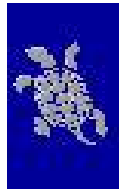




Lithuanian Fund for Nature



Project LIFE05NAT/LT/000094 “Protection of European pond turtle and threatened amphibians in the North European lowlands”

## **ACTION A.2: EVALUATION OF PONDS**

### **THE CHARACTERISTICS OF POND TURTLE *EMYS ORBICULARIS*, GREAT CRESTED NEWT *TRITURUS CRISTATUS* AND FIRE-BELLIED TOAD *BOMBINA BOMBINA* POND HABITATS**

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## INTRODUCTION

An ongoing decline of the European Pond turtle, *Emys orbicularis* and the Fire-bellied toad, *Bombina orientalis*, is now documented throughout the North European lowlands. Additionally, the decline of the Great crested newt *Triturus cristatus* is recognized in Estonia, Finland, Germany and Denmark and is suspected in Poland and Lithuania.

Based on population genetics theories, each population of these species has to have at least 500 adults to survive. However, today very few turtle populations meet this criterion as most of them are between 10 and 50 adults. One of the ways to reach the goal is to restore, improve or even create their population habitats, especially within intensively-used landscapes.

During terminated *B.bombina* LIFE projects (Consolidation of *B.bombina* in Denmark, Management of *B.bombina* in the Baltic region) and terminated *T.cristatus* LIFE project (Protection of *T.cristatus* in the eastern Baltic region) there is considerable knowledge available for the design and improvement of pond landscapes of high quality for amphibians. With basic experience on turtle conservation existing already in Lithuania, Poland and Germany, there is a solid foundation for the implementation of protective measures on herpetological sites of European interest and to develop a concept of active protection of sites of high herpetological diversity in the North European lowlands. The active protection of aquatic habitats of *E.orbicularis*, *B.bombina* and *T.cristatus* will also support a number of annex IV amphibian species on the same sites.

Main threats to the targeted habitats/species in general are: habitat fragmentation and migration barriers, loss of water bodies, overgrowth of water bodies, loss of turtle nesting areas, lack of hibernation sites, loss of terrestrial habitats, intensive agriculture, predation on turtle nests, fish introduction, lack of public environmental awareness, lack of international co-operation.

The main objective of the investigation is to find out optimal aquatic habitat characteristics for the three species to ensure the possibility of creating and restoring the population sites in the North European lowland, especially in the areas where *T.cristatus* and *B.bombina* occur together with *E.orbicularis*.

The habitat improvement as expected will increase population sizes which are necessary for the structure of viable populations of *E.orbicularis*, *B.bombina* and *T.cristatus* and the successful preservation of all three species in the investigated region.

## DESCRIPTION OF THE SPECIES' ECOLOGY

*Emys orbicularis* (Linnaeus, 1758)

*Emys orbicularis* is a species, which needs two different habitat types. It requires aquatic and terrestrial habitat. This species can only feed in water, so it is completely dependent on water bodies. This species inhabit smaller and bigger ponds with submerged and floating plant cover in Lithuania, Poland and Germany. While turtles hibernate in small areas close together, in late spring and summer the individuals spread upon different ponds. Consequently, if the water bodies are not suitable for the whole year, turtles move to different ponds in summertime. Therefore pond systems with different types of ponds are very suitable for *Emys orbicularis*. In the water bodies turtles need sun exposed places for basking e.g. deadwood or at the shore. Basking is an important behaviour and the occurrence of suitable basking sites can be a limiting factor in the northern species range. *Emys orbicularis* feeds on invertebrates, amphibians and their larvae and on plants. In March or April after hibernation the turtles start with mating period. 4 up to 8 weeks after mating the nesting season begin in the End of May and lasted 2 or 3 weeks up to the Middle of June. In this time females leave the water bodies and move on land up to several kilometers for finding a suitable nesting site. They need open, south exposed, sunny areas with sandy ground for egg laying. Usually, females reproduce once a year and produce an average number of 12 or 13 eggs. The availability of suitable nesting sites is especially in the northern distribution range of the species a critical factor for species survival. The duration of incubation depends on weather conditions and lasts about 3 month. In the End of August or in September hatchlings leave the eggs. Some of them leave the nests, too, but most of the hatchlings remain in the nest cavity during the first winter and hatch in spring or early summer. In cold summers with low temperatures the development of the embryos is prolonged or even embryos can die off. After leaving the nests hatchlings walk to water bodies. The juveniles are not good swimmers and divers, so they need to stay in shallow, highly structured areas during their first weeks of life. The occurrence of suitable ponds for hatchlings (without predators, good water temperatures and good food resources for a fast growth) can be a critical factor, too, because the northern populations of *Emys orbicularis* have a lower reproduction rate than the southern populations and the survival of northern the juveniles is more important for the viability of the populations than in the southern regions.

*Triturus cristatus* (Laurenti, 1768)

*Triturus cristatus* is a species, which needs both several aquatic habitat and also few terrestrial habitat types. *Triturus cristatus* can only bred in water, so it is completely dependent on water bodies. The adult newt migrates in early spring from hibernation site to the breeding pond. The ponds chosen must have an underwater vegetation of plant with soft leaves suitable for the female to lay eggs in. Further the pond must have fairly clear water and preferably pond bottom areas without vegetation in order for the males to carry out the mating courtship towards the females. In order for the pond to a successful breeding pond there must be no fish that can easily predate the very pelagic larvae of *Triturus cristatus*. Further the pond must have a rich underwater vegetation mixed with open water areas and with plenty of small invertebrate pray and tadpoles that the larvae can feed on. Some breeding ponds can be deeper and at the same time function as foraging ponds for the adult *Triturus cristatus* during the summer. Also juvenile one year old *Triturus cristatus* may enter ponds for foraging during spring and summer. Hibernation places is as we know on dryer terrestrial habitats that never floods during spring and that are frost free during winter. It can be forest, hedgerows, stone fences and stone piles and house cellars. *Triturus cristatus* feed on invertebrates and their larvae both under water and on land. From July to September and very site dependent a most of the population leaves the water and seek shelter and food in terrestrial habitats rich in structure and hiding places as forest with laying dead and alive tree trunks or in cultural

landscape with a mixture of meadow stone fences and hedgerows. The terrestrial habitat does not have to be sun exposed.

*Bombina bombina* (Linnaeus, 1761)

*Bombina bombina* is a species, which needs two several aquatic different habitat types and also few terrestrial habitat types. *Bombina bombina* can only breed in water, so it is completely dependent on water bodies. *Bombina bombina* breed both smaller and bigger ponds in Lithuania, Poland and Germany with submerged and floating plant cover. The terrestrial habitat depends on what the local conditions offer and often the terrestrial habitat type and its capacity for feeding and hibernation also have a high influence on the population's viability and population size. Foraging ponds can have all types of water quality except the acidic one. It's not unusual that these ponds have muddy bottom and have very eutrophic water or are partly overgrown or shadow covered. Ponds rich in structures with different vegetation zones and dead wood and tree trunks. Hibernation places are on dryer terrestrial habitats that never floods during spring and that are frost free during winter. It can be forest, hedgerows, stone fences and stone piles and house cellars, *Bombina bombina* migrates between these habitat components and thus its possible with 5 to 6 migrations between habitat components from early spring to late fall. Some time one pond can contain all the necessary pond habitat components which limits the migration. *Bombina bombina* feeds on invertebrates and their larvae. In March or April after hibernation the *Bombina bombina* seek a pond for sun basking and feeding. In May and June the breeding and egg laying takes place in carefully selected ponds, where the males forms large chorus, when they call for the females. After calling season the frogs disperse in to feeding grounds and ponds are often not enough for the larger populations who also seek food in moist terrestrial habitats as fens and meadows. In the End of August or in September the frogs seek towards a hibernation places that can not be flooded by water in winter time. Often they feed during warm days in September and October near the hibernation place.

