the Project LIFE-HerpetoLatvia, releasing of reared *Emys orbicularis* will be continued in the following years. Therefore, it is important to assess the condition of biotopes on the territories, potentially suitable for renewal of populations of *Emys orbicularis*. It is known about 6 cases of meeting *Emys orbicularis* in the valley of the river Dzeguze (Pupins, Pupina 2008), out of that 2 turtles were caught directly in the river, traces of a turtle were found on the bank at a distance of less than 300 m from the river, three findings of turtles have been made at a distance of less than 1,5 km from the river Dzeguze. The river Dzeguze flows through numerous swampy biotopes, rich on reservoirs territories, it is shallow and strongly overgrown by vegetation and can serve as a route of migrations. At the same time, findings of *Emys orbicularis* were made several years ago, therefore, it is important to study up-to-date condition of the river Dzeguze, as a territory of potentially use of *Emys orbicularis* for habitation and migration, and distribution.

Material and methods. The study was carried out in 2011-2012 in Dagdas region, Latvia. Measurements were carried out in 26 points on the whole length of the river. An every point the following criteria were defined: shadiness, degree of development of vegetation (in percentage), width of the river, the maximal and average depth (m) and maximal and average speed of flow (m/sec).

Results. At the present moment the river is little used in economical activities of people. In the result of the study it was ascertained that the banks of the river are comparatively heavily overgrown by bushes and trees, shadiness of the river by bushes comprises in average 44% (Median 47; St.dev. ±37). The water surface is also covered by plants in average on 44% (Median 43; St.dev. ±36). An average width of the river is 7 m (Median 7,2 m; St.dev. ±3,5 m), the maximal depth is 0,87 m (Median 0,86 m; St.dev. ±0,86 m). The river flows slowly with the maximal speed in average of 0,04 m/sec, in many places flowing cannot be registered by used equipment.

Discussion. The river Dzeguze is heavily overgrown by plants, with plenty of shelters and substratum for sun-basking that corresponds to the needs of *Emys orbicularis*. The river is heavily polluted, but it has got separate open areas. The depth of the river allows it not to freeze in winter till the bottom. The low speed of flowing does not hinder potentially possible distribution of *Emys orbicularis* both along and against the flowing. Possibly, the river Dzeguze can be used by *Emys orbicularis* for migration and distribution.

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IDENTIFYING EVOLUTIONARY LINEAGES AND HYBRID INDIVIDUALS USING MTDNA AND NDNA MARKERS: IMPLICATIONS FOR CONSERVATION OF *EMYS ORBICULARIS* IN POLAND

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The aim of the analyses was to determine the genetic differentiation within Polish populations of *Emys orbicularis* and to identify hybrid individuals.

We collected mtDNA (*cytb* gene) and nDNA (13 STRs: msEo41, GmuD107, msEo21, GmuD16, msEo2, GmuD88, GmuD93, Emys2, Emys4, Emys5, Emys6, Emys11, msEo29) data from 146 European pond turtles from 28 locations in 5 regions of Poland (NEP: north-eastern Poland, EP: eastern Poland, CP: central Poland, SEP: south-eastern Poland, WP: western Poland). The MEGA v 5.02 was used to construct dendrogram using the *N-J* algorithm. Population differentiation was calculated by pairwise F_{st} from STRs data using the ARLEQUIN. STRUCTURE was used to estimate the most probable number of clusters (K) and to classify each turtle into clusters (admixture model, correlated allele frequencies).

Three mtDNA lineages and five haplotypes were identified. All turtles from NEP, EP, CP and SEP formed a highly supported cluster – eastern lineage. The most turtles from WP formed a distinct cluster – western lineage. STRUCTURE results showed that the most probable number of clusters agreed with the number of autochthonous mtDNA lineages ($\Delta K = 2$). Two geographically cohesive groups were identified (92.46% of turtles assigned to their sampling locations with a $Q > 0.95$). The analysis of the eastern group identified two genetic groups: 1) NEP, EP, and SEP; 2) CP. The results suggest that the NEP and EP are younger splits than are the CP and NEP/EP divisions. Pairwise values of F_{st} ($P < 0.01$) also indicating their deriving from the same historical gene pool.

