Managing Bombina bombina in the Baltic Region

Best Practice Guidelines

Experiences from the LIFE-Nature project 'Management of firebellied toads in the Baltic Region' LIFE04NAT/DE/000028

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Contents

1. Introduction

The firebellied toad (*Bombina bombina*) is a characteristic animal of the north European lowlands. In former times it was widespread all along the Baltic countries. It occupies a place in the collective cultural memories of humans with its melodious call as a distinct feature of summer afternoons and nights. It is part of our natural heritage. However, the rapid land use change has resulted in loss of original breeding sites and summer habitats. Increasingly fewer cows were kept outside, ponds disappeared from the landscape and the use of heavy machines threatened many firebellied toad populations. In 2004 only 13 animals was left on the island of Fehmarn – although many people still remembered the songs of the

Juvenile *Bombina bombina.*

The firebellied toad is a typical amphibian of the north European lowlands. Here, a calling male is doing his best to attract females.

Arial view of the project site in Stodthagen (Germany), where a network of ponds has been created for the firebellied toad during the project.

"Fehmarner Nachtigall" (the nightingale of Fehmarn) quite well.

The trend on Fehmarn was repeated in the other partner countries in this LIFE-project: Denmark, Germany, Sweden and Latvia. In order to contribute to the protection of the firebellied toad and to secure its future within the northern distribution range we decided to launch the LIFE-*Bombina* project. Eight partners from four countries joined efforts and knowledge during five years (2004-2009). Financial resources from the European Union enabled us to carry out various measures for protection of the firebellied toad, which is listed on the red lists of all partner countries. Key features in the project were construction and renovation of new breeding ponds, construction of hibernation quarters, instalment of grazing in *Bombina* areas and creation of an ecological network between small isolated populations. In addition, population management activities were implemented; *Bombina* eggs were collected in the wild and reared under controlled conditions in our breeding stations. The young toadlets were released at sites which were prepared for them in advance.

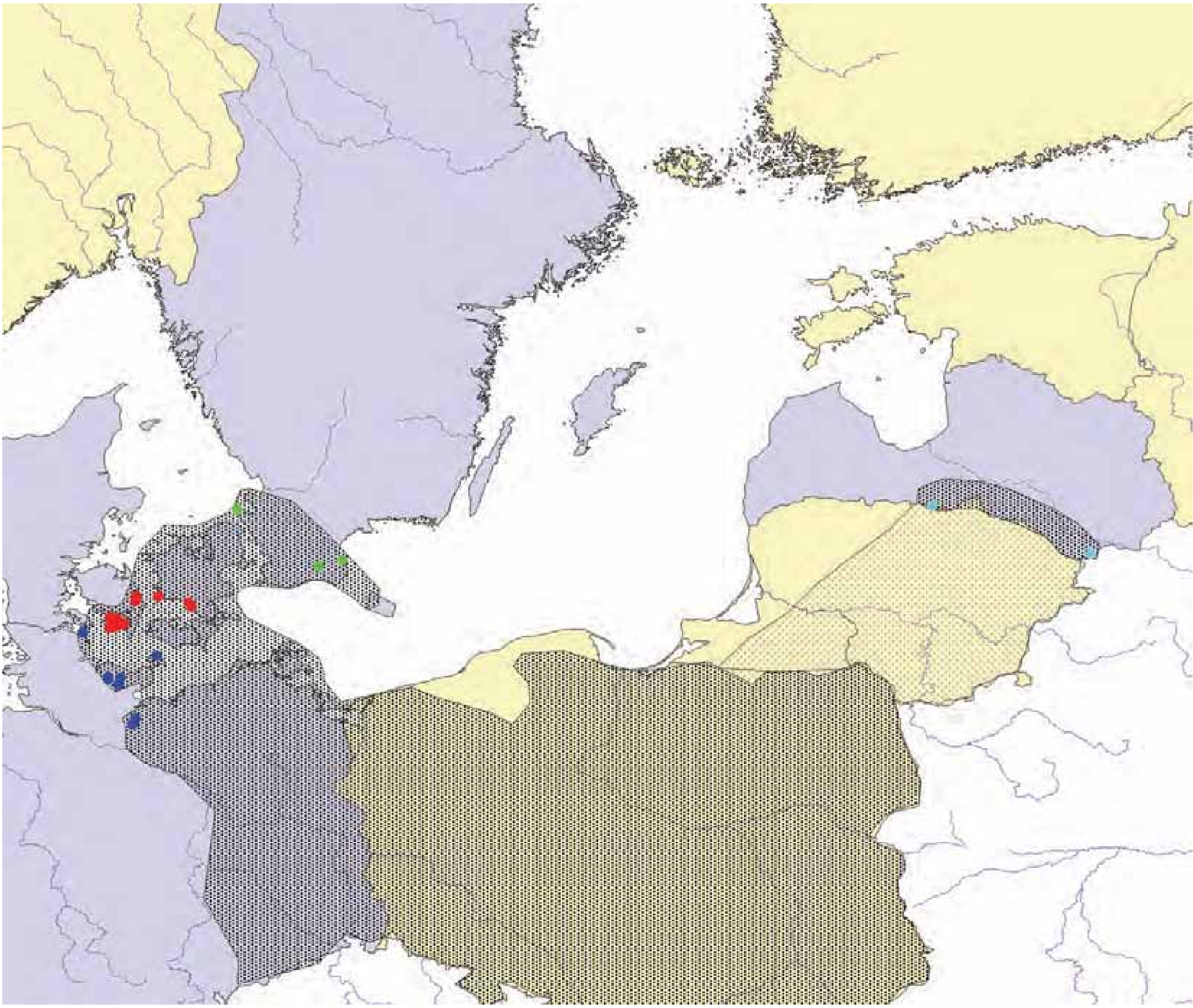
Corresponding to the conservation work, special focus was placed on public relation work focusing on *Bombina*. Numerous talks with landowners, guided tours and two 'European *Bombina* song contests' helped returning the threatened species to the public mind. And the results? The Fehmarner Nachtigall is calling again. In 2010 more than 40 calling toads were registered on the island – approximately 150 animals inhabit Fehmarn again. And the other project sites? Please read our final report; the presented details generate positive outlooks for the future of the firebellied toad in the Baltic countries.

In the following chapters we present our practical and technical knowledge concerning ecology and management of *Bombina bombina* to an interested audience, hoping that our experiences will help others engaged in the protection of this highly endangered species.

Project Sites

Four countries (Germany, Denmark, Sweden and Latvia) were involved in the project and a total of 27 project sites around the Baltic Sea were targeted for the protection of the fire-bellied toad.

Project sites at the Baltic Sea. The involved countries have a blue colour. The hatched area shows recent and historic recordings of *Bombina bombina*. Project sites are marked with blue dots in Germany, red dots in Denmark, green dots in Sweden and turquoise dots in Latvia.



2. Biology and Morphology

2.1. The Genus Bombina

The genus *Bombina*, firebellied toads, belongs to a relatively primitive group of anurans and differs from more advanced anurans in several respects. For instance, the tongue is short and barely protrudes the mouth, limiting available food sources.

Worldwide, the genus *Bombina* is composed of eight species. Five of these live in East Asia, mainly China, and three live in Europe.

The best known east Asian species is *Bombina orientalis* (Boulenger), which is often held in captivity. It resembles *B. bombina*, but the skin warts on the dorsal side are much more acute, and the ventral side is smoother, nearly glossy with a red coloured pattern without white spots.

The European species are:

- Apennine firebellied toad, *Bombina pachypus* (Bonaparte), which only lives in Italy south of the Po valley.
- Yellow-bellied toad, *Bombina variegata* (L.), which lives in mountain regions throughout central Europe and the Balkan Peninsula.
- Firebellied toad, *Bombina bombina* (L.), which lives in lowlands in east-central Europe.

2.2. Morphology

2.2.1. Description of *Bombina bombina*

Bombina bombina is a toad-like creature with brownish, warty skin and short legs. It has many small round tubercles. The tubercles on the ventral side are white and they have a central black pin when the toad is submerged in water. These central black pins are invisible when the skin is dry. The eyes are positioned rather high on the head, obviously an adaptation to a mostly aquatic feeding habitat. The pupils are not quite rounded, but instead inverse drop shaped with the acute angle turning downwards.

Behind the eyes, the tympanum is difficult to discern, and in the neck region, a pair of indistinct glands resembles the parotid glands of toads. They are often slightly more chestnut or reddish than the rest of the dorsal side.

In general, the dorsal side is greyish, grey brown or nearly black, with darker spots. The ventral side is almost black, with distinct spots in a bright colour; typically orange but may vary from yellow to red. More details on the colour pattern are given in the section about

Above: dorsal view of a female *Bombina bombina* - notice the slim triangular neck. Below: ventral view of a male *B. bombina*; every animal has a unique, never-changing pattern. The male vocal sac is clearly visible.

Freshly metamorphosed animals basking in the sun.

hybrids.

When newly metamorphosed specimens first enter land, they are typically about 16 mm long (variation: 10 to 23 mm). The dorsal side resembles the adult colour pattern. The neck region is often distinctly marked with green duckweed spots, and some individuals have more extensive green colouration on the back. This green colouration may disappear rather soon, or be preserved for a year or more. The ventral side is greyish, and the characteristic orange spot pattern takes several months to form. Once the pattern is formed, it is practically unchanged for the rest of the animal's life.