## INVESTIGATION AREA AND METHODS

The investigation was carried out in three European countries: Poland, Lithuania and Germany. Three main regions chosen to be investigated in Poland were the following: North-eastern (Olsztyn), Eastern (Bialowieza) and Western (Poznan); per one region were chosen in Lithuania and Germany. There were investigated 258 ponds in total: 88 ponds checked in Poland (42 in Olsztyn, 30 in Poznan and 16 in Bialowieza), 47 in Lithuania and 123 in Germany.

Ponds and surrounding habitats were checked for a variety characteristics such as physical parameters of the ponds, geology and water quality, composition of surrounding habitats, biotic factors etc.

Data collection was carried out in 2006-2009 in late spring-early summer seasons. The main method for investigating of the pond turtle was observation the pond edges and the water mirror with binocular, in some cases telemetry was used. The main method for investigating the great crested newt was checking the eggs in folded leaves and dipnetting of larvae if eggs were not found. Checking of calling males in the ponds was the main method for investigating the fire-bellied toad as well as dipnetting of larvae of the species too.

A special data form for each pond was filled out featuring the mentioned characteristics.

The gathered data were categorized and analysed by means of data mining that summarises the categories' data and presents the main patterns. In addition to data mining the hypotheses of the researchers were checked using Chi-square test. Analyzing the significance of gathered and categorized data we used chi-square test based on the null hypothesis theory. The null hypothesis is that the observed data are sampled with the frequencies, which in our case are expected to be equal for all categories. Combining together the discrepancies between the observed and expected data distribution, and then calculate a P value we answer the question, if the null hypothesis were true? In other words if the theory that generated the expected values were correct, what is the probability of observing a discrepancy between observed and expected values? The smaller P value we get the higher evidence that the data are not sampled from the distribution you expected.

We can combine the observed and expected counts into a variable – chi-square. To calculate chi-square:

1. For each category compute the difference between observed and expected counts.
2. Square that difference and divide by the expected count.
3. Add the values for all categories. In other words, compute the sum of  $(O-E)^2/E$ .
4. Use computer program (or a table) to calculate the P value. It is important to take into account the number of degrees of freedom. It equals the number of categories minus 1.

We didn't use the Yates' correction in our calculations (it is used sometimes when there are only two categories).

## RESULTS AND DISCUSSION

### *Emys orbicularis*

#### 1. Physical parameters of the ponds

##### 1.1. Pond type

There were found several types of ponds in Poland occupied by the pond turtle (lakes, ponds, natural depressions, swamps), but the conditions seem to be more optimal for adult *Emys orbicularis* if they inhabit lakes (50% occupancy) and at less extent ponds and depressions (33%). Quite opposite preference peculiar to young which are more common in ponds and depressions (17%) and less in lakes (13%).

Natural depression as a pond type seems to be the most numerous and the most occupied comparing to the others by adults of *Emys orbicularis* in Lithuania (48%) what is proved to be extremely significant ( $\chi^2 = 33.500$ ,  $p = 0.0001$ ). Similar, but not significant trend show young of *Emys orbicularis* (10%). Germany shows the same preference (6%), but no significance to be taken into account as almost all the records of *Emys orbicularis* were made from natural depressions (N=117) compare to the others (N=6).

Summarizing the results in all investigated areas together analysing different pond types with *Emys orbicularis* occurrence we significantly found out that the adults prefer pond/lake-like ponds to the others (32%) ( $\chi^2 = 30.310$ ,  $p = 0.0001$ ). The young are recorded more often in ponds and natural depressions to the same extent (11-12%) ( $\chi^2 = 2.669$ ,  $p = 0.2632$ ).

##### 1.2. Size of the pond

The sizes of investigated ponds in Poland were in range of 25 sq m up 640 000 sq m. The conditions of size of the pond seem to be more optimal for adult *Emys orbicularis* if it is at least 500 sq m what is considered to be statistically significant ( $\chi^2 = 10.462$ ,  $p = 0.0333$ ).

Data collected in Lithuania show the same tendency: both young (38% occurrence) and adults (88%) of *Emys orbicularis* prefer 500-2000 sq m ponds what is especially proved as significant for the adults ( $\chi^2 = 11.333$ ,  $p = 0.0231$ ).

Pond sizes checked in Germany seem to be optimal for the pond turtle if its area is more than 10 000 sq m (13% occurrence) while the result is not proved to be significant ( $\chi^2 = 5.750$ ,  $p = 0.2186$ ).

Generalizing all the results, we have clearly shown that the most occupied pond size type is 500-2000 sq m (28-31%) in the whole area, what is proved as significant for the adults of *Emys orbicularis* ( $\chi^2 = 12.000$ ,  $p = 0.0174$ ).

##### 1.3. Maximum depth of water

The analysed data from Poland show the highest occupancy of *Emys orbicularis* in ponds with depth more than 1 m both for young (18%) and adults (53%) compare to shallower waters. The result is considered to be extremely significant for adults ( $\chi^2 = 24.332$ ,  $p = 0.0001$ ) and not quite significant for young ( $\chi^2 = 5.205$ ,  $p = 0.0741$ ).

On the contrast, *Emys orbicularis* was found to be more frequent in 0,5-1 m deep ponds in Lithuania (46% for adults and 13% for young). High level of significance is proved only for the adults ( $\chi^2 = 11.386$ ,  $p = 0.0034$ ).

Preference of *Emys orbicularis* to the deep ponds is clearly shown in Germany also. The highest occupancy was found in ponds with more than 1m water depth (20%) with extremely high level of significance ( $\chi^2 = 17.000$ ,  $p = 0.0007$ ).

The total results show that water depth of the pond for *Emys orbicularis* is very significant factor and has to be no less than 0,5 m. Preferably more than 1 m (12% occurrence of youngs and 34% of adults). In case of adults it is considered to be extremely significant ( $\chi^2 = 30.911$ ,  $p = 0.0001$ ) (Tabl. 1).

Table 1. Maximum depth of water of all the investigated ponds in relation to the occurrence of *Emys orbicularis*

Pond depth, m	Number of investigated ponds	Presence of <i>Emys orbicularis</i> youngs, %	Number of investigated ponds	Presence of <i>Emys orbicularis</i> adults, %
Less than 0,5	-	-	37	0
0,5-1	43	9	90	20
More than 1	42	12	93	34

#### 1.4. Slopes

The inclination of the pond slopes in Poland seem to be more optimal for adult *Emys orbicularis* if it is between 11 and 25 grades (36% occupancy). The result is proved as very significant ( $\chi^2 = 14.385$ ,  $p = 0.0024$ ).

Lithuanian data show the same tendency (50%), especially for young *Emys orbicularis* which were found only in ponds with low inclined slopes (0-25 grades) (15%) ( $\chi^2 = 4.000$ ,  $p = 0.2615$ ).

Generally it was shown while not proved significantly that the trends of slopes inclination for occurrence of young and adult *Emys orbicularis* seem to be opposite: youngs prefer more lower slopes as their occurrence in the ponds is increasing with decreasing of the slopes inclination (from 8 to 15%), adults – more steeper as their occurrence is decreasing with the decreasing of the pond slopes inclination (from 62% to 30%).

#### 1.5. Zones with shallow water

Data collected in different regions in Poland show the different trends: north-eastern populations of *Emys orbicularis* were found in higher numbers (40%) in the ponds with 10-25% of shallow water ( $\chi^2 = 4.774$ ,  $p = 0.0919$ ), but west polish populations of *Emys orbicularis* were found mostly in less than 10% of shallow water ponds (60% occurrence), while it could be simply explain with lack of such category ponds in our investigation in the west Poland.