Mitochondrial and STRs markers indicated largely congruent differentiation patterns in European pond turtles inhabiting Poland. The discordance of mtDNA and nuclear data sets was observed only for 4 turtles – hybrid individuals. Turtles from Karpicko in WP with the haplotype Ia were assigned to the CP based on STRs data, which indicated that this location is a natural habitat of turtles of the lineage Ia. Combined data applied to Polish populations of the European pond turtles allowed to delineate two main units for conservation (eastern and western lineage) and showed sub structuring in eastern Polish populations. Our study showed that the management of the particular populations (introduction, relocation, etc) should be done with special caution. Particularly important is to preserve the relict *E. orbicularis* lineage IIb.

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PRELIMINARY STUDY AND POPULATION MANAGEMENT PLAN FOR *BOMBINA BOMBINA* (DEMENE, LATVIA, FUTURE NATURA 2000 TERRITORY)

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Introduction. *Bombina bombina* is a rare and protected species of amphibians in Latvia, it is placed in the Red Book of Latvia and is listed among especially protected animals in Latvia, for which it is allowed to create Microreserves. Latvia is the most northern country, in which passes the northern boundary of the European area of *Bombina bombina* (Kuzmin et al. 2008). In the course of many decades in Latvia were known only two little (up to 10 vocalizing males) populations of *Bombina bombina*. Found in Latvia in recent years some new populations of *Bombina bombina* are located on non-protected territories, including the largest population of Latvia called “Demene”. Conservation of that boundary population is very important as like a bridgehead for distribution of *Bombina bombina* in the northern direction in Latvia. In 2010-2014 conservation of the largest population of *Bombina bombina* in Latvia is realized by the Project LIFE-HerpetoLatvia. This project provides carrying out of preliminary study, realization of population management plan, creation of new Natura 2000 territory.

Material and methods. We inspected the territory of Demene in 2011. Distribution and quantity of *Bombina bombina*, location and types of reservoirs, structure of water, conditions of biotopes (water and coastal) and their suitability for habitation of *Bombina bombina* were considered. In the study took part students of Daugavpils University and volunteers. Preliminary study was carried out in 2011, partially monitoring was continued in 2012. Studies of the quantity of vocalizing males of *Bombina bombina* we carried out by audial method, as well as by interrogation of local people. Reservoirs were found by means of searching on the route transects, we studied their size, depth and type (temporal, constant), flowage, degree of development of vegetation in reservoirs and on the banks, presence of invasive and local fish, interconnection of reservoirs with each other, etc. By the development of the management plan the quantity and distribution of *Bombina bombina* on the territory, condition and suitability of reservoirs for habitation and presence ground biotopes of wintering were taken into account, especially in the border with Belarus part, important for creation of corridors.

Results. In the result of the study a system of small reservoirs in Demene, inhabited by *Bombina bombina* was described, indicated their problems, as well as the problems of environmental pollution of *Bombina bombina* due to development of regional dump in Demene. On the basis of obtained results the population management plan was created, in which 3 main the most optimal biotopes for restoration of reservoirs and improvement of quality of areas for wintering were singled out, documents for organizing of microreserve and a new territory Nature 2000 were prepared. These biotopes will serve for releasing of young *B.bombina* into nature since 2014 and function as corridors for connections with stronger southern populations of Belarus through a system of small reservoirs.

Discussion. In the result of the study, strong negative factors for the largest Latvian population of *B.bombina* were detected (creation and development of the dump in Demene, invasion of *Perccottus glennii* into reservoirs, overgrowing of reservoirs). Therefore the project LIFE-HerpetoLatvia is very urgent and timely for conservation of the largest Latvian population of *B.bombina* and creation of border corridor for genetic contacts. Creation of a new territory Nature 2000 (Pupina et al. 2010) and realization of the Project will allow to conserve the given population as a bridgehead for distribution of the species, thus, providing of lasting successful existence of *B.bombina* in Latvia.