During their first autumn, the toadlets may typically grow to a length of 20 to 25 mm. During the winter they hibernate. Next year (second calendar year), they feed intensively and go through a period of rapid growth. If food is not limiting, they reach adult size during the summer. After the second hibernation, in the beginning of the third calendar year, they are sexually mature. In dense populations where food may be limiting, it may take one more year to reach sexual maturity. Sexual maturity is reached when the toad is approximately 37 mm (Denmark) or 30 mm (east Germany) in both sexes. Growth continues during the following years, and older animals may reach lengths up to 56 mm (Denmark) or 60 mm (Poland). Very old animals shrink somewhat. The oldest known animals observed in nature were (at least) 13 years old, but most individuals die before the age of 10.

2.2.2. Animals with hybrid features

In areas where the distributions of *B. variegata* and *B. bombina* overlap, the two species interbreed. The hybrids are viable and fertile, but have reduced fitness, thus the species barrier does not collapse.

Interbreeding results in *B. variegata* genes entering the *B. bombina* gene pool, and *B. bombina* genes entering the *B. variegata* gene pool. The colour pattern differs between typical specimens and may be intermediate in hybrids:

Dorsal side

In *B. bombina*, dark coloured (nearly black) tubercles form a more or less symmetrical pattern along the dorsal midline. Two elongate, curved, sausage-shaped spots on each side of the neck are particularly characteristic. Each spot can be divided in two shorter spots. Next to these on each side of the midline are a pale spot, which is often bright green, especially in juveniles ('duckweed spots' - they look like leaves of duckweed adhering to the skin).

In *B. variegata*, the tubercles are not much darker than the rest of the back, and not noticeably elongated. They are not especially symmetrically arranged along the midline, and the duckweed spots are absent.

Belly

In *B. bombina*, the ventral side is dark (bluish-black) with many small white points, and rather large orange coloured spots with irregular shapes. These spots rarely cover more than 50% of the belly surface. In the breast region, i.e. between the forelegs, there are two separate spots, one on each side of the midline. Likewise, the pelvic region almost always has two separate narrow spots.

In *B. variegata*, the yellow or orange spots usually merge into one large adjoining pattern. The spots in the breast region and the pelvic region are not separate. Rather, a continuous area of yellow stretches from the arms via the breast to the pelvic region and the hind legs, without interruptions of black.

Forelegs

On the underside of the forelegs, *B. bombina* has orange spots under the humerus and the lower arm; they often merge. There is a spot on the palms, which does not extend to the fingers. The finger tips are dark or weakly coloured. In *B. variegata*, the spots on the arm merge with each other and the spots on the breast. The spots on the palms extend to the first finger, which is completely yellow.

In *B. bombina* specimens with some *B. variegata* genes, any combination of the *B. variegata* colour pattern may be expressed on the dorsal side, on the belly, and on the underside of the arms and fingers. Typically, for instance, the pair-wise spots in the breast and pelvic regions are not isolated from the other spots; the outer part of the first finger may be orange; or the dorsal spots may not be elongate or arranged along the midline.

2.2.3. Sex differences

It is only possible to distinguish the sexes in adult animals. The males have a vocal sac under the skin in the throat region resulting in a somewhat bulbous hind part of the throat. When the sac is not extended, the skin on the throat is relatively loose. The head is fairly broad, as if to make room for a broad vocal sac.

During breeding season, males have a patch of rough skin, nearly like sandpaper, on each lower arm. This patch borders the orange and the black part, and the colour is generally brownish. It is usually rather easily distinguishable, but in some sexually active males it is weakly developed and very hard to discern.

Females differ in having more narrow heads, and in lacking the sandpaper patch on the lower arm in the breeding season. It can be very difficult to tell the sexes apart out of breeding season.

2.2.4. Toxicity

The skin secretes toxic substances. Handling firebellied toads and then touching one's own mucous membranes e.g. the eyes, will result in a painful extended experience. Therefore, it is recommended to wash hands after handling the animals.

If the animals are stressed, they secrete white foam which is particularly toxic and may kill other anurans.

The conspicuous colour pattern on the ventral side is obviously an alarm signal, but it is usually not seen. When an animal feels threatened, it will raise its hind- and forelegs and show the orange spots there. It will also curve its back, and maybe even turn around to show the whole belly as a warning.

It is not known precisely which predators are kept away by the toxicity and/or the warning signal.

2.2.5. Eggs

Eggs are deposited in clutches on aquatic plants some distance below the water surface, usually - but not always - on vertical straws, where the elongate clutch is placed all around the straw. The number of eggs per clutch can vary, but often 20 and 40 eggs are laid, and rarely more than 100 are found. Each egg nucleus has a diameter of 1.2 - 2.2 mm, typically 1.8 mm. The top half is brownish clay coloured. Downwards there is a gradual, but narrow transition to the pale colour of the bottom half, which is not quite white, but rather somewhat yellowish. Around each nucleus is a relatively firm gel with a diameter of 4 - 8 mm. All eggs are connected by a very thin and loose gel.

Bombina bombina egg clutch characteristically arranged around a *Juncus* stem.

The eggs may be confused with eggs from green frogs (*Rana esculenta*). Egg clumps from green frogs are extremely variable with respect to nearly all features (placement; egg number; egg size; colour on top half; colour on bottom half; width of transition zone), and in some cases it may be nearly impossible to differentiate *R. esculenta* eggs from *B. bombina* eggs. The most constant feature is probably the structure of the connecting gel. This is very thin and without structure in *B. bombina*, whereas in *R. esculenta*, it is somewhat fibrous when stretched.

Shortly before the eggs hatch, the foetuses are easily seen. In *B. bombina*, the foetus has a broad U-shape. In *R. esculenta*, on the other hand, the shape is more like a J, with a relatively short part of the tail bent around. In *R. esculenta* the head is relatively large and the tail relatively short.

2.2.6. Newly hatched larvae

Newly hatched larvae have a length of 6 - 7 mm. The dorsal side is bright greyish brown, and the belly (the yolk sac) is bright yellowish. In this stage, they are very similar to larvae of some other anurans, especially some individuals of *R. esculenta*. There are some differences, however. Compared to *R. esculenta*, the head of *B. bombina* larvae is shorter and broader. The eyes are smaller, and the tail is longer. A diagnostic feature is the adhesive glands (two dark papillae beneath the mouth), which may be seen with a magnifying glass when the larva is adhering to a glass wall. In *B. bombina*, these are two relatively large chestnut coloured papillae which are situated closely together near the ventral midline. In *R. esculenta*, they are usually dark brown or black, and they are often (but not always) situated much further apart.

This stage lasts for about five days. Toward the end of this period, the adhesive glands change their shape and become two traversal bands some distance behind the mouth. The colour is still dark chestnut.

Bombina bombina tadpole, 3 cm. Distinct white stripes are clearly visible from the nostrils along the spine.

2.2.7. Tadpoles

When the mouth and anus are formed and the animal starts feeding, it has become a tadpole. At this point it is yellowish with three longitudinal black bands, one through the eye on each side, and one along the dorsal midline. When it grows, the characteristic longitudinal bands remain visible. The ground colour is grey brown with a characteristic bluish violet lustre. In a magnifying glass, two sets of very fine black lines crossing each other are perceptible within the tail fins.

The body is short and rounded, the tail is relatively short, and on the back, the tail fin stretches forward and almost reaches the head.

2.3. Distribution and genetic variation

2.3.1. Details on the distribution of *Bombina bombina*

The distribution of *Bombina bombina* is seen in Figure 2.1. In the east, the species reaches the Ural Mountains and the regions east of the Ural River in Kazakhstan. It lives at the north slopes of the Caucasus range and on parts of Crimea Peninsula. It follows the coast of the Black Sea westward into Rumania, Bulgaria and Thrace, i.e. the European part of Turkey, and easternmost Greece. In addition, it occurs in a restricted area in the north-western part of Asian Turkey.

In the west, the distribution stretches from the westernmost part of Bulgaria into Serbia and Bosnia; continues through central Croatia and Slovenia to southeast Austria. The distribution continues into the Pannonian region, where *B. bombina* is distributed all over the Czech Republic, Slovakia and Hungary. The populations in these countries are separated by mountain ranges (Carpathians etc.) from the populations living further to the east and north.

East of the Carpathians, the distribution extends all the way from the Black Sea to the Baltic Sea and into eastern Germany. West of Magdeburg it was formerly distributed in the Aller valley as far west as the river Weser east of Bremen, but the populations here have disappeared during the 20th century.

The former extensive distribution along the river Elbe has also declined, but surviving populations persist as far west as Niedersachsen. *Bombina bombina* extends into Schleswig-Holstein south of Lübeck. Recent extinctions have separated these populations from those in the central parts of the hilly east Holstein. Westward, it extends almost to Kiel. Formerly a few relict populations existed north of Kiel near the coast of the Baltic Sea; in Dänischer Wohld, in Schwansen, and at Gelting in Schleswig. Of these, only the population in Dänischer Wohld has survived. Further north, in Denmark, the species used to live on the island of Als.