The conditions seem to be more optimal for *Emys orbicularis* in Lithuania if the pond shallow zone is 11-25%. In this case occurrence is the highest and makes 70% for adults and 20% for youngs.

Almost all the records of *Emys orbicularis* from Germany were made from ponds with presence of shallow zone (N=117) compare to the others (N=6).

Taking into account all the local data we could make a conclusion that the conditions seem to be optimal for *Emys orbicularis* youngs if the pond shallow zone area is 11-25% (17% occurrence) and up to 25% for adults (44%). So we can just suppose that the mentioned species need shallow water to some extent.

## 2. Geology and water quality

### 2.1. Sediment



The sediment recorded in the ponds all over the investigated area was mainly mud, sand and peat. Polish populations of the pond turtle split again after this criteria and show very significant preference to mud pond bottom in west Poland (55%) ( $\chi^2 = 20.482$ ,  $p = 0.0001$ ) and to sand one in north-east Poland (35%) ( $\chi^2 = 14.385$ ,  $p = 0.0024$ ).

Whereas Lithuanian *Emys orbicularis*, especially youngs (17%) occur mostly in peat bottom ponds (42%) compare to the others ( $\chi^2 = 7.500$ ,  $p = 0.0576$ ).

So, drawing a resumptive conclusion we could only say that no general preference to pond sediment type was shown in the investigated area as it depends on local geological and soil conditions which totally prevail in each of the cases, while formally we found out the significant preference of adults of the pond turtle to mud (41%) and sand sediments (38%) ( $\chi^2 = 9.059$ ,  $p = 0.0108$ ).

## 2.2. Water quality

Water quality in each investigated pond was checked. The main categories to describe this parameter were the following: clear, brown (which is often caused by humus rich sediment) or muddy.

Choice between clear and muddy waters looks to be with slight preference of muddy waters while not significant in case of Polish *Emys orbicularis* (50%) ( $\chi^2 = 3.504$ ,  $p = 0.1735$ ).

The occupancy of *Emys orbicularis* in muddy water was rather low in Lithuania. The highest occupancy for the pond turtle there was recorded in brown and clear waters both for adults and youngs (40-42% and 8-13% accordingly).

So, we can say in general that water quality doesn't have any significant influence upon the occurrence of adult *Emys orbicularis* ( $\chi^2 = 0.890$ ,  $p = 0.6409$ ) while clear water ponds have a bit higher frequency (44%).

## 3. Surrounding habitats

### 3.1. Buffer zone

The width of the buffer zone around the pond was measured. The buffer zone was defined as an area of any type of habitat around the pond, except cultivated land.

It became obvious that in Poland youngs of *Emys orbicularis* prefer ponds with wide buffer zone (50 m and more) and occur only there (56% cases) ( $\chi^2 = 10.010$ ,  $p = 0.0067$ ). Adults inhabit ponds with different width of buffer zone and their occupancy was rather neutral to the given criteria ( $\chi^2 = 7.546$ ,  $p = 0.0230$ ).

Lithuanian populations of *Emys orbicularis* also don't show any significant dependence on this criteria.

But presence of buffer zone around the ponds seems to be important for German *Emys orbicularis* (8% compare to 0% (absence of buffer zone)) what is proved as highly significant ( $\chi^2 = 8.000$ ,  $p = 0.0047$ ).

So, totally, the highest occupancy of *Emys orbicularis* youngs was recorded in the ponds with wide buffer zone (more than 50 m) (42%) what is considered to be extremely significant ( $\chi^2 = 19.920$ ,  $p = 0.0001$ ). Adults are shown to occupy both narrow (28%) and wide buffer zone ponds (26%) almost to the same extent ( $\chi^2 = 16.136$ ,  $p = 0.0003$ ) (Tabl. 2).

Table 2. Width of buffer zone for all the investigated ponds in relation to the occurrence of *Emys orbicularis*

Minimum buffer zone, m	Number of investigated ponds	Presence of <i>Emys orbicularis</i> youngs, %	Number of investigated ponds	Presence of <i>Emys orbicularis</i> adults, %
0-9	28	11	54	28

10-49	6	17	60	10
50 and more	38	42	111	26

### 3.2. Grazing of pond

Grazing in the surroundings of the ponds was also observed. Three types of grazing were taken into account: extensive, normal and intensive. There were only 8% of ponds found under extensive grazing in Poland. Majority of the individuals of the investigated species was recorded in ponds without grazing compare to extensive one where single *Emys orbicularis* were noticed. The differences are significantly proved ( $\chi^2 = 4.455$ ,  $p = 0.0348$ ).

Grazing absence has very significant positive trend ( $\chi^2 = 12.888$ ,  $p = 0.0016$ ) for adult *Emys orbicularis* (44%) and for young too (11%) in Lithuania.

Summing up, absence of pond grazing was found to be significantly important for adults of *Emys orbicularis* (40% occurrence to 27% with grazing) ( $\chi^2 = 8.333$ ,  $p = 0.0039$ ) in all investigated areas.

### 3.3. Terrestrial habitat within a 50 m radius around the pond

The habitat complexes within 50 m radius around the pond were analysed and compared to the occurrence of *Emys orbicularis* in the ponds.

Adult *Emys orbicularis* were found mostly in the ponds the closest surroundings of which arranged by shrub, dead wood, deciduous forest and common dry grass (35-59% occurrence) in Poland. Such the distribution is considered to be significant ( $\chi^2 = 16.400$ ,  $p = 0.0217$ ). Dispersal of young is not significant.

No clear significance in close habitat type frequencies distribution for *Emys orbicularis* was found in Lithuania, except dead wood for the youngs (25%), probably ( $\chi^2 = 7.182$ ,  $p = 0.4102$ ).

So, in general, looks that dead wood (58%), shrub (44%) and deciduous forest (46%) are the most important habitat types within 50 m zone for occurrence of *Emys orbicularis* adults ( $\chi^2 = 21.803$ ,  $p = 0.0027$ ). Youngs, whereas not significant ( $\chi^2 = 7.992$ ,  $p = 0.2387$ ), could be supposed to appear frequently in urban (15%) and common dry grass (17%) close terrestrial surroundings.

### 3.4. Terrestrial habitat in the radius of 50 to 500 m around the pond

The most often habitat type in radius 50-500 m of the inhabited by adult *Emys orbicularis* ponds in north-eastern Poland are common dry grass (53% occurrence), field (40%) and urban (39%). The distribution of youngs is more or less regular like in previous case (3.3), but has some insignificant shift to the common dry grass habitat type (24%). The composition of terrestrial habitat in radius 50-500 m does significantly matter for *Emys orbicularis* in west Poland as it was more frequent in the ponds with forest in the surroundings (52%) ( $\chi^2 = 20.370$ ,  $p = 0.0048$ ).

The Lithuanian results are similar to those from west Poland and show significant trend for presence of coniferous forest (50% occurrence) and meadow/fen (41%) as habitat type in radius 50-500 m for adult *Emys orbicularis* ( $\chi^2 = 19.847$ ,  $p = 0.0029$ ).

Finalizing the analysis for the whole investigated region, we assume that coniferous forest (42%), meadow/fen and common dry grass (39% each) are more frequent habitat types in 50-500 m radius from the ponds where occurrence of *Emys orbicularis* adults is proved on very significant level ( $\chi^2 = 20.431$ ,  $p = 0.0023$ ). Youngs have shown the same dispersal on the same low significance level as in previous case of habitat type within 50 m (3.3): urban (13%) and common dry grass (12%) are the highest values for them.