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LONG-TERM EXPERIENCE AND INTENSIVE TECHNOLOGY OF CONSERVATIONAL ZOO CULTURE OF *EMYS ORBICULARIS* IN DAUGAVPILS, LATVIA

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Introduction. *Emys orbicularis* is a very rare animal in Latvia that dwells there on the extreme northern boundary of its European area. *E. orbicularis* is listed among the specially protected animals of Latvia and in the Red Book of Latvia (in “zero” category). In 2008 was created and officially approved by the Ministry of environment "Plan on protection of *Emys orbicularis* in Latvia". Among other arrangements it recommends to create zooculture of *E. orbicularis* for their rearing, growth and release in nature. In 2010-2014 those actions are realized by the Project LIFE-HerpetoLatvia. At the same time, creation of a breeding group, study and keeping of zooculture are carrying out continuously in Daugavpils, Latvia in the framework of a private background project of the authors Emys-Latvia-MAP since 1985. Thus, the aims of our studies were the following: 1) detection of species' tolerance in zooculture; 2) search for suboptimal conditions; 3) intensification, efficiency and minimization of technology of *E. orbicularis* zooculture.

Material and methods. Creation of the breeding group and empirical development of technologies of zooculture began from our first obtainment of the first individual of *E. orbicularis* in 1985. The breeding group was completed from the individuals that were found in Latvia and checked genetically. A high value for a population of every natural and reared individual served the following restrictions for methods of elaboration of technologies of zooculture: 1) proficiency in forming of conditions for keeping and growth; 2) absence of checking and experimental groups; 3) minimization of risk in experiments; 4) comparative subjectivity of estimation of reaction of *E. orbicularis*; 5) variation of conditions in the framework of ecological tolerance of the species. The goal of the research was the development of technologies of intensive and economical growth «from egg to egg». In the course of the research we tried out practically different methods of keeping of *E. orbicularis*, different types of forage and diets, day and year activities, etc. The criteria of optimality of technologies were the absence of pathology, successful reproduction, speed of growth and development, low mortality.

Results. As a result of the research the following methods of keeping were selected: we keep the breeding group year-round in an outdoor terrarium in natural climatic conditions; the concrete pool is equipped with aeration, in some areas there are places woods for sun-basking, *Acorus calamus* and other water plants; filtration is missing. Forage - cut sprat, in the pool there dwell natural types of forage (invertebrates, plants). Laid eggs are incubated in incubators under the temperature of 28-30°C on a foam rubber substance. On the first year of living *E. orbicularis* are grown in a recirculation system; the water temperature is 29°C, air – 23-24°C, forage – dry *Gammarus* sp. (proteins - 56,2 %, fats - 5,8 %, carbohydrates - 3,2 %); food for cats “Viskas”(proteins 7%, fats 4,5%, minerals 2,5%, fibers 0,3%, water 82%), included meat 4%, meat sub-products, plants proteins, grains plants, minerals, vitamins etc. Local heating, illumination and UV are twenty-four-hour long. We get 20-40 young turtles yearly (females do not lay eggs every year in Latvian climate). In 2011 and 2012 we got eggs from turtles for the first time that hatched out and were grown in our zooculture, i. e. a complete cycle of zooculture of *Emys orbicularis*: “from egg to egg” passes.

Discussion. At the present moment we dispose of effective, intensive and economical technology of zooculture of *E. orbicularis*. At the same time, a question of unidentified infectious diseases still remains urgent. We managed to solve it by placing turtles into more natural “natural pond biotope”.

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PRELIMINARY STUDY AND POPULATION MANAGEMENT PLAN FOR *EMYS ORBICULARIS* IN NATURE PARK SILENE, LATVIA

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Introduction. Latvia is situated on the northern boundary of *Emys orbicularis* habitation in Europe. The Plan on protection of *E.orbicularis* in Latvia singles out the main natural limiting factors for *Emys orbicularis* in Latvia: 1) the coldest climate in the area of *E.orbicularis*; 2) overgrowing of biotopes; 3) influence of natural predators. The main negative factors of anthropogenic origin are the following: 1) capture and removal from nature of adult *Emys orbicularis* by people; 2) destruction of biotopes (amelioration); 3) influence of transport (traumatism and probable death of *Emys orbicularis* on the roads); 4) introduction into Latvia of exotic predators (*Nyctereutes procyonoides*, *Neovison vison*, possible *Ondatra zibethicus*) and others. Reduction of populations of *Emys orbicularis* and fragmentation of the area may result from the influence of such factors. That leads to limitation of contacts and genetic interexchange among groups of turtles. Such interexchange of genes and contacts of border Latvian groups with stronger maternal southern populations of Belarus and Lithuania seems to be especially important. The most southern protected natural territory of Latvia, on which the authors repeatedly registered *Emys orbicularis*, is the territory of Natura 2000 - Nature Park Silene. In 2010-2014 conservation of *E.orbicularis* on that territory is realized by the project LIFE-HerpetoLatvia. This project provides implementation of preliminary study and development and realization of a population management plan.