The original northern distribution border followed approximately the 56° degree of

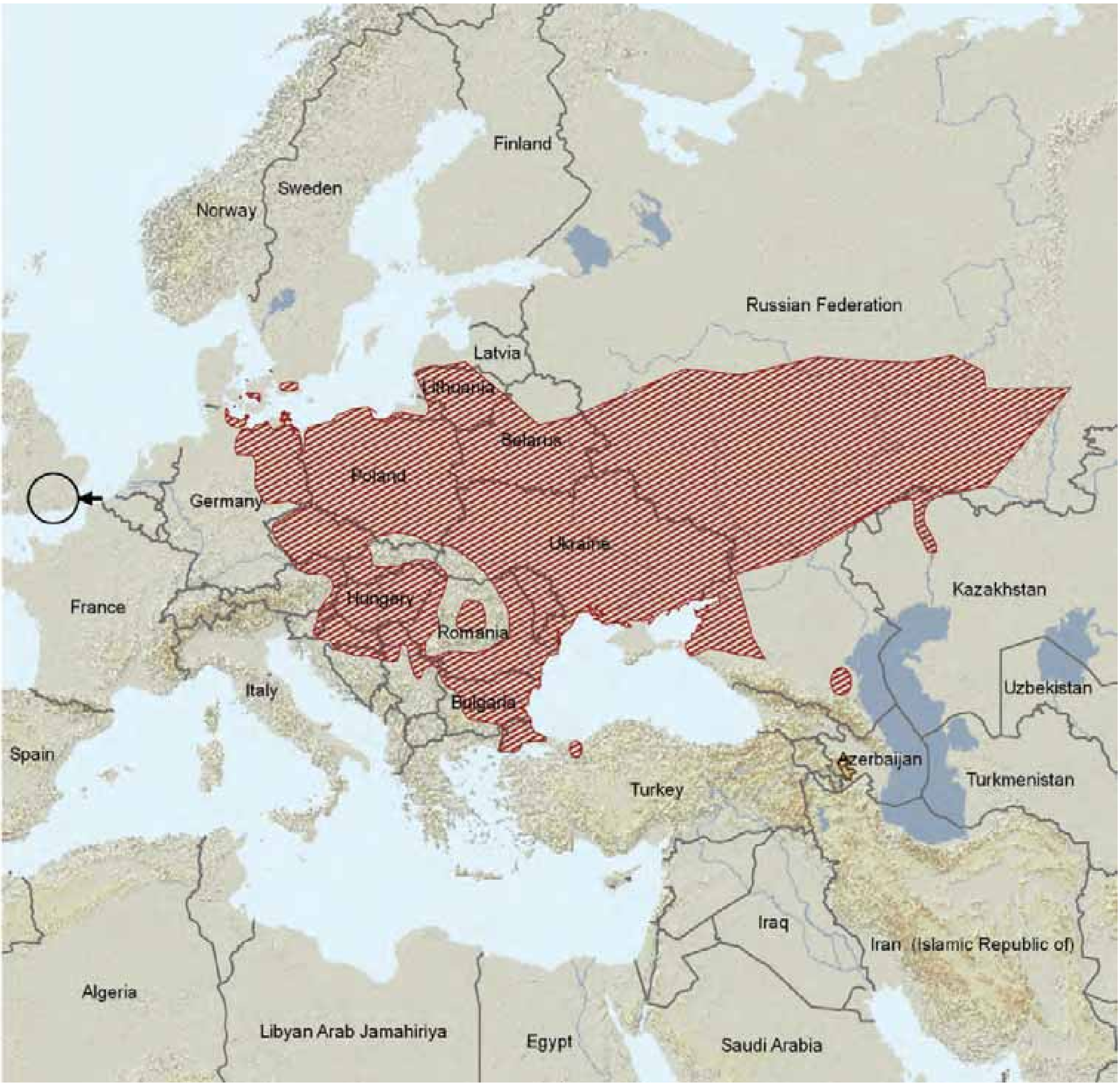


Figure 2.1. Distribution of *Bombina bombina* marked with red hatching. (From IUCN).

latitude, but many of the northernmost populations have gone extinct. The northernmost naturally surviving population in Scandinavia is on the Danish island of Nekselø (55°48').

East of the Baltic Sea, in Lithuania and Latvia, it avoids the coastal regions. In Latvia, it is thought to have lived as far north as Riga (57°), but today it is only found in southernmost Latvia south of Bauska and south of Daugavpils. The north border continues eastwards through Russia, where it can be found as far north as 56-57° N. East of Moscow, it still persists in several places near the former north limit of 57° in Tatarstan.

In conclusion, the northernmost latitude is nearly constant all the way from Denmark to the Ural Mountains. This indicates that the north border is determined by summer temperatures or the influx of sunshine.

2.3.2. Genetic variation

The genetic variation within *Bombina bombina* has been investigated by various scientists. In the 1980s, variation in enzyme systems (isoenzymes) was investigated. In these systems, the number of differing alleles is relatively small. Later, mitochondrial DNA was studied in many eastern Europe populations.

During the LIFE *Bombina* project, a study group at Potsdam University led by Ralph Tiedemann has investigated samples from the northern part of the species' range. They analysed various types of DNA. These were:

1) Mitochondrial DNA, which mutates relatively rarely, and thus provides information about diversification on a relatively wide scale in time and space.

2) Microsatellites of the cell nucleus DNA. These have a high rate of mutation and shows variation on a narrow scale in time and space.

3) DNA of the Major Histocompatibilty Complex in the cell nucleus (MHC). The MHC

Presentation of the genetic laboratory at Potsdam University with professor Tiedemann (second from the right).

codes for essential proteins in the immune system and thus has adaptive value to the animal. The rate of mutation is intermediate.

The studies on variation in isoenzymes have shown that there is a relatively high genetic variation (heterozygosity) in southern populations, and a very low variation in northern populations (Poland, Russia). This is corroborated by mitochondrial DNA studies. The highest variation is found in regions where populations survived during the ice age. Apparently, at least two refugia were present during the last ice age. One was in the Romanian lowlands, probably extending along the river Donau into the Pannonian basin. Populations from this refugium later colonized the northwest including most of the Pannonian basin and the northeast including parts of Ukraine. Another possible refugium is found at the north bank of the Black Sea. Populations from this refugium have spread northwards east of the Carpathians as far as into Denmark, Sweden and the Baltic countries. The distribution extended to the west, along the north side of the Sudeten Mountains, via southeast Germany into the Czech Republic.

According to these results, there are two main groups (clades) of *Bombina bombina* in Europe; one inhabiting most of the Pannonian basin, the Romanian lowlands and parts of Ukraine, and another inhabiting the remaining parts of the distribution area. Today, these two clades meet in Ukraine and possibly eastern Czechia, but previously, they have been separated for long, even during the last glacial maximum.

The studies performed by the Potsdam group provide more detail on the genetic variation in the north and northwest. After the end of the ice age, animals have migrated from Ukraine and Belorussia northward into the Baltic countries, and westward through Poland into eastern and northeastern Germany. The distribution of mitochondrial DNA haplotypes demonstrates what happened next. One widely distributed genotype is common in Holstein, especially in west Holstein. Another genotype, differing by one mutation, replaces it to the east, in northeast Germany and parts of Poland. The common haplotype in Holstein is also quite widespread further north in Denmark. This suggests that the Danish animals have emigrated from Holstein. A different local mutation has resulted in a local genotype which is found only in the Fyn region in Denmark. Another genotype, differing from the more eastern (German-Polish) type by one mutation, is endemic to the Great Belt region in Denmark, i.e. on the islands near the coast of Sjælland, and quite rarely also in a population on the Fyn side of the belt.

Altogether, the Danish animals contain three or probably four mitochondrial genotypes. Such a wide variety of genotypes in Denmark indicates that the migration from Holstein into Denmark has involved many animals, not just a small founding colony. Furthermore, it must have happened long ago, since local evolution and diversification is registered in Denmark.

Bombina bombina went extinct on Bornholm around 1930 and in Scania (south Sweden) around 1960. We do not know which genotypes were present in these extinct populations.

2.3.3. Illegal introductions

At the last Swedish *B. bombina* locality, Mölle in NW Scania, the species went extinct in 1960. Two illegal reintroductions are known, one from Denmark in 1977, and one allegedly from eastern Austria a few years later. The number of calling males was low during the late 1980s. After 1987, juveniles originating from Denmark were introduced legally, and the population grew. It would thus be credible to assume that the genetic composition reflected the Danish origin of the animals, possibly with a very small contribution of genes from Austria.

However, analysis of samples taken in 2004 offered a completely different result. A mitochondrial genotype, which occurs in the Pannonian region (Austria) and differs widely from north European genotypes, had a frequency of no less than 87%.

Surprisingly, the Pannonian mitochondrial genotype has been found in three other localities. First, at Fredriksberg in central Scania, Sweden, with a percentage of about 50% - the most likely explanation is that the animals were introduced illegally from Mölle to this locality. And second, at two localities in

Holstein. At one of these localities, near Bungsberg, other unexpected amphibians are encountered, and it seems obvious that the landowner has introduced amphibians from elsewhere in Europe.

Thus, the genetic analyses have proved to be a powerful tool to demonstrate the origin of populations and to detect illegal introductions.

2.3.4. Legal introductions in Sweden

The legal introductions in Scania in south Sweden have typically been established by releasing offspring from three different Danish localities.

For instance, 250 individuals (tadpoles and metamorphs) originating from Agersø were released at 'Skogshuset' in central Scania in 1983. In 1984 the same number of individuals, originating from Nekselø were released, and in 1985 another 250 individuals from Knudshoved. Later, additional animals were released from the rearing station in Göteborg, all originating from the same three source localities.

A genetic analysis of the population at Skogshuset in 2004 allowed for mapping of the surviving alleles. The microsatellite data unequivocally shows that all individuals present at Skogshuset have inherited their genes almost exclusively from Nekselø and that offspring from Agersø and Knudshoved practically has become extinct.