### 3.5. Distance between the pond and the forest

Analysing the results of distance between pond and the forest in Poland we found them to be not significant, but it could be noticed some shift for *Emys orbicularis* preference of occupancy the ponds not far than 100 m from forest (37% on average for adults and 13% on average for young) compare to those are far.

The shorter distance between the pond and the forest is (0-50 m) the higher occupancy for adult *Emys orbicularis* in ponds we found out (75-83%) ( $\chi^2 = 13.833$ ,  $p = 0.0078$ ) in Lithuania. Distribution of *Emys orbicularis* youngs is the same (17-25%) as they were not recorded at all in ponds located far than 50 m from forest.

The occupancy of ponds with close distance to the forest (10-50 m) was the highest also for *Emys orbicularis* (43%) compare to the other investigated distances in Germany.

So the final conclusion is that occupancy of *Emys orbicularis* was rather higher if the distance to the forest didn't exceed 50 m. This is considered to be very significant in case of adults ( $\chi^2 = 11.913$ ,  $p = 0.0077$ ), but not proved as significant for youngs (Tabl. 3).

Table 3. Distance between the pond and the forest for all the investigated ponds in relation to the occurrence of *Emys orbicularis*

Distance to the forest, m	Number of investigated ponds	Presence of <i>Emys orbicularis</i> youngs, %	Number of investigated ponds	Presence of <i>Emys orbicularis</i> adults, %
0-9	14	14	38	40
10-49	24	17	44	43
50-200	15	7	34	24
201 and more	22	5	47	9

### 3.6. Distance between the investigated pond and other ponds

Presence of other ponds in vicinity of several hundred meters to the occupied pond seems not to be very important for *Emys orbicularis* in Poland due to its mobility compare to amphibians, but we can notice some slight preference to the pond clusters anyway (42-47% to 33-34% for adults and 15-16% to 11% for young). The results are considered to be not statistically significant.

Presence of other ponds seems to be indifferent factor at all for *Emys orbicularis* in Lithuania as the distribution we got is uniform for almost all the categories of criteria and is rather close to expected distribution as the null hypothesis predicts.

So, no significant difference was observed in frequencies dispersal for the other ponds presence in different distances. The only thing we can consider there are bit higher occurrence values in 0-200 m zone for youngs (20-21%) and in 0-500 m for adults (54-62%) compare to the longer distances.

## 4. Biotic factors

### 4.1. Shade provided by trees over the pond

Shade provided by trees over the pond was recorded in five different categories: 0%, 25%, 50%, 75% and 100% shade. The most optimal level of shade over the water surface looks to be 25-49% both for adult *Emys orbicularis* (39% occurrence) ( $\chi^2 = 7.546$ ,  $p = 0.0230$ ) and for the youngs (17%) in Poland.

*Emys orbicularis* is likely to occur mostly in 25% shaded ponds (33% for adults and 17% for young) in Lithuania, but the result is considered to be not statistically significant.

*Emys orbicularis* likes 50% shaded ponds in Germany (19%) ( $\chi^2 = 10.750$ ,  $p = 0.0295$ ).

Generalizing the analyses we can assume that shade provided by trees over the pond is considered to be significantly important criteria for *Emys orbicularis* youngs as their occurrence is

comparatively higher (17-25%) if the shade is 25-50% ( $\chi^2 = 9.222$ ,  $p = 0.0265$ ). Adults seem to occur more often in 50%-shaded ponds (32%), but that is not significantly proved.

## 5. Other fauna

### 5.1. Fish

The highest occupancy of adult *Emys orbicularis* was recorded in ponds with fish (33%) in Poland. Quite opposite in case of young, which were found mostly in ponds without fish (14%).

Presence or absence of fish in the investigated ponds in Lithuania was not proved as significant in any case, better say this factor seems to have almost theoretical (expected) frequencies according to the null hypothesis used in the analyses.

Presence of fish in the ponds seems to be important for *Emys orbicularis* (24% occurrence compare to 1% when fish is absent) what is proved as significant ( $\chi^2 = 4.500$ ,  $p = 0.0339$ ).

Taking into account all the local results we can consider that fish looks indifferent criteria for young *Emys orbicularis* ( $\chi^2 = 0.000$ ,  $p = 1.000$ ), but occurrence of adults looks to be much higher in the ponds with fish (36%), compare to ponds without fish (12%), although the result is considered to be not statistically significant ( $\chi^2 = 0.556$ ,  $p = 0.4561$ ).

## *Triturus cristatus*

### 1. Physical parameters of the ponds

#### 1.1. Pond type

The distribution of *Triturus cristatus* among the mentioned pond type categories is considered to be not statistically significant, but in spite of that we can see the comparatively very low occupancy of larvae and adults in village ponds in east Poland. In west Poland there were found mostly natural depressions as a pond type. They like to be the only possible places for occurrence of *Triturus cristatus* (32% larvae and 16% adults) as they were not recorded at all in other pond types ( $\chi^2 = 8.000$ ,  $p = 0.0047$ ).

The similar occupancy of the species was recorded in Germany.

In general, the occupancy of *Triturus cristatus* was significantly lower in pond-like type of ponds (0% larvae, 13% adults) as in depression-like ones (39% and 15% accordingly) ( $\chi^2 = 12.103$ ,  $p = 0.0024$  and  $\chi^2 = 24.922$ ,  $p = 0.0001$ ) while the rest of the pond types were chosen as a habitat even a bit often (45%, 21%).

#### 1.2. Size of the pond

The distribution of *Triturus cristatus* larvae was found to exist in wider range of pond sizes (from less than 100 sq m up to more than 2000 sq m), but the highest occupancy of them (50%) was noticed in the smallest ponds (less than 100 sq m) ( $\chi^2 = 8.250$ ,  $p = 0.0828$ ) in Poland. Analysing pond sizes we also found out occupancy trend for *Triturus cristatus* adults towards smaller ponds (less than 100 sq m to 500 sq m) - 38% ( $\chi^2 = 8.500$ ,  $p = 0.0749$ ).

Quite opposite result came from Germany where pond sizes seem to be optimal for *Triturus cristatus* if the area is more than 10000 sq m (37% occurrence) ( $\chi^2 = 23.111$ ,  $p = 0.0001$ ).

So, analysing them altogether, the frequency distribution of pond size criterium for *Triturus cristatus* all over the investigated area is considered to be not significant neither larvae nor adults, but some tendency to prefer smaller ponds (less than 100 m) can be noticed in larvae occupancy (42%).

### 1.3. Maximum depth of water

The maximum pond depth was analysed as one of the criterium of pond characteristics. Its analysis shows that *Triturus cristatus* occupies all the depth spectrum (from less than 0,5m-up to more than 1 m) in Poland, but the highest number of them was found in 0,5-1 m deep ponds, although it is not proved to be statistically significant ( $\chi^2 = 3.000$ ,  $p = 0.0833$ ). Shallow ponds seem to be more optimal for larvae of *Triturus cristatus* (42% occurrence) compare to the deep ones (13%).

The highest occupancy of *Triturus cristatus* in Germany was found in deep ponds (more than 1 m) with extremely high level of significance (46%)( $\chi^2 = 39.778$ ,  $p = 0.0001$ ).

Pond depth on average was found to have clear optimal parameters for both *Triturus cristatus* larvae and adults. The general analysis show extremely significant preferences for 0,5-1 m deep waters for larvae (46% occurrence)( $\chi^2 = 19.770$ ,  $p = 0.0001$ ) and more than 1 m depth for adults (28%) ( $\chi^2 = 17.661$ ,  $p = 0.0001$ ) (Tabl. 4).