Material and methods. The territories of Nature Park Silene were inspected. Location and condition of system of reservoirs, occurrence of *E.orbicularis*, condition of biotopes (water and potential biotopes of laying of eggs) and their suitability for habitation of *Emys orbicularis* were studied. Students of Daugavpils University and volunteers took part in the study. Preliminary study was carried out in 2011, monitoring of temporal reservoirs was continued in 2012. We carried out the study of occurrence of *E.orbicularis* by means of visual search, using of traps, and interrogation of local people. Reservoirs were detected by means of searching on the route transects, we studied their size, depth, type (temporal, constant), flowage, degree of development of vegetation in a reservoir and on the banks, presence of substratum for sun-basking, interconnection of reservoirs with each other, etc. During the inspection of the ground biotopes their suitability for laying of eggs was assessed according to the following criteria: type of ground (sandy soil), orientation, nearness to water biotopes. During the elaboration of management plan the occurrence of *Emys orbicularis* in a park was taken into account, condition and suitability for habitation of *Emys orbicularis* in reservoirs and ground biotopes an important part for creation of corridors.

Results. As a result of the study a system of small reservoirs in Nature Park Silene, which included 165 main reservoirs, basically on the south-eastern part of the park was described. During the course of the study we have not caught any *Emys orbicularis*; at the same time, we got some communications from local people about seen single individuals. Sandy biotopes that are suitable for laying of eggs were found out. Population management plan was created, in which 3 main the most optimal biotopes for restoration of reservoirs and improvement of quality of areas for laying of eggs were singled out. These biotopes will be the areas for releasing of young *Emys orbicularis* into nature since 2014 and will function as corridors for connection with stronger southern populations of Belarus, other populations in Latvia through a system of small forest reservoirs and large lakes Ricu and Sita.

Discussion. In the result of the study the present project is a very urgent and timely for recruitment of practically vanished population on the given territory and for creation of border corridor for genetic contacts. Probably, conserved on the territory single individuals already cannot restore independently its own quantity. Thus, functioning of the Project enables to save and restore the given population, as well as provide corridors for the contacts of populations.

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LACERTA AGILIS, POTENTIAL FOOD SOURCE FOR HERPETOPHAGOUS SPECIES, DISTRIBUTION IN DAUGAVPILS CITY TERRITORY, LATVIA

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Introduction. *Lacerta agilis* is rare reptilian of Latvia and an important food source for many herpetophagous species (*Coronella austriaca* and other). For complete herpetophagous species reservation in Latvia is needed to know more about *Lacerta agilis* and its living habitat space. Especially interesting is *Lacerta agilis* surviving in changed natural biotopes like it is in city.

Material and methods. The city was chosen, because of Daugavpils city's placement in Latvia as more in south being city. Latvia is placed near to northern border of *L. agilis* areal of living. Research was started in year 2009 and still continues. In researches were used transect, observation, visual registration and geomatic methods. At field studies *Lacerta agilis* specimens were caught by hands for measuring, and released in same place. Digital photo-camera Olympus FE-5000 was used for documenting.

Results. Observation is done in 70 expeditions. In result was acquired information about *Lacerta agilis* placement in Daugavpils city territory, more suitable biotopes and other Reptilia species in same biotopes as well. In our observation *Lacerta agilis* were found in four biotopes with overall 37 deposits. In same biotopes with *Lacerta agilis* live some other protected in Latvia species.

Discussion. In close future will be made factor analyse of *Lacerta agilis* locations and most important environment factors: as relief, soil and flora. The results can be used in Daugavpils city planning, *Lacerta agilis* monitoring analyse and educational causes of society.

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THE ASSESSMENT OF THE METHODOLOGY FOR THE FEEDING OF THE EUROPEAN POND TURTLES (*EMYS ORBICULARIS*) JUVENILE UP TO ONE YEAR

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Introduction

The Lithuanian Zoological Gardens participated in a project of rearing European pond turtles in captivity. One of the project tasks is to prepare the methodology for rearing of this species, which includes the preparation of the methodology of feeding the juvenile.