Similar investigations have been performed at Bäckhalladalen in easternmost Scania, where animals were released from several Danish localities during the period 1983-2002. Again, microsatellite analysis clearly showed that the dominating alleles originated from a single locality (Avernakø), with contribution of alleles from one other locality in some individuals.

Thus, the genetic analyses have demonstrated that although animals from at least three localities were released in each locality, the surviving genetic material mainly originates from just one of these.

3. Ecology

3.1. Behaviour

3.1.1. Yearly activity cycle

At some point during the summer season, the animals leave the ponds and enter land. If the ponds dry out, this may happen as early as around midsummer. Otherwise, it may happen from late July well into August. They migrate to suitable nearby localities and hide in holes, e.g. between stones in stone fences, under tree roots, or in rodent holes. The place is often several hundred meters from the pond. After spending some months here, they seek deeper into suitable cavities. Big aggregations of se-

Artificial hibernation quarters.

Ideal spring habitat with shallow water that heats up fast.

A calling male tries to attract females for spawning. Visible waves are produced during calling. Males in the vicinity sense the waves and keep their distance.

veral hundred individuals are known from exceptionally favourable places. Normally, the species hibernates on land, but hibernation in ponds is mentioned for a few sites in Russia and Kazakhstan.

The animals reappear from their hibernation sites during spring; usually in the latter half of April but it may vary between late March (Germany) and late May.

Typically, shallow temporary ponds that are heated up relatively fast by the sun, are favoured by the animals in April in order to get warm. These pools eventually dry up, and the animals move on to the breeding ponds. Here they will stay in May and June during the breeding season. They may stay there to feed for the rest of the summer. However, many individuals move on to other ponds unsuitable for breeding, but suitable for feeding, and stay there during summer.

3.1.2. Breeding activity

The males start to call when the water surface temperature is at least 14-15°C. Males are stimulated to call by pronounced weather improvements, e.g. when grey and cold weather is succeeded by several warm and sunny days. Not only temperature, but also precipitation is important. Rainfall may trigger calling.

The males undergo physiological change when they enter the mood for calling. Their bodies absorb water which makes them swell and the skin hang loosely. In this condition, they are able to take in air and become inflated prior to calling. The air is pressed out into the vocal sac, and when the air is squeezed back into the abdomen, the sound comes out. Simultaneously, surface waves are produced in the water. Usually, the male also makes jerky movements with the hind feet, which creates additional waves. The waves may be perceived by other individuals through the sideline system. Calling volume varies. Sometimes it can only be heard 200 meters from the source, but on rare occasions it may be so loud that it can be heard more than 1 km away.

Males stimulate each other to call; usually, they form a chorus. The chorus can be active for a period of one to two weeks, and then turn silent, either because the weather cools, or because the males get tired. After a resting period, which may last 1-2 weeks, a new round of calling may be initiated. The calling periods are not necessarily synchronized between ponds.

The calling activity seems to be necessary to prepare the females for spawning. As long as a female's eggs are unripe, it will stay away from the calling males. Typically, it will spend time near the water's edge, partly to warm up in the shallow water, and partly to feed.

Deposition of eggs requires higher temperatures than calling. Usually, the water temperature during the night must be at least 17°C before any eggs are deposited.

When a female is ready for spawning, its behaviour will change. Now, it will stay in the vicinity of a calling male – typically a large intensely calling male. Typically, it will keep a distance of about 1 meter from the male, and stay calmly in the water surface. The calling male will often be aggressive toward other individuals nearby, and if the intruding individual happens to be a female, it will chase the female and eventually grasp it.

The male grasps the female right in front of its hind legs. The pair is now in amplexus. Often, the female will try to free itself from the male, e.g. by crawling through dense vegetation, or between stones. Sometimes the male gives up, and mating does not occur. Sometimes the female gives up, and resigns. The male will then retain its grasp for a long time. If the female is not able to spawn, the male will abandon the female e.g. the following day, but if the female is able to spawn at least a few egg clumps, the male stays and fertilizes these.

Typically, a pair will enter amplexus in the afternoon, and spawning will occur during the following night. Every time a clump of eggs is about to come out, the female, with the male attached, dives into the water and uses its forearms to find a suitable straw. The female holds the straw with one of its hands, and swims in a spiral pattern around the straw. Simultaneously, the eggs come out, and the male spawns semen into the water around the eggs. The clutch is wound in a spiral around the straw. Then the pair returns to the surface. This procedure is repeated several times. When the female has no more eggs to spawn, the male detaches himself. He will often start to call for a short period immediately after this.

The total number of spawned eggs and the number of separate egg clumps vary widely. Young, small females may spawn only once during a season, and produce no more than 100 eggs. Older, larger females may often spawn many clutches with a total of 200-300 eggs per mating. They typically mate twice per year.

The number of eggs per clump is very variable – it may be anywhere between 1 and 130 eggs. The eggs are usually deposited where the water depth is at least 20 cm.

3.1.3. Development of the eggs and tadpoles

The speed of egg development until hatching depends on the water temperature. It takes about three days at 23°C, four days at 20°C, and seven days at 15°C. In fairly warm weather, the average time is about four days. The newly hatched larvae swim away and find a substrate to which they can adhere. They will remain rather inactive and feed on the contents of the yolk sac. After a period of approximately five days, the intestine system has developed, and they will start feeding and moving around.

Unlike tadpoles from other species, *B. bombina* tadpoles are not very efficient at filtering planktonic algae from the water. They mainly feed by rasping microorganisms from surfaces, especially on aquatic plant straws. Usually, algae (green algae, diatoms and others) make out the bulk of the diet, but protozoans and various types of bacteria, which are better

Amplexus of *Bombina bombina*. The male graps the female around the belly or waist. The couple can remain in amplexus for several hours or even days. As soon as the female starts laying her eggs, the male will add its sperm. Notice that the male's head is wider than the female's head.

protein sources, are important supplements.

The tadpoles complete their development in c. 90 days in cool summers and c. 60 days in warm summers. In warm summers, the first metamorphosed individuals will appear around July 15th, and in average summers around August 1st. If the summer is cool, the toadlets may not appear until early September, and during the extremely cold year 1987 metamorphosing toadlets were observed at the water's edge as late as early November. Some of these late metamorphosing animals apparently survived.

Normally, the toadlets will stay and feed at the water's edge for some time – maybe a few weeks, maybe a month or more. Eventually, they will migrate and find a hibernation site on land.

3.1.4. Food

Newly metamorphosed toadlets feed on a variety of small insects present at the pond edge – typically collemboles and small dipterans – sometimes small worms are available (tubificids).

Adults mainly feed at or just below the water surface. Food items may be water lice (*Asellus*), spiders, drowning insects, various insect larvae (e.g. pyralids), mosquito pupae, various worms, and some snails. The toads may also feed on insects on land. In all investigations, insects make out 82-95% of the food. Ants constitute up to 15% of the diet.

3.2. Predators

3.2.1. Predators on eggs

The only documented predators on *Bombina* eggs are crested newts.

3.2.2. Predators on tadpoles: invertebrates

Some invertebrate predators, especially leeches (*Haemopsis sanguisuga*) and water beetle larvae (*Dytiscus marginalis*) eat a significant part of the tadpoles. Crayfish (*Astacus fluviatilis*, *Pacifastacus leniusculus*) may or may not be important predators. It probably depends on the population density of the crayfish and/or the shape of the pond.

3.2.3. Predators on tadpoles: fish

Fish of all kinds are by far the most important predators. Carps of all kinds eat *Bombina* tadpoles and their presence usually totally prevents breeding success of *Bombina*. Adult *Bombina* do not spawn if fish are present in the water. The animals somehow sense the presence of fish in the water, and this provokes a reluctance to breed. Large populations of *Bombina* may build up in ponds with carp rearing. This does not mean that *Bombina* will breed in ponds with large fish populations. The explanation is that carp ponds are emptied at regular intervals to harvest the adult carps. The following year, the emptied pond is stocked with carp brood, which in April-May is too small to constitute any threat. Actually the *Bombina* larvae may feed on the carp food.

If the pond contains sticklebacks (*Gasterosteus* or *Pungitius*), few *Bombina* larvae survive to a size where they are no longer in danger of being eaten. As a main rule, if sticklebacks enter, *Bombina* will leave.

3.2.4. Predators on tadpoles: other vertebrates

Bombina tadpoles are eaten by other vertebrates. Newts (*Triturus vulgaris* and *T. cristatus*) readily feed on *Bombina* tadpoles. A very high density of large newts in a pond decreases the chance of *Bombina* tadpole survival, and it is likely that adult *B. bombina* refrain from spawning in such a pond. Tadpoles are eaten by some reptiles, including turtles (*Emys orbicularis*) and probably grass snakes (*Natrix natrix*).

Some birds, for instance herons (*Ardea*) and ducklings (*Anas platyrhynchos*), eat tadpoles.

3.2.5. Predators on newly metamorphosed and adult *Bombina*

It is generally believed that the poisonous skin secretions and the warning colours protect *Bombina bombina* from being eaten by predators. This, however, is not always true.

Some fish eat *Bombina*. Pike (*Esox lucius*) may sometimes eat *Bombina*, and sometimes not. Perch (*Perca*) eats them.

Evidence concerning green frogs as predators is mixed. When green frogs (*Rana esculenta*, *R. ridibunda*) catch *Bombina*, either newly metamorphosed or up to one year old, they sometimes spit them out again, and sometimes swallow the prey.

Grass snakes often move among calling *Bombina* males without attacking them. Occasionally, however, grass snakes may eat small specimens or even adults.