Table 4. Maximum depth of water of all the investigated ponds in relation to the occurrence of *Triturus cristatus*

Pond depth, m	Number of investigated ponds	Presence of <i>Triturus cristatus</i> larvae, %	Number of investigated ponds	Presence of <i>Triturus cristatus</i> adults, %
Less than 0,5	4	25	39	3
0,5-1	39	46	74	11
More than 1	32	16	67	28

### 1.4. Slopes

*Triturus cristatus* larvae ponds have 0-45 grades average slopes inclination in general, but the most occupied ones were the ponds with 11-25 grades slopes (60%). Adults were not observed in the ponds with the lowest slope inclination (0-10 grades) and it looks to prefer higher pond slopes (11-45 grades)(40-50%) ( $\chi^2 = 2.200$ ,  $p = 0.5319$ ) in Poland, but in general, pond slopes inclination analysis doesn't show any significant dispersal for larvae and adults of *Triturus cristatus* ( $\chi^2 = 0.286$ ,  $p = 0.8667$  and  $\chi^2 = 1.078$ ,  $p = 0.5833$  accordingly) in all the investigated area.

### 1.5. Zones with shallow water

The percentage of zone with shallow water in the ponds seems to be one of the important criterium for amphibians and in our research in Poland we show that the occupancy of *Triturus cristatus* was the highest under 11-25% values (60% cases for larvae and adults).

Almost all the records of *Triturus cristatus* in Germany were made from ponds with presence of shallow zone (N=117) compare to the others (N=6). So we can only suppose that the mentioned species need shallow waters to some extent.

The highest occupancy of *Triturus cristatus* adults (25%) was recorded in ponds with 11-25% shallow zone ( $\chi^2 = 18.619$ ,  $p = 0.0001$ ) all over the investigation sites, while larvae not significantly ( $\chi^2 = 0.876$ ,  $p = 0.6454$ ) looks to be a bit frequently in those with more than 26% of shallow area (33%).

## 2. Geology and water quality

### 2.1. Sediment

Most of the observed ponds in eastern Poland have mud bottom. The occupancy of *Triturus cristatus* larvae was rather high (64%) only in the ponds with mud bottom. This result is extremely significant ( $\chi^2 = 14.014$ ,  $p = 0.0009$ ). Adults of *Triturus cristatus* were found in ponds with mud and clay bottom (50% and 36%, accordingly), but that result was proved as not quite significant.

Sediment type was found to be indifferent for *Triturus cristatus* larvae in west Poland ( $\chi^2 = 1.001$ ,  $p = 0.6062$ ), but adults of *Triturus cristatus* were recorded only in peat bottom ponds (57%) ( $\chi^2 = 8.008$ ,  $p = 0.0182$ ).

So in total, *Triturus cristatus* was found mostly in the ponds with clay and mud bottom giving some insignificant preference to clay sediment.

## 2.2. Water quality

Ponds with muddy water, compare to brown and clear was found to be the most inhabited with *Triturus cristatus* in eastern Poland. The occupancy of larvae in such ponds is higher than adults (75 to 50%) and is considered to be statistically significant ( $\chi^2 = 8.866$ ,  $p = 0.0119$ ).

Whereas, the highest occupancy of *Triturus cristatus* in west Poland was found in brown water ponds (40%) ( $\chi^2 = 8.008$ ,  $p = 0.0182$ ) as they were found only in peat bottom ponds (see 2.1.).

So, larvae of *Triturus cristatus* with the highest frequency were found in clear water ponds (38% occurrence) while the highest occupancy of adults was recorded in brown waters, while such results are considered to be not statistically significant.

## 3. Surrounding habitats

### 3.1. Buffer zone

Importance of wide buffer zone (more than 50 m) is clearly proved as significant for *Triturus cristatus* larvae ( $\chi^2 = 10.761$ ,  $p = 0.0046$ ) in Poland. Presence of buffer zone around the ponds seems to be important for *Triturus cristatus* adults in Germany as well (15% occurrence compare to 11% when the buffer zone is absent) what is proved as highly significant ( $\chi^2 = 10.889$ ,  $p = 0.0010$ ). Summing up, we can say that the occupancy of *Triturus cristatus*, both larvae and adults was rather low even absent in case of narrow buffer zone (0-9 m) (0% and 7% accordingly), what significantly prove the necessity of wide buffer zone for the species, especially on larvae stage ( $\chi^2 = 15.663$ ,  $p = 0.0004$ ) (Tabl. 5).

Table 5. Width of buffer zone for all the investigated ponds in relation to the occurrence of *Triturus cristatus*

Minimum buffer zone, m	Number of investigated ponds	Presence of <i>Triturus cristatus</i> larvae, %	Number of investigated ponds	Presence of <i>Triturus cristatus</i> adults, %
0-9	9	0	27	7
10-49	13	31	65	19
50 and more	35	37	88	14

### 3.2. Grazing of pond

Grazing of ponds was absent mostly among the investigated ponds in Poland, and the presence of *Triturus cristatus* larvae in them was 50%, what is considered to be very significant ( $\chi^2 = 7.000$ ,  $p = 0.0082$ ). Majority of adults of the investigated species was recorded in ponds without grazing (N=26) compare to extensive one (N=2). The differences are also significantly proved ( $\chi^2 = 4.000$ ,  $p = 0.0455$ ).

But, in general, pond grazing looks to have a bit negative influence over the occurrence of *Triturus cristatus* while it is not proved as significant conclusion in the end ( $\chi^2 = 3.240$ ,  $p = 0.0719$ ).

### 3.3. Terrestrial habitat within a 50 m radius around the pond

Terrestrial habitat within 50 m around the ponds were investigated and analysed. *Triturus cristatus* larvae were found quite often (55-70%) in the ponds surrounded with dead wood, meadow/fen and deciduous forest in Poland; the result is very significant ( $\chi^2 = 18.923$ ,  $p = 0.0084$ ). Adult *Triturus cristatus* also have significant distribution ( $\chi^2 = 15.947$ ,  $p = 0.0256$ ) and were present mostly in the ponds with common dry grass (56%), dead wood and shrub (39-40%) surroundings.

So, such a habitat types within 50 m around the ponds as dead wood (39%, 21%), meadow/fen (41%, 21%), common dry grass (46%, 28%) for both *Triturus cristatus* larvae and adults and field (22%) for adults only, have the highest occupancies what is considered to be highly statistically significant ( $\chi^2 = 28.892$ ,  $p = 0.0002$ ).

### 3.4. Terrestrial habitat in the radius of 50 to 500 m around the pond

Terrestrial habitat composition in radius 50-500 m was also confirmed as important for *Triturus cristatus* existence in Poland, but the distribution of investigated categories is more uniform: the highest occupancy of larvae in the ponds was 43-44% and 36% for adults. In all the cases meadow/fen, shrub, common dry grass and deciduous forest are present. The analyses considered to be significant in both cases ( $\chi^2 = 20.704$ ,  $p = 0.0042$  and  $\chi^2 = 17.476$ ,  $p = 0.0146$ , accordingly).

The only visible but insignificant result ( $\chi^2 = 7.592$ ,  $p = 0.2695$ ) in case of terrestrial habitat composition in radius 50-500 m from the pond all over the investigated area is the lowest occupancy of *Triturus cristatus* if coniferous forest is present (14% for larvae and 7% for adults).

### 3.5. Distance between the pond and the forest

The conditions seem to be more optimal for *Triturus cristatus* when distance between the pond and the forest doesn't exceed 50-100 m in Poland (most of the individuals including larvae were recorded for these categories).

In spite of insignificance of the total analysis for *Triturus cristatus* ( $\chi^2 = 5.159$ ,  $p = 0.0758$ ), we can say that short distance from pond to the forest (0-9 m) seems to be quite important for the species, especially for adults (23% occurrence).