We decided to examine the mixed feeding option – to feed both natural foods (beef, fish and other) with supplements and commercial kitten food. In preparing the primary feeding methodology, the requirement of foods for juvenile turtles was calculated by the basic energy rate necessary for growing animals by weight.

The purpose of this investigation is to assess how a theoretical calculation of the feed requirements meets the juvenile growth rates and to identify a feeding correlation within the primary methodology of feeding the juveniles depending on their weight and life cycles.

Methods

The metabolic energy requirements of juvenile turtles were calculated using the formula: $BMR = 0,32W^{0,77} \cdot 2.5$ (Jansen W.L., Nijboer J.D. 2003), applying the animals' activity coefficient of 2.5. This formula applies to the keeping of animals at 30°C. The metabolic energy requirements were calculated from 5 to 200 g at increasing intervals between 5 and 50 g.

All juveniles were weighed monthly up to 1 year of age and the amounts of consumed feed were totaled. We aimed to feed them optimally and didn't overfeed.

Energy value (kcal) of the consumed food was calculated using a created database in MS Access base for assessment of feed. A monthly energy requirement was also calculated using the above formula for the average weight of the animals.

Results

European pond turtle juveniles consume an average 4.5% (range from -20.5% to 36%) less kcal for weight gain in August – November and March – May months than calculated in the feeding methodology. The animals were kept at an ambient temperature of 20-24°C. Animals are less active at a lower temperature resulting in a lower energy consumption (Parmenter, Robert R. and Harold W. Avery 1990). During December – February they were prepared for hibernation and hibernated. During June – August some of them were kept in an outdoor adaptation enclosure in natural conditions where they found a natural diet.

Conclusions

The prepared methodology of the feeding of European pond turtle juveniles essentially satisfies calculations of the gathered data in rearing practice. More reliable data will be gathered in further studies of rearing European pond turtles in captivity.

Literature

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A NON-DESTRUCTIVE SAMPLING METHOD OF HIGH QUALITY DNA EXTRACTION FOR PCR ANALYSIS IN AMPHIBIAN CONSERVATION GENETIC

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Modern studies of population genetics rely increasingly on DNA markers amplified by polymerase chain reaction (PCR) for detecting genetic variations within and among populations. Such information is useful for planning conservation strategies for great number of species.

Liver and muscles are the most common tissue used as sources of DNA but for collection of it the animal needs to be sacrificed. Such destructive a lethal sampling has the potential to seriously impact to genetic makeup of populations under investigation and should be avoided when never possible.

Conservative genetic programs whenever possible have to use the least destructive and least invasive DNA sampling methods to limit disruption and damage to the endangered, vulnerable or declining study organisms.

Some small animals has been investigated using DNA from hair, blood, feces, ear tissue.

A standard method of collecting DNA with minimal invasiveness from human involves buccal swabbing to dislodge epithelial cells from which the DNA can then be extracted. Among the advantages of this method are rapidly and simplicity.

In this study, to avoid disturbance and stress to the animals, which negatively affect their subsequent survival rates, breeding behaviour and reproductive success, the minimally invasive, non-destructive DNA sampling method using buccal swabs was tested. Buccal samples were taken from several animals in the field and also captivity in the Latgales Zoo. After sampling, the genomic DNA was extracted using salting out the cellular proteins with a saturated ammonium acetate solution. The quantity, quality and suitability of the isolated DNA samples for PCR were checked by using spectrophotometric measurements, agarose gel electrophoresis and PCR amplifying a 1200-bp large fragment of *Bombina bombina* cytochrome *b* gene. The obtained results show that such easy, non-destructive DNA sampling method as buccal swabbing provides a reliable source of good quality and quantity DNA, thus enhancing molecular studies in the context of *Bombina bombina* conservation and population genetics. To investigate *Bombina bombina* genetic origin, mitochondrial DNA (mtDNA) polymorphism was studied.

THE PROBLEM OF COEXISTENCE OF *BOMBINA BOMBINA* WITH ANOTHER REPRESENTATIVES OF ANURA CENTRAL EUROPE

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In the article there is an analysis of an adaptation of *Bombina bombina* to living together with another species (*Lissotriton vulgaris*, *Triturus cristatus*, *Pelobates fuscus*, *Bufo bufo*, *Hyla arborea*, *Rana temporaria*, *Bufo viridis*, *Rana lessonae*, *Rana ridibunda*, *Rana esculenta*, *Rana arvalis*). An important role is given to the period of reproduction and preparation to stay the winter.