Many birds eat *Bombina*. Scraps of several *Bombina* specimens have been found in stomachs of mallard (*Anas platyrhynchos*), grey heron (*Ardea cinerea*) and white stork (*Ciconia ciconia*). Many other bird species may feed on them, including bitterns (*Botaurus stellaris*).

Many mammal species have been recorded as predator of *Bombina*, including hedgehog (*Erinaceus europaeus*), muskrat (*Ondatra zibethicus*), raccoon dog (*Nyctereutes procynoides*), polecat (*Putorius putorius*), badger (*Meles meles*) and otter (*Lutra lutra*). Mammal species under suspicion of having eradicated *Bombina* populations are stoats (*Mustela erminea*) and rats (*Rattus norvegicus*).

3.2.6. Parasites

Parasites in *Bombina* include several species of protozoans, trematodes, and nematodes, and one species of acanthocephalan. The number of parasite species as well as the number of individuals present is much lower than in *Rana esculenta*.

Two generations of breeding success.

3.3. Population ecology

3.3.1. Fecundity

The number of eggs spawned by one female during mating is very variable and depends on the size of the female. In a study in southern Poland, an average of 466 eggs per mating was recorded, varying between 35 and 1102. In a Danish study, based on 55 matings, the average was 179 eggs, with a variation from less than 50 up to 450. The observed record in Denmark is 619 eggs in one mating.

In small Danish populations where females have been monitored carefully, most females spawn twice per season. The number of eggs spawned the second time is usually, but not always, smaller than the first time. Two young females (2-3 years old) both produced a total of c. 300 eggs in a season, whereas older females (> 4 years) produced between 200 and 650 eggs per season, with an average of about 450.

3.3.2. Breeding success

Breeding is considered a process that includes many steps. Lack of breeding success may be due to failure in any step. Therefore, whenever breeding success is monitored, all steps are important.

First, males must call. To check that males do indeed call in the pond, one must visit the pond during optimal weather conditions several times during the season. Males sometimes call even if the pond is not suitable for breeding. For instance they may call in highly eutrophic ponds and in ponds with high salinity.

Although males call and females are willing to mate and spawn, mating may fail. Insufficiently agitated males during calling could be an explaination.

Even when males and females go into amplexus, spawning may still fail. This usually happens if the female at that particular moment does not have ripe eggs in its ovaries, but other explanations may be plausible. Most importantly, if fish are present in the water, the female will not spawn no matter what. Usually spawning requires weather with at least some rain, and/or presence of spring floodings forming shallow water. If spring is dry without floodings and the weather is dry, sunny and windy, spawning will be sparse.

Yearly differences in breeding success in any particular pond are very large, and apparently these differences are highly dependent on variations in the number of eggs spawned. In connection with rearing programmes some ponds have been examined thoroughly approximately twice a weak during several months in order to find eggs, but sometimes hardly any eggs were spawned during the season, even though many males and females were present.

If eggs have been fertilized by male sperm (this may sometimes fail), the eggs will develop, and most of them will hatch. When water quality is good and the population is genetically healthy, the percentage of hatching eggs is close to 100. However, many eggs may die in polluted water with decaying organic matter. Even if the water quality is OK, eggs may fail to develop for genetic reasons (inbreeding). In some populations it is not uncommon that only 70-80% of the eggs hatch in even the best conditions. The average of hatching eggs in Danish populations is 94%.

Growth and survival of hatched larvae requires sufficient food. In newly dug ponds without vegetation the growth rate of tadpoles may be much reduced.

The survival rate of free swimming tadpoles has been estimated in 12 cases in Denmark, distributed among seven ponds. In these cases, the number of metamorphosed toadlets in relation to the number of hatched larvae is 0.2-6.1%. The best estimates are typically in the range 2-3%. The data indicate that predators typically reduce tadpole populations with 95 to 98%.

3.3.3. Mortality data of *Bombina*

3.3.3.1. Survival of newly metamorphosed toadlets

Survival of newly metamorphosed toadlets depends on their number. In very numerous populations only few toadlets survive. Observed mortality rates during the first year in huge populations are as high as 98%.

Under more normal conditions, the survival rate during the first two years after metamorphosis range from 2.4% to 68% in Danish studies; in most cases, the values lies around 40%. The survival rate for reared toadlets released into nature is usually less than this. Observed survival of reared toadlets during the first winter is 7-69%.

Breeding pond surrounded by 50% arable fields.

3.3.3.2. Survival of juveniles

Juveniles are animals that have survived their first winter, but are sexually immature. Their rate of survival is higher than newly metamorphosed toadlets, although sometimes not quite as high as the adults. There is practically no difference in survival rate between artificially reared one year olds and natural breeding one year olds.

3.3.3.3. Survival of adults

The average yearly survival of adults has been estimated in many localities in Denmark. All numbers are based on observations of marked animals. The average yearly survival varies from 0 to 94%, but is usually above 50%. Whenever the yearly survival rate is markedly below 50%, intense predation, for instance from herons, seems to be the cause.

3.3.3.4. Factors affecting survival

The character of the landscape surrounding the breeding pond appears to have a strong relationship with differences in survival rate between different localities in Denmark. Localities with the highest survival (often 80 to 90% per year) also had the highest occurrence of rather natural vegetation around the ponds. Ponds in agricultural landscapes with large fields had survival rates of 46 to 60%. A general – but somewhat blurred – trend showed that a higher proportion of fields in the surroundings resulted in a lower survival rate.

3.3.4. Predicting growth of populations based on fecundity and survival

In order to get an overview of the importance of fecundity and survival data for the prospects of populations, population life tables, which are a standard tool in demographics and population dynamics, may be constructed.

Two life tables (3.1 and 3.2) are illustrated here, one for a population with low mortality, and one for a population with high mortality.

The first life table (3.1) is mainly based on data from Hjortø (1990-1991). Hjortø was at that time a locality with favourable surroundings and hence high survival. We assume a yearly survival of 0.9 for adults and a fecundity of 300 eggs per year for 2-year olds and 450 eggs per year for older animals.

The table is created under the assumption that we consider 2,000 newly spawned eggs, of which 1,000 are female eggs. During each step

Table 3.1. Life table for *Bombina bombina* on Hjortø c. 1990-1991. Start: 2,000 eggs of which 1,000 are females. Generation time G (average of mothers) = 5.28 yrs.

Year/Stage	Fraction surviving	Number	Of these breeding	Fecundity	No. of eggs
Start (year 0)		2,000			
Of these females		1,000			
Hatched eggs	0.94	940			
Metamorphosed	0.01	9.4			
Year 1	0.40	3.8			
Year 2	0.90	3.4	0.9	300	914
Year 3	0.90	3.0	1	450	1,371
Year 4	0.90	2.7	1	450	1,234
Year 5	0.85	2.5	1	450	1,110
Year 6	0.80	2.1	1	450	944
Year 7	0.75	1.7	1	450	755
Year 8	0.70	1.3	1	450	566
Year 9	0.65	0.9	1	450	396
Year 10	0.60	0.6	1	450	258
Year 11	0.55	0.3	1	450	155
Year 12	0.50	0.2	1	450	85
Year 13		0.1	1	450	42
In total for mature females		18.0			7,828
R_0					3.91

we record how many females are still alive. When the last female is gone, the total number of eggs spawned during their lifetime is 7,828. Half these are female eggs and the reproduction rate from one generation to the next is 7,828/2,000 = 3.91. Thus, if the parameters are unchanged for some time, each generation will be nearly four times larger than the preceding generation.

To calculate the growth rate of the population, we need to know the generation time. This is approximately the same as the age of the mother averaged over all eggs. The average age of a mother is calculated, and the result is G = 5.28 years. The exponential rate of growth is then

$r = \ln(R_0)/G = 0.258$.

Thus, the population will have increased by a factor of 1.29 after one year, 1.68 after two years etc.

The second life table (3.2) is mainly based on data from Avernakø around 1990. At that time, the ponds were surrounded by cultivated fields, and the yearly survival was low. We assume a yearly survival of 0.5 for adults up to 5-years old. The data on fecundity is the same as before, except that we assume that all females spawn already at the age of two years. Survival of tadpoles is somewhat better than before, but that does not quite make up for the low survival of adults.

In this life table, when the last female is gone, the total number of eggs spawned is 3,105. The reproduction rate from one generation to the next is 3,105/2,000 = 1.55. Once more, the generation time is calculated as the average age of the mothers, and here the mothers in average are much younger than before, only 3.09 years. The exponential rate of growth is then

$r = \ln(R_0)/G \Rightarrow \ln(1.55)/3.09 = 0.142$.

Thus, the population will have increased by a factor of 1.15 after one year, 1.33 after two years etc.

The life tables may be used for several purposes.

First, it is possible to calculate the rate of population growth under the assumption that

Table 3.2. Life table for *Bombina bombina* on Avernakø c. 1990. Start: 2,000 eggs of which 1,000 are females. Generation time G (average of mothers) = 3.09 yrs.

Year/Stage	Fraction surviving	Number	Of these breeding	Fecundity	No. of eggs
Start (year 0)		2,000			
Of these females		1,000			
Hatched eggs	0.94	940			
Metamorphosed	0.03	28.2			
Year 1	0.30	8.5			
Year 2	0.50	4.2	1	300	1,269
Year 3	0.50	2.1	1	450	952
Year 4	0.50	1.1	1	450	476
Year 5	0.50	0.5	1	450	238
Year 6	0.45	0.2	1	450	107
Year 7	0.40	0.1	1	450	43
Year 8	0.35	0.03	1	450	15
Year 9	0.30	0.01	1	450	5
In total for mature females		8.3			3,105
R_0					1.55

parameters will remain unchanged.