### 3.6. Distance between the investigated pond and other ponds

Other ponds in the vicinity of investigated ones seem to be important in east Poland, and the analysis shows higher occupancy of *Triturus cristatus* adults (46-56%) in those ponds where there at least 1 pond was found in range of 500 m. Presence of other ponds close to the investigated ones was shown to be important for *Triturus cristatus* on highly significant level both for larvae ( $\chi^2 = 16.400$ ,  $p = 0.0009$ ) and adults ( $\chi^2 = 12.000$ ,  $p = 0.0074$ ) in west Poland. In case of larvae the highest occupancy (88%) was observed in ponds neighbouring others in the distance less than 100 m.

So, the only significant general conclusion can be as follows: *Triturus cristatus* larvae have the highest occupancy in the ponds (47%) in case when distance to the closest ponds is less than 100 m ( $\chi^2 = 11.414$ ,  $p = 0.0097$ ).

## 4. Biotic factors

### 4.1. Shade provided by trees over the pond

Shade over the water surface is considered to be optimal for *Triturus cristatus* on 25% level, especially for larvae as they were not found at all in 0% shaded ponds in east Poland. But the less shade is the more occupied ponds are in case of *Triturus cristatus* larvae in west Poland (32% occupancy) what is considered to be statistically significant ( $\chi^2 = 11.000$ ,  $p = 0.0117$ ).

Shade over the water surface has another influence over the occurrence of investigated species in Germany, where it prefers 25-50% shade level (19-22%) ( $\chi^2 = 10.333$ ,  $p = 0.0352$ ).

General conclusion about the shade over the water surface can be drawn that the conditions seem to be more optimal for *Triturus cristatus* if shade over the water surface is between 25-50%. The conclusion is considered to be very significant for both larvae (35-50% occupancy) and adults (19-23%) ( $\chi^2 = 21.000$ ,  $p = 0.0003$ ,  $\chi^2 = 18.194$ ,  $p = 0.0011$ ) (Tabl. 6).

Table 6. Shade provided by trees over the pond for all the investigated ponds in relation to the occurrence of *Triturus cristatus*

Shade over the pond, %	Number of investigated ponds	Presence of <i>Triturus cristatus</i> larvae, %	Number of investigated ponds	Presence of <i>Triturus cristatus</i> adults, %
0	47	28	69	16
25	17	35	53	23
50	6	50	32	19
75	3	33	30	7
100	2	50	14	0

## 5. Other fauna

### 5.1. Fish

*Triturus cristatus* was found mostly in ponds without fish (60% occupancy for larvae and 40% for adults) in east Poland. Ponds with fish were occupied twice-three times less. While such the distribution for larvae considered to be not quite significant ( $\chi^2 = 3.571$ ,  $p = 0.0588$ ).

Presence of fish significantly reduces the occupancy of *Triturus cristatus* in west Poland too ( $\chi^2 = 4.500$ ,  $p = 0.0339$  for larvae and  $\chi^2 = 4.000$ ,  $p = 0.0455$  for adults).

The opposite result was obtained in Germany where presence of fish in the ponds seems to be positive factor for *Triturus cristatus* (48% occurrence compare to 4% when fish is absent) and was proved to be significant ( $\chi^2 = 5.556$ ,  $p = 0.0184$ ).

So, the only clear conclusion about fish presence in the ponds can be made for *Triturus cristatus* larvae which higher occurrence in fishless ponds (39%) compare to those with fish (13%) is proved on extremely significant level ( $\chi^2 = 14.727$ ,  $p = 0.0001$ ).

## ***Bombina bombina***

### 1. Physical parameters of the ponds

#### 1.1. Pond type

There were found mostly natural depressions as a pond type in Poland. The distribution of *Bombina bombina* is considered to be not significant with some possible preference of other pond types unlike natural depressions for adults (60% to 40% accordingly).

*Bombina bombina* in Lithuania is likely to occur often in village ponds (80%), but it is considered to be not significant.



Almost all of the records of *Bombina bombina* was made from natural depressions in Germany (N=117) compare to the others (N=6).

So, summing up, adult *Bombina bombina* is shown to have higher occupancy in pond-like pond type (67%) compare to depression pond type and other types where occupancy is significantly lower (36-37%) ( $\chi^2 = 73.926$ ,  $p = 0.0001$ ).

### 1.2. Size of the pond

Analysing pond sizes in Poland we found out that *Bombina bombina* larvae were recorded only in the smallest ponds while not quite significant ( $\chi^2 = 8.000$ ,  $p = 0.0916$ ) (25% occupancy).

*Bombina bombina* doesn't show any preference to the analysed criteria in Lithuania ( $\chi^2 = 0.364$ ,  $p = 0.9853$ ).

*Bombina bombina* in Germany was found mostly in ponds with area more than 2000 sq m (53-56%) ( $\chi^2 = 22.939$ ,  $p = 0.0001$ ).

So, finalizing, big ponds (more than 2000 sq m) are found out to be preferable for adult *Bombina bombina* (57-59% occurrence) what is considered to be very significant ( $\chi^2 = 17.489$ ,  $p = 0.0016$ ). Larvae of the species were recorded in different sized ponds and didn't show significant preference in the investigated area in total ( $\chi^2 = 5.286$ ,  $p = 0.2592$ ), while with some slight shift to the larger ponds (500-10 000 sq m).

### 1.3. Maximum depth of the water

Ponds with maximum depth 0,5-1m seem to be more optimal for larvae of *Bombina bombina* (8%) compare to the deeper ones (0%) in Poland. Adult *Bombina bombina* doesn't show any significant preference to the criteria (42 and 44% accordingly) in Poland.

Adult *Bombina bombina* in Lithuania significantly likes deep ponds (more than 1 m)(40% occurrence) ( $\chi^2 = 8.918$ ,  $p = 0.0116$ ).

*Bombina bombina* in Germany likes deep ponds (more than 1m) (51% occupancy), and also middle deep ponds (0,5-1 m) (57%). This result is considered to be very significant ( $\chi^2 = 15.408$ ,  $p = 0.0015$ ).

So, in general, water depth has to be at least 0,5 m for adult *Bombina bombina* what is proved with more than twice higher occurrence frequency (45-49%) compare to shallow ponds (21%). Such a distribution is considered to be extremely significant ( $\chi^2 = 23.912$ ,  $p = 0.0001$ ). In case of species' larvae we can see that 0,5-1 m depth is probably optimal while the result is not significant ( $\chi^2 = 1.333$ ,  $p = 0.2482$ ).

Table 7. Maximum depth of water of all the investigated ponds in relation to the occurrence of *Bombina bombina*

Pond depth, m	Number of investigated ponds	Presence of <i>Bombina bombina</i> larvae, %	Number of investigated ponds	Presence of <i>Bombina bombina</i> adults, %
Less than 0,5	-	-	39	21
0,5-1	27	30	86	45
More than 1	30	13	85	49

### 1.4. Slopes

Any significant difference in the pond slopes inclination frequencies distribution for *Bombina bombina* in Poland wasn't show.

Small slopes' inclination (less than 10 grades) is found to be preferable and significantly proved for *Bombina bombina* in Lithuania (59% occurrence).

So it looks that general frequency trend of slopes inclination in the whole investigation area show the lower slopes are the higher *Bombina bombina* occurrence is ( $\chi^2 = 6.891$ ,  $p = 0.0319$ ).

### 1.5. Zones with shallow water

The percentage of shallow zone in the ponds is likely to be optimal on 11-25% level for *Bombina bombina* in Poland. Almost all the records of *Bombina bombina* from Germany were made from ponds with presence of shallow zone (N=117) compare to the others (N=6). So we can only suppose that the mentioned species need shallow waters to some extent.

In spite of the fact that the analysis of pond shallow zone coverage in total is considered to be not significant, we can say that the conditions seem to be more optimal for both *Bombina bombina* adults (73% occurrence) and larvae (40%) if it exceeds 25%.