GROWING OF EUROPEAN POND TURTLE (*EMYS ORBICULARIS*) JUVENILES IN LITHUANIAN ZOOLOGICAL GARDEN

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The aim of the paper is to present a new European pond turtle incubation and growing methodology in captive conditions designed in Lithuanian Zoological Garden.

Results of the Life+ Nature Project “Protection of the *Emys orbicularis* and amphibians in the North European lowlands (LIFE05NAT/LT/000094)” (2005 – 2009) showed that some turtles’ females choose unsuitable places for eggs laying. As an extension of the project, the second Life+ Nature Project “Development of a Pilot Ecological Network through Nature Frame areas in South Lithuania” (2010 – 2014) was developed. One of the project’s aim is to collect eggs from unsafe nests and raise the juveniles which will be returned into the wild after some time. Second task is to create a methodology for incubation of the eggs and growing of the juveniles.

127 European pond turtle’s eggs were collected from the wild during expeditions in 2011 and 2012. Incubation temperature was 28-29⁰ C in day time and 22-25⁰ C at night. Humidity was about 90-95 %. The juveniles of the same nest were put together in a special aquarium depending on size of the nest. For successful growing, photoperiod time and temperature ranges were created. Juveniles were hibernating in laboratory conditions for 6-8 weeks in the first year. Weight and body size of all the juveniles were measured once a month. Was made special wood platforms that help to study behavior of the juveniles.

From 127 eggs 110 were hatched, 14 were unfertilized and 3 embryos died in 2011- and 2012 season. Incubation success was 86.6 % (97.3 % without unfertilized eggs). It shows that incubation methodology was very successful. Was built a successful options to grow juveniles in captivity (107 juveniles from 110 survived).

Was created a food diet of 85-90 % animal products and 10-15 % plant-based products for juveniles. Hibernation lasted for 6-8 weeks and all juveniles survived. Second hibernation should last for 3 month. After hibernation group of juveniles lived in an outdoor enclosure simulating natural habitat. Growing conditions were optimized for turtles’ health and to create competition between individuals. According to results, we can state that was created good methodology for *Emys orbicularis* juveniles’ growing in laboratory conditions, but we are hoping to improve it even more.

STATUS OF *TRITURUS CRISTATUS* AND *PELOBATES FUSCUS* IN ESTONIA

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In frame of the Estonian *Triturus cristatus* action plan, 9 Provinces in Estonia were systematically surveyed in 2007-2012 to define the actual distribution and status of the populations. 2512 Sites were dipnetted on larvae and surveyed for eggs in June-July by the same person, materials and methodology. The status of the habitat for two threatened species was evaluated where populations were detected: *Triturus cristatus* (*T.cri*) and *Pelobates fuscus* (*P.fus*). The criteria used were reproduction success, isolation and habitat conditions (shade, fish, dumping, pesticides, etc.). Five categories of status of the breeding sites were defined. The status of both species is visualized showing the percentage of sites with each status.

Results

Both species were commonly recorded in southeastern Estonia, the eastern region (Peipsi lake region) and more isolated in northern regions. A wider distribution area of both species was recorded, with populations of *T.cri* nearly 100 km more to the West. The overall status of both species is threatened. Throughout the country 29% of *P.fus* and 17% of *T.cri* breeding sites were in optimal conditions and require no active management, whereas 71 % of sites with *P.fus* and 83% with *T.cri* require direct active management or within a few years to avoid extinction. Due to the method of dipnetting the results for *Pelobates fuscus* are positively biased for the species could only be detected in sites with relatively favorable conditions (with larvae).

The mayor threats for both species vary per region: in the southeast fish introduction, fish pond digging and overgrowing are the mayor threats whereas in the other regions these are overgrowing or intensification of land use.

Management

Since the year 2000 management actions were undertaken in frame of National projects and LIFE NATURE projects (LIFE2004NAT/EE/000070 and DRAGONLIFE LIFE08NAT/EE/000257). Both species have shown to respond positive to management actions (pond restoration and new ponds). At the same time repeated surveys in sites where no actions were undertaken show a fast deterioration as was expected. Large scale management and education will be required to maintain the large metapopulations and to avoid regional extinctions.