Second, a population's vulnerability to changes can be checked. For instance, what would happen if the survival of tadpoles in the second life table were the same as in the first, i.e. if it dropped from 0.03 to 0.01? If that was the case, the number of individuals reaching sexual maturity would be one third, and the number of eggs spawned and the value of R_0 would also be one third, i.e. the rate of reproduction would be 0.52, which is far below one. The generation time would remain unchanged, and the population growth rate would be

$r = \ln(R_0)/G \Rightarrow \ln(0.52)/3.09 = -0.213$.

Thus, the population would be reduced to 81% after one year, 65% after two years etc.

3.3.5. Migration

Firebellied toads do not stay in one place all year. In the spring, they are often seen in shallow temporary floodings, before they move on to the breeding pond. Later in the season, they sometimes abandon the breeding pond for a foraging pond, where will they stay until it is time to find a hibernation site.

Migrations between different ponds during the season are probably performed by a large fraction of a population. In one well studied case, a cliff is facing the sea, and above the cliff a breeding pond of 300 m^2 is surrounded by a field. Quite a large fraction of the population may remain in the cliff pond during late summer in densities of about one individual per m^2. The toads are believed to hibernate in holes in the cliff.

Another case features a small deciduous wood (2.4 ha) with a pond. The pond is quite shaded and muddy, but it serves as a foraging

Spring habitat in Salem.

Breeding pond at Dannau.

site, and there is evidence that the toads hibernate nearby under tree roots. During a dry period in mid July 1993, 20 adults (and some juveniles) were found in the pond. These 20 adults were found in breeding ponds, in subsequent years (1994 and/or 1995). The ponds are situated 240 m, 260 m, and 400 m apart, respectively.

Alternations between closely situated breeding ponds are not quite as frequent. Data from three different localities in Denmark all indicated that between 23 and 28% of the adults alternate between ponds. This percentage does not depend on number of years passing between registrations.

Migrations over longer distances are much rarer. Knudshoved in Denmark have some groups of ponds, with distances of 300 m, 800 m, and 900 m respectively between the groups. Only 5 % of animals labelled in 1988 had crossed the distance of 300 m between two pond groups. The other intervals had not been crossed at that time, but the 800 m interval was crossed by one animal between 1991 and 1992, and another animal between 1991 and 1994. The 900 m interval was crossed in the spring 1994 as part of a colonisation event.

3.3.6. Dispersal and colonisation

Dispersal

Whereas migration refers to movements within a pond system which is already occupied by the toad population, dispersal and colonisation refers to movements away from the area occupied by the population, and into other areas either occupied by remotely related populations, or previously unoccupied.

Dispersal may occur when newly metamorphosed animals reared in captivity are released in nature. Such animals may not yet have developed site fidelity and may thus leave the pond and move in random directions in the surrounding landscape. Later, they may be found in ponds some distance from the release sites. In Denmark, such animals have dispersed distances between 400 and 1200 m in various localities.

Colonisation of sparsely populated localities
If toads move to a pond far away from their origin, and start founding a new population, it is called colonisation. In some cases, a pond has very few surviving animals. When the pond is restored and markedly improved, the survivors may call extremely loud and attract individuals from far away. In one case in Denmark, several individuals were attracted from another pond 900 m away; in another case, several individuals immigrated from ponds 2440 to 2550 m away.

Colonisation of previously void localities
If a new pond is created close to a breeding pond, it will often be colonised within a few years. Some data on how often this happens may be found in the first Danish LIFE *Bombina* project which ran from 1999 to 2003. During these years a number of previously uninhabited ponds were improved, and new ponds were dug. According to the maps in the project report, 65 of these ponds had not been colonised by firebellied toads during the project, whereas 22 previously unoccupied ponds had been colonised by immigration of dispersing animals.

Large *Bombina* populations quickly built up at the most successful locality in the Danish LIFE project. Initially, toads dispersed to a lake 650 m to the east and started founding a large breeding population. A few years later, firebellied toads discovered a flooding in a field 3170 m to the east of the lake, and bred there in 2007.

Colonisation may occur over even greater distances. In Holstein in Germany, a population, proliferating due to measures taken during the international LIFE project 2004-2009, managed to colonise a new locality approximately 6 km from the nearest possible source.

The highest rate of natural colonisations has been recorded in Scania in south Sweden. In one of the release areas, *Bombina* has proliferated greatly. During the first years, from 1983 to 1989, one new pond was colonised per year on average. After the first years, the rate of colonisation increased, and in 2006-2007, approximately 100 new ponds were colonised each year. In the first years, the extreme colonisation distances were about 1 km. In 2007 the toads had spread greatly outside the previous distribution limit, dispersing in one step 5.5 km to the north, east, and south, 8 km to the west, and 9 km to the southwest. In 2005, 94 ponds were colonised naturally, and 67% of these had breeding success.

3.3.7. Metapopulation structures

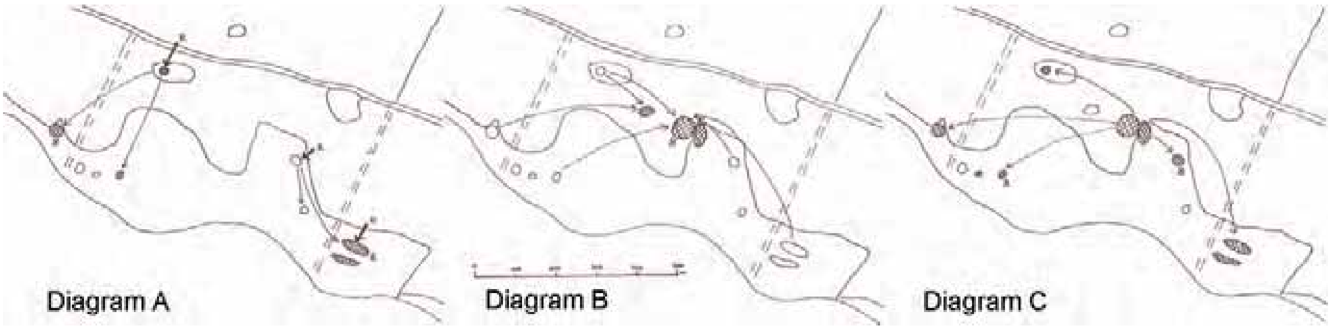
A metapopulation is a system where the animals can migrate to and from several breeding ponds, and where the population status in one pond depends on what happens in the other ponds.

For example, the occurrence of *Bombina* at Ilgas in southeastern Latvia close to the Belorussian border is part of a metapopulation that extends into Belorussia. In their present state, the habitats on the Latvian side of the border probably would be unable to sustain an isolated population of *Bombina*. The species only persists there because animals constantly immigrate from two large populations on the Belorussian side of the border. If the connection were somehow severed, *Bombina* would very likely be extinguished on the Latvian side.

The situation may be far more complex. This is illustrated by the three diagrams in Figure 3.1. The population has alternated between different breeding sites in different years, and if any of these sites were not available, the continuous chain of breeding possibilities might have been broken, and the population might not have survived.

The locality in the figure is Knudsskov in

Figure 3.1. Migration between breeding sites at Knudsskov in Denmark. R marks release ponds, B breeding ponds, and ponds with occurrence of *Bombina bombina* are cross-hatched. Migrations are explained in the text.



southwestern Sjælland, Denmark. Approximately 10 ponds were made or restored around 2000 in the first *Bombina* LIFE project. *Bombina* was introduced by release of reared toadlets from 2001 onward.

The situation around 2003 is shown in diagram A. Many animals have migrated away from the release ponds and colonised other ponds. One of the release ponds was empty – the toads had all migrated elsewhere. Some years later, the first naturally produced juveniles were observed, especially in the easternmost locality (the two parallel elongated ponds). This locality had breeding success in 2005 and 2006, but was subsequently flooded with salt water, and the animals migrated.

Meanwhile, unintended deterioration of a drainage ditch created shallow floodings in the centre of the area. These floodings are marked in diagram B. Some animals bred in these floodings in 2006. In 2007, the main part of the animals bred in the floodings, and breeding success was good. Diagram B shows the situation in 2008. This year, all other ponds had been abandoned, and the entire population was concentrated in the floodings.

The last diagram C shows the situation in 2009. The preceding winter had been unusually dry, and the water level was low in the central floodings. Water was too scarce to allow breeding success, and most of the animals had left. The easternmost locality had been pumped dry to remove sticklebacks and salt water in the preceding autumn, and it was now colonised again. All nearby ponds were also colonised, and for the first time breeding success was recorded in two of them.

This alternation resulted in build up of animals in temporary floodings where they produced plenty offspring in 2007. Later, they were forced away again and thus colonised and bred in the ponds they had left in the first place. If the floodings had been permanent, the animals might not have colonised the other ponds at all.

The conclusion must be that contiguous investigations may be necessary to understand how a locality functions. The situation may change dramatically from year to year, and only repeated studies of how populations survive good and bad years allow understanding of what is required for long term survival.

3.4. Habitat requirements

3.4.1. Difference from *Bombina variegata*

The two species of *Bombina* in central Europe have somewhat different habitat requirements. *Bombina variegata* mainly prefers water bodies surrounded by forest. These water bodies are usually puddles, often with no or very little vegetation. *Bombina bombina*, on the other hand, prefers larger ponds, frequently with more and higher vegetation. These ponds are generally situated in open land.