## 2. Geology and water quality

### 2.1. Sediment

Sediment type was found to be indifferent for *Bombina bombina* larvae ( $\chi^2 = 1.001$ ,  $p = 0.6062$ ), but adults of *Bombina bombina* were recorded only in mud bottom ponds in Poland (41%) ( $\chi^2 = 8.008$ ,  $p = 0.0182$ ).

The strong preference to sediment type in *Bombina bombina* was found out in Lithuania. Adults of the species were recorded in most of the cases in mud bottom ponds (42% occurrence) and absent in peat bottom ones at all ( $\chi^2 = 25.727$ ,  $p = 0.0001$ ).

So, we are disposed to think that mostly mud and clay (43-50%) are those sediment types of the ponds to be occupied frequently by *Bombina bombina* ( $\chi^2 = 26.116$ ,  $p = 0.0001$ ).

### 2.2. Water quality

Choice between brown (50%) and muddy (43%) waters looks to be almost equal while not significant in case of *Bombina bombina* in Poland.

*Bombina bombina* was found only in muddy (50%) and clear waters (33%) in Lithuania with a sufficient degree of significance ( $\chi^2 = 12.636$ ,  $p = 0.0055$ ).

But, the best water quality for *Bombina bombina* unambiguously is clear, what is proved with the highest occurrence frequencies both for larvae (32%) ( $\chi^2 = 6.149$ ,  $p = 0.0462$ ) and adults (55%) ( $\chi^2 = 7.690$ ,  $p = 0.0214$ ) in the whole range of investigations and considered to be statistically significant (Tabl. 8).

Table 8. Water quality of all the investigated ponds in relation to the occurrence of *Bombina bombina*

Water quality, visual	Number of investigated ponds	Presence of <i>Bombina bombina</i> larvae, %	Number of investigated ponds	Presence of <i>Bombina bombina</i> adults, %
Brown	13	23	28	25
Clear	28	32	40	55
Muddy	18	11	32	47

### 3. Surrounding habitats

#### 3.1. Buffer zone

Importance of wide buffer zone (more than 50 m) is not clearly proved as significant, but it is noticeable in case of *Bombina bombina* larvae (10%) which never occur in the ponds with the narrower buffer zone in Poland.

On the contrast, in Lithuania all of the investigated ponds with *Bombina bombina* adults occurrence had narrow buffer zone (0-9 m) (55%) what is considered to be extremely significant ( $\chi^2 = 22.022$ ,  $p = 0.0001$ ).

*Bombina bombina* adults were found in both cases in Germany with some predominance of no pond buffer zone (56% to 37%) ( $\chi^2 = 34.306$ ,  $p = 0.0001$ ).

Summing up, we can say that only *Bombina bombina* larvae analysis shows extremely significant positive dependence between the width of buffer zone and occurrence of the larvae (34% in case of more than 50 m wide buffer zone) ( $\chi^2 = 17.732$ ,  $p = 0.0001$ ). Result for adult *Bombina bombina* doesn't show clear dependence ( $\chi^2 = 2.390$ ,  $p = 0.3028$ ).

#### 3.2. Grazing of pond

Majority of the individuals of the investigated species were recorded in ponds without grazing (N=26) compare to extensive one (N=2) in Poland, while single *Bombina bombina* adult was noticed once. The difference is significantly proved ( $\chi^2 = 7.364$ ,  $p = 0.0067$ ).

Grazing, especially normal regime of pond grazing seems to be important factor for *Bombina bombina* pond occupancy in Lithuania (63%) ( $\chi^2 = 0.728$ ,  $p = 0.6949$ ).

Analysing influence of grazing on *Bombina bombina* occurrence in the ponds in general, we can only assume that the species is likely to be more frequent in grazed ponds while the results are considered to be not significant ( $\chi^2 = 0.572$ ,  $p = 0.7512$ ).

#### 3.3. Terrestrial habitat within a 50 m radius around the pond

Differences for terrestrial habitat within 50 m radius for both *Bombina bombina* larvae and adults in Poland were considered to be not statistically significant ( $\chi^2 = 2.667$ ,  $p = 0.9140$ ).

At the same time, field (55%) and urban (75%) seem to be the most frequent habitat type nearby the ponds occupied by *Bombina bombina* adults in Lithuania ( $\chi^2 = 22.805$ ,  $p = 0.0018$ ).

Requirements for the habitat types within 50 m for *Bombina bombina* are more or less clear after the total analysis and show that high larvae occurrence is in case of meadow/fen, shrub, deciduous forest and common dry grass presence (24-29%), and field and shrub (56-57%) possibly urban too (64%) for adults. This is considered to be very significant for both life stages ( $\chi^2 = 20.061$ ,  $p = 0.0054$  and  $\chi^2 = 30.051$ ,  $p = 0.0001$  accordingly).

#### 3.4. Terrestrial habitat in the radius of 50 to 500 m around the pond

No significant preference to any habitat type in the radius 50-500 m was highly proved for adult *Bombina bombina* in Poland ( $\chi^2 = 19.429$ ,  $p = 0.0069$ ).

Similar result was obtained for Lithuania, except of little prevalence of urban (64%), shrub (53%) and deciduous forest (80%) habitat types ( $\chi^2 = 10.922$ ,  $p = 0.0908$ ).

So, the final analysis of terrestrial habitat composition in radius 50-500 m for adult *Bombina bombina* occurrence doesn't show any significant dependence ( $\chi^2 = 8.061$ ,  $p = 0.2337$ ) except may be urban and deciduous forest habitat types, that have a bit higher frequencies (59% and 55% accordingly).

### 3.5. Distance between the pond and the forest

Distribution of the analysed frequencies of the distance between the pond and the forest in Poland was shown to be insignificant for *Bombina bombina*.

*Bombina bombina* was found only in ponds that were far than 50 m from forest in Lithuania ( $\chi^2 = 13.714$ ,  $p = 0.0083$ ).

The occupancy of ponds with close distance to the forest (10-50 m) was the highest in *Bombina bombina* (71%) compare to the other investigated distances in Germany, while the result is not proved as significant ( $\chi^2 = 1.882$ ,  $p = 0.7574$ ).

Summing up, even the result is not proved as significant ( $\chi^2 = 4.433$ ,  $p = 0.1090$ ) we got clear growing occurrence frequency trend towards longer distance to the forest from ponds (more than 50 m) for *Bombina* larvae (35%). Adult *Bombina bombina* show almost the same preference as the larvae as their highest occupancy (92%) was recorded in ponds on 50-200 m far from the forest ( $\chi^2 = 8.027$ ,  $p = 0.0906$ ).

### 3.6. Distance between the investigated pond and other ponds

The highest occupancy of *Bombina bombina* adults (73%) was recordered in the ponds with neighbour ones in 100-200 m ( $\chi^2 = 9.889$ ,  $p = 0.0195$ ) in Poland only.

## 4. Biotic factors

### 4.1. Shade provided by trees over the pond

Analysis of the results of shade over the water surface can be drawn that the less it is the more occupied ponds are, especially for adult *Bombina bombina* (47%), what is considered to be statistically significant ( $\chi^2 = 13.769$ ,  $p = 0.0032$ ).

Absence of shade over the water surface seems to be important for *Bombina bombina* in Lithuania too, as occurrence in this case is the highest in the ponds (33%), moreover the species was not recorded in the ponds with shadow over 50% at all ( $\chi^2 = 19.909$ ,  $p = 0.0002$ ).

*Bombina bombina* in Germany prefers 0-25% (46-50%) ( $\chi^2 = 13.551$ ,  $p = 0.0089$ ).

So, general conclusion is that conditions of shadow over the water surface seem to be the best for *Bombina bombina* larvae and adults occurrence if it is 0% at all (29% and 50% accordingly) what is proved as highly significant ( $\chi^2 = 7.143$ ,  $p = 0.0075$  and  $\chi^2 = 33.830$ ,  $p = 0.0001$ ) (Tabl. 9).

Table 9. Shade provided by trees over the pond for all the investigated ponds in relation to the occurrence of *Bombina bombina*

Shade over the pond, %	Number of investigated ponds	Presence of <i>Bombina bombina</i> larvae, %	Number of investigated ponds	Presence of <i>Bombina bombina</i> adults, %
0	41	29	91	50
25	12	17	60	45
50	-	-	33	39
75	-	-	33	27

## 5. Other fauna

### 5.1. Fish

Presence of fish reduces the occupancy of *Bombina bombina* larvae ( $\chi^2 = 2.000$ ,  $p = 0.1573$ ) while *Bombina bombina* adults looks to be more or less indifferent to the criteria in Poland.

Presence or absence of fish in the investigated ponds in Lithuania was not proved as significant in case of *Bombina bombina*, better say this factor seems to have almost theoretical (expected) frequencies according to the null hypothesis ( $\chi^2 = 0.818$ ,  $p = 0.3657$ ).

*Bombina bombina* was found in both cases with predominance of ponds with fish in Germany (55% to 35%) ( $\chi^2 = 5.898$ ,  $p = 0.0152$ ).

So, total analysis show that *Bombina bombina* larvae were found only in ponds without fish (21% occurrence) what is considered to be very significant ( $\chi^2 = 9.000$ ,  $p = 0.0027$ ), whereas adults show little preference for ponds with fish (49%) inhabiting at the same time ponds without fish too (36%) ( $\chi^2 = 14.412$ ,  $p = 0.0001$ ).

## Conclusions

From our inventories of 274 ponds in the project areas of the 3 countries and from 31 ponds created or restored in Denmark and Schleswig-Holstein targeting *B.bombina* and *T.cristatus* in previous LIFE projects we could take recommendations for our own work on pond creation and restoration. The recommendation was used in practice in this ongoing LIFE project. We can recommend our best practice and to pay attention to the following criteria and parameters for aquatic habitat restoration for the 3 target species *E. orbicularis*, *B.bombina* and *T.cristatus* in North European lowlands. In general its very important for all 3 species to have pond clusters with different size ponds and rich in the biological structures (see chapters on *Emys orbicularis* and previous best practice guidelines for LIFE bombina and LIFE cristatus projects) needed for each species.

These biological structures of plant communities and dead wood develop with time but during the restoration and creation process its possible easy to directly influence physical parameters of the ponds, water color and quality, Shadow on pond, distance to other habitats, fish presence and grazing.

### **POND CLUSTERS:**

It is found to be significant that the distances between ponds in pond cluster should preferably be 100 m for *Triturus cristatus* and 200 m for *Bombina bombina*. While pond clusters is probably also important for *Emys orbicularis* areas despite that fact that in other areas the whole population live in on one larger wetland or ponds. We suggest increasing pond density will benefit for all three species.

### **SIZE OF POND:**

In each pond cluster it's seem is better to make less some ponds of less than 500 m<sup>2</sup> ponds for *Triturus cristatus* and some larger than 2000 m<sup>2</sup> for *Bombina bombina*. *Emys orbicularis* was found most often in ponds of 500 m<sup>2</sup> -2000 m<sup>2</sup> and thus if possible some of the created ponds should have this size.

### **DEPTH OF POND:**

It is most optimal to create 0,5-1,0 m deep ponds for *Triturus cristatus* and deeper than 0,5 m for *Bombina bombina*. *Emys orbicularis* shows similar tendency to *Bombina bombina* so there should be some ponds in the pond cluster with more then 0,5 m.

**SLOPE OF POND:**

Pond slope inclination has no real negative influence on *Triturus cristatus* in this study while *Bombina bombina* prefers ponds with flat slopes. *Emys orbicularis* adults looks like to have preference to ponds with some steep unexposed slopes and juvenile and subadults occur more ponds with flat slopes. So its recommended to have many ponds with a variation in slopes in order to benefit all 3 species.

**SHALLOW WATER ZONE:**

Shallow water zone area doesn't show any preference for *Triturus cristatus*. It is better to make ponds with shallow zone more than 25% cover for *Bombina bombina*. It looks that *Emys orbicularis* doesn't show tight attachment to shallow zones in the ponds. It's recommended to always create some ponds with shallow zone in order to have *B.bombina* in the ponds.

**WATER CLARNESS:**

It is better to have clear water in the ponds both for *Triturus cristatus* and *Bombina bombina*, especially in case of larvae, but adults can also live in brown or muddy water. Water quality has no significant influence on *Emys orbicularis* presence. It's recommended to have several ponds with clear water in each cluster to secure breeding of *Triturus cristatus* and *Bombina bombina*.

**BUFFER ZONE:**

*Triturus cristatus* and *Bombina bombina* larvae show clear preference to the aquatic habitats with wide uncultivated buffer zone (over 50 m around the pond). The same is the case for *Emys orbicularis* adults and even more for the juveniles and subadults.

**DISTANCE FROM POND TO FOREST:**

The results of this study show that preferable distance from the pond to the nearest forest for *Triturus cristatus* and *Bombina bombina* larvae is no more than 200 m, better less for *T. cristatus*. Presence of forest not so important for *Emys orbicularis*, despite the fact that *Emys orbicularis* last remaining populations often occur in small open areas inside or on the edge of larger forested areas. The recommendation is that several of the ponds in the pond cluster should be within 200 m distance of the forest in order to have all 3 target species in the clusters.

**GRAZING:**

Grazing found slightly negative influence on *Triturus cristatus* larvae, but *Bombina bombina* prefers it. So, grazing should be planned in details not to harm the rest of the species including *Emys orbicularis* where wrong planned grazing on the nesting sites and some pond slopes can be negative.

**SURROUNDING HABITATS:**

It is important to have permanent grasslands close to *Triturus cristatus* and *Bombina bombina* aquatic habitats (within 50 m around); additionally – dead wood nearby in case of *Triturus cristatus*. It looks to be important also to add dead wood to especially newly created a pond banks for *Emys orbicularis* and of most importance is maintain and create nesting sites as close as possible to the ponds.

**SHADOW ON PONDS:**

It is better to make moderate shaded ponds for *Triturus cristatus*, and the less shade the better for *Bombina bombina*. No significant preference for pond shade was noticed for *Emys orbicularis*, however there must be a minimum of 25-50 % sun in summer ponds (not hibernation ponds which

can have much shadow) and each pond must be evaluated separately by experts before management before a change in shadow and sun influx is made.

**FISH PRESENCE:**

*Triturus cristatus* and *Bombina bombina* larvae can normally survive only in fishless ponds, exemptions are sometimes ponds with a large fluctuating shallow zone, while adults can live also in ponds with fish. It seems not important to *Emys orbicularis* if there is fish in the pond or not according to the pond inventory data. It's known that turtle can feed on dead fish, and thus its recommended to have some ponds with fish and some without in the pond cluster.

**WATER BIRD PRESENCE:**

Try to avoid water birds, especially ducks for larvae of *Triturus cristatus* and *Bombina bombina*, while the birds do not harm adults according to this pond study. However its known that heron can reduce number of adults of *Triturus cristatus* and *Bombina bombina* significantly. Water birds seem no problem for *Emys orbicularis* in this study but again there can be cases where some bird are predating on especially small turtles. To avoid birds in the pond cluster is not needed but it's important not to do actions to attract birds as feeding birds or setting up nest boxes or platforms for ducks.