3.4.2. Pond type in relation to behaviour

Firebellied toads use different types of ponds during the seasons. Right after leaving hiber-

Glyceria fluitans dominates the pond edges. In this vegetation the females find hiding and places for warming up.

Young *Bombinas* fouraging in shallow warm water.

nation, they may look for very shallow temporary floodings with warm water. After a few days, they migrate to larger water bodies and warm areas near these. The females dwell here some time to feed and utilise the warmth to mature their eggs. Examples of such sites are shallow water bodies in the vicinity of the breeding ponds, typically flooded grass, or shallow extensions of a deeper pond with an average depth of 30-60 cm. In semi-natural river landscapes, flood zones in the valleys form vast extensions of female habitats. Optimally, females can spend the necessary time undisturbed by males and predators. The females will not leave the shallow zone until they are ready to spawn.

Next, the toads may search for permanent fish-free ponds with good water quality. Here, they can call and breed during most of May and June.

When a female approaches the calling males and is taken by a male, she locates a spawning locality. Most eggs are attached to 1-3 mm thick stems of strong vertical vegetation, such as stems of *Potamogeton*, *Eleocharis, Glyceria* or *Scirpus.* Stems covered with algae or slimy films are not used. Generally, some open water around the stems is required for the spiral movements of the spawning couple. However, eggs may also be placed on horizontal structures e.g. in dense *Batrachium* mats. The depth at which the eggs are attached varies. In early spring eggs are normally attached 20 to 40 cm under the water surface, but if spring is warm, many eggs are placed at a depth of more than 70 cm. Later in the season, eggs are spawned at greater depths closer to the bottom.

Bombina bombina tadpoles mainly forage microflora on vertical structures in the ponds. Sometimes, a layer of bacteria (iron oxidising bacteria) on basal parts of plant stems in the bottom layer is the most nutritious food. Unlike most other tadpole species, the tadpoles cannot filter microorganisms from water. They are rather inefficient escaping potential predators and thus rely on a low predator density in the water.

The newly metamorphosed juveniles forage along the banks, in shallow water and in humid places on land. They need open, sun exposed habitats, e.g. mud, with plenty small insects, but the habitat must also provide good hiding opportunities. They can use e.g. cattle foot prints for hiding, but optimally they hide in dense, low, sun exposed vegetation. *Eleocharis* stands along the water's edge provide optimal microhabitats. Optimal microhabitats should be present on a large scale in direct vicinity of the breeding ponds to ensure survival of a large number of newly metamorphosed individuals.

3.4.3. Important features of breeding ponds

Breeding ponds can be natural depressions or man-made ponds. They may or may not be permanent. Statistics on breeding success of introduced *Bombina* populations in 88 water bodies in Scania reveals that five are temporary floodings, 20 are semi-permanent ponds that may dry out during draughts, and 64 are permanent ponds. Thus the majority of these breeding ponds are permanent. A large percentage of the ponds have been excavated in order to improve their value to amphibians.

The size of breeding ponds is very variable. On Enø in Denmark, a male is regularly calling

Three examples of optimal ponds are found below:

Large shallow waterbody with deeper parts, fully sun-exposed.

Reactivated kettle hole in natural depression.

Very shallow, temporary grazed pond on Hjortø.

Two examples of suboptimal ponds are found below:

Old eutrophic village pond in Latvia.

Old marl-pit overgrown by *Scirpus maritimus*, *Typha angustifolia* and *Ceratophyllum emersum.*

in an approximately 1 m^2 garden pond, and breeding has been recorded in a 10 m^2 pond. In Scania, four small semi-permanent ponds have an area of 60-100 m^2. All other ponds are 100 m^2 or larger, up to 35,000 m^2 (3.5 ha).

Bombina may breed in deep ponds. For instance the clay pit at Tårup Strand on Fyn, which used to be a very important breeding pond, has a water depth of 4-5 m. Optimal ponds, however, are very shallow; late May water depth can be as low as 30 cm or even less, provided that these ponds do not dry out until the end of summer. This is only possible when no drainage systems that lower the ground water table are present. Furthermore, a steady supply of ground water from neighbouring hillsides is important.

Predominantly, *Bombina* prefers breeding ponds with fully sun exposed surfaces. The ponds can be surrounded by forest or other types of high vegetation, but usually the water surface is less than 25% shaded. Since 15°C is the approximate threshold for the males to start calling, this temperature must be reached no later than early May.

Man-made ponds, e.g. cattle watering holes and marl pits, usually have rather steep sides, which allow trees to grow close to the water's edge. Previously, farmers regularly cut down willow trees near ponds for firewood and fencing material. Today, the trees are left standing inevitably resulting in shaded ponds. This makes them unsuitable for *Bombina*. Natural depressions may have waterlogged surroundings preventing growth of most trees, but willow bushes (*Salix cinerea* and others) and alder (*Alnus glutinosa*) may still cause shading. Furthermore, the shallow water zone, which is vital for rapid warming in spring, may be overgrown with dense stands of reed (*Phragmites*), bulrush (*Typha sp.*) or other wetland plants shading the water and lowering the water temperature by several degrees.

Therefore, in most cases vegetation needs to be kept down and this is often achieved by cattle grazing. A good example is Schleswig-Holstein where nearly all *Bombina* ponds are grazed by cattle.

Grazing retains a suitable vegetation structure in breeding ponds.

Termination of grazing may cause *Bombina* to disappear in a few years. For instance a pond at Getryggarna in Scania was fenced by the owner in 1988. In 1988 and 1989 the pond had several calling males and breeding success up to 1989. From 1990 to 1992 only a few callers and no breeding success were confirmed. After 1992 no animals have been recorded in the pond. By now, the pond is overgrown with *Alnus* and some *Salix*. Thus lack of grazing has had an almost immediate effect. In other words, *Bombina* disappears before obvious shading of the water occurs. This indicates that cattle are important in other ways too; for instance by creating flooded grass where the females can bask to mature their eggs and mud flats with many insects providing food for newly metamorphosed toadlets.

Although overgrowth with reed, bulrush, willow bushes and alder is a natural process, the rate of overgrowth is not natural. Today's input of plant nutrients, especially nitrogen, is enormous relative to pristine conditions thus the rate of growth has sped up greatly.

Even though cattle grazing generally are positive for *Bombina*, occasionally watering ponds are rendered unsuitable by heavily use of cattle. Massive amounts of cow dung and urine may deteriorate the water quality. These highly affected ponds may still have some breeding success provided that the cattle are not released into the pen before mid May. Prior to the release of cattle, the shallow water may be overgrown with dense stands of *Sparganium*. If the firebellied toads manage to spawn in this vegetation before mid May, the eggs will hatch, and the small larvae will feed well. Later in the season, when vegetation along the banks is heavily affected by cattle, the *Bombina* tadpoles may survive in the *Potamogeton* vegetation in the middle of the pond. Thus good breeding success is possible as long as the *Potamogeton* vegetation can survive.

Potamogeton natans, a valuable plant for egg-laying and as shelter for tadpoles.

3.4.4. Degree of eutrophication

The best breeding ponds are naturally eutrophic, typically rich with aquatic vegetation. However, the term eutrophication describes the process where unnaturally large amounts of plant nutrients are supplied to the pond. As a result, the pond is no longer naturally eutrophic but rather hyper-eutrophic. The excess nutrients are utilised by vegetation to produce more biomass that are easily turned over. During the night, the respiration of all this plant biomass lowers oxygen concentrations dangerously. A few plant species thrive on these conditions and thus outcompete other species. Some examples are bulrush (*Typha*) which may take over the edge zone and duckweed (*Lemna*) which may shade all other aquatic vegetation. During summer, accumulation of decaying leaves on the bottom consumes more oxygen than supplied, rendering conditions in the top mud layer anoxic. Gradually this will spread to the water above the mud. The anoxic conditions

Bulrush (*Typha sp.*) starts dominating the center of this pond in Niedersachsen, indicating eutrophic conditions.

Naturally eutrophic ponds with well-developed aquatic vegetation are excellent summer habitats for the firebellied toad.

Duckweed (*Lemna sp.*) is covering the surface of the pond and outcompeting other aquatic plants.

change the solubility of phosphorus. Insoluble ferric phosphates are changed into soluble ferrous phosphates causing phosphate ions to enter the water. This escalates with the supply of plant nutrients growing and thus increasing the amount of decaying plant material.

There is no simple way to measure the degree of eutrophication. Measurement of plant nutrient concentrations in the water phase is inefficient, because nearly all is taken up by vegetation leaving nothing in the water. Nitrate levels are at a maximum in January and February because nitrate is released from rotting vegetation. However, microscopic algae start to grow in March, and soon nitrate has been removed from the water. During March, April and May, nitrate and phosphorus levels are close to zero in the water. From June onwards, anoxic conditions at the bottom mud bring phosphates into solution. During the rest of the summer, plant growth is limited by nitrogen, not by phosphorus. Thus, supplies of nitrogen from the air or via drainage pipes will result in further eutrophication and deterioration of the pond ecosystem.

The degree of eutrophication is indicated by accumulated organic matter, including the total amount of plant and animal biomass and the amount of organic matter in the bottom mud. A qualitative evaluation can be achieved by judging the sludge thickness at the bottom, and/or by the amount of vegetation; i.e. the height and density of emergent vegetation, and the density of aquatic vegetation.

Many flora and fauna indicator species can indicate the degree of eutrophication. For instance, when levels of eutrophication are low, the aquatic vegetation consists of plant species with relatively narrow leaves (e.g. *Potamogeton pusillus*) or somewhat larger and broader leaves (e.g. *Potamogeton obtusifolius* and *P. natans*). The amount of insect larvae of *Zygoptera* is a very good indicator eutrophication. In mesotrophic or naturally eutrophic ponds, many individuals and many species of e.g. the genera *Lestes*, *Ischnura* and *Coenagrion* are present.

Some degree of eutrophication may be beneficial for *Bombina*. Fairly lush films of microflora on plant stems, preferably with high protein content, are beneficial to *Bombina* tadpoles for grazing. Such substrates develop in ponds influenced by moderate amounts of cattle dung, thus moderate eutrophication from cattle dung is beneficial. Higher degrees of impact from

A desiccating mudflat is a good fouraging area for metamorphosed and sub-adult *Bombina.*

dung will render the water turbid and too 'rotten'. The eggs often die from infection of *Saprolegnia* (mold) or other pathogens, and the tadpoles cannot survive.

3.4.5. Soil type and pH

Some ponds on sandy or peaty soil may function as breeding ponds for firebellied toads, but these are exceptions. Normally, clay is optimal since water on clay is naturally rich in nutrients and calcium. Ponds on calcareous soil can be good breeding ponds. The water pH must be above six, otherwise breeding success are impossible.

3.4.6. Salinity

Breeding success has been recorded in moderately brackish water. In one case (Knudshoved, Denmark), eggs were spawned in a rearing case in a swamp with 3.3‰. Most eggs perished at this salinity, but a few survived to become healthy tadpoles.

Adults tolerate much higher salinities. A pond at Knudshoved had a large chorus of calling males in both 2008 and 2009, even though the salinity was 8‰ and 5‰ respectively. A winter flooding in another pond at Knudshoved caused an increase in salinity to 11‰, and all *Bombina* fled the pond.

Sometimes sea water floodings of the habitats are tolerated, but this is not always the case. Experiments have demonstrated that adult *Bombina* may survive in 15‰ sea water for 20 hours.

3.4.7. Foraging ponds

When breeding activities have ceased, typically in early July, the adults seek good foraging habitats. These habitats may not necessarily be good for breeding. Examples of foraging habitats are small pools, hyper-eutrophic ponds, desiccating mudflats, pond edges grazed by cattle and sun exposed ponds in woodlands. Often very small ponds with turbid water and dense low vegetation or ponds with abundant bramble, branches, lumber etc. are chosen. The habitat needs to provide plenty hiding places. These ponds usually offer ample insect larvae and insects as food source.

Firebellied toads are occasionally found in completely turbid water bodies, such as muddy cattle watering holes. The grazing cattle create a mosaic of trampled bare ground with lots of resting insects. The trampling of cattle or horses generates holes in the soft soil at the pond edges and this offer hiding places for juvenile firebellied toads. Large amounts of cattle dung produce numerous flies for firebellied toads to feed on.

3.4.8. Habitats in late summer

In dry summers, the adults may leave the ponds by midsummer and seek hiding places on land. When the weather is more normal, many individuals leave the ponds in August, and the rest in September. The adults migrate on warm days and seek moist places rich in insects and other invertebrates.

On the Danish island Agersø, firebellied toads are known to migrate distances up to 400 m regularly to reach a partially shaded pond in a wood. When this and other ponds dried out during the dry summer in 1993, many adults were found under logs in the vicinity in mid July. The pond surroundings are muddy, with decaying litter and many dead branches.

3.4.9. Hibernation habitats

Firebellied toads use a variety of habitats for hibernation. Known locations are deciduous woodlands rich in leaf litter and dead wood, under the bark of willow trees, under tree roots, holes in cliffs or hedge walls, rodent holes, stone heaps partly covered by soil, stone walls in grasslands, under stony railway embankments, and in human buildings (in cellars or under a staircase).

Up to several hundred individuals may hibernate together in cavities 20-60 cm under the soil surface.

Absence of hibernation sites, often the case in flat landscapes with intensive agriculture on clay soils, seems to influence survival during the winter. In landscapes like these, hibernation sites such as a mouse hole might easily be flooded by winter rain. Observations in captivity show that hibernation in waterlogged soil may be fatal due to fungal infections, thus the hibernation site must be well drained. The di-

Flooding in woodland as summer habitat. Occasionally, males are heard calling during warm August nights.

stance and the land use between the hibernation site and the breeding pond also influence the survival rate of adults. A short distance between breeding pond and hibernation site as well as extensive grazing between the habitats offers optimal conditions for survival.

4. Threats

4.1. Landscape changes

The extensively utilised agricultural landscapes of the past provided good habitats for *Bombina bombina*. Around 1750, the species occurred all over a 40 km stretch of the southern plains in Scania (south Sweden). At that time, all farms and fields were clustered together in and around villages, whereas the rest of the land was extensively grazed, non-ditched commons. Water bodies on these commons must have been shallow, sun-exposed and extensively grazed. However, in the years after 1803 agriculture in Scandinavia was reformed resulting in ploughing and draining of the former commons; in other words a dramatic change. By the late 19th century, the species had declined seriously in Scania, evidently because of this reform.

The last remaining localities on Bornholm, a Danish island south of Scania, were in the former large common in the centre of the island. These localities were lost around 1930; one to a conifer plantation, another due to dumping of litter.

In the rest of Denmark the species disappeared from inland localities before 1900, e.g. the central parts of Sjælland. Almost all of the remaining populations were found along the coasts and on small islands. The reason for this could be climatic or because the extensively grazed commons persisted longer in these areas. Most populations still present today live in such extensively grazed commons. However, far into the 20th century the species could also be found at many localities in the cultivated agricultural landscape. Somehow the species managed to survive in rather intensively utilised agricultural landscape for decades. The rate at which populations were extinguished in the agricultural landscape is only known after the 1940s. By then, the species was declining fast, and some naturalists warned that it would go extinct if no measures were taken to prevent this. Still, even though the ponds were protected, the species disappeared from most of them.

The earliest information about *Bombina bombina* from Schleswig-Holstein is from 1897. Here, the story of the species is not one of constant decline. After establishment of some fish ponds at Wallnau in west Fehmarn, the

Open grassland with a scattering of ponds has disappeared outside nature reserves.

population increased to at least one thousand. Except for Fehmarn, however, the species was not very common anywhere around 1930 and mainly occurred in isolated ponds here and there. This changed markedly at the end of the Second World War. The populations in the east Holstein lake district increased by a factor of a hundred within a decade and spread to many new localities. This was probably due to the numerous new small pools formed in bomb craters and to the deterioration of agricultural infrastructure. Even drainage systems deteriorated in many places and more cattle were grazing the inefficiently drained low lying fields. After the 1950s the populations have declined again.

In Niedersachsen, the species occurred along the Elbe valley and in the agricultural landscape east of Lüneburg around 1950. In the latter area, *Bombina* has disappeared completely after 1980. Many ponds have been backfilled, and others are overgrown or have dried out.

The Elbe valley biotopes have been thoroughly studied. Each spring and early summer, the water level in the river rises and the surrounding areas are flooded. Dikes have been built along the river to prevent flooding of fields, but water usually penetrates the dikes causing water levels to rise in the extensively grazed meadowland inside the dikes. Germans call it 'Qualmwasser', i.e. water leaking out of the meadow soil. Inside the dikes, some permanent and semi-permanent ponds are found and certain fish species survive there.

The well grazed 'Qualmwasser' ponds at the river Elbe represent a core area for *Bombina bombina* in the north European low-lands.

These ponds are also dwelling place for adult firebellied toads. Come spring floods, the firebellied toads are stimulated to breed. They suddenly call loudly, and disperse in the extensive shallow water bodies. They mainly breed in temporary floodings without fish. Most of the water bodies dry out during summer steadily limiting the amount of fish. The adults migrate back to the permanent ponds as soon as the water level sinks again, whereas the newly metamorphosed toadlets concentrate in small pools left after the floodings.

Recently, the distribution of *Bombina* in the western Elbe valley has declined drastically. If spring floods are reduced slightly, the water dries out too early and *Bombina* breeding success fails. The situation is worsened by reduced grazing as this result in denser overgrowth. Digging of new ponds will not solve the problem, since fish survives in permanent ponds.

4.2. Climatic changes

All the areas mentioned in the preceding paragraph – Scania, Denmark, Schleswig-Holstein and Niedersachsen – are situated at the northwestern border of *Bombina bombina* distribution. Therefore, small climatic changes could potentially have profound effects on the abundance of *Bombina* in these areas. Some biologists have expressed concern that climatic changes are causing the decline and that nothing can be done to prevent the decline. This conclusion is dangerous because it justifies inactivity. Furthermore, the conclusion is probably wrong. For instance, during the

1940s *Bombina* declined markedly in Denmark while proliferating greatly in east Holstein and experiencing fairly stable populations on Fehmarn. Consequently, these differences cannot be explained by overall climate change.

It is still evident, however, that climate changes are important. We have knowledge of the species' temperature demands. For instance, for spawning to occur during the night, water surface temperatures must have reached at least 19-23°C during the preceding day. During spawning in the night, the water temperature must be at least 14-16°C. These temperatures must be reached no later than the end of May. Development from egg until metamorphosis depends on water temperature. In warm summers in Denmark and Scania, it takes approximately 60 days, and in cold summers about 90 days. In Poland, metamorphosis took place after 95 days at a constant rearing temperature of 20°C. If eggs are spawned at 1st June, and development takes 90 days, the toadlets cannot leave the water until 1st September. After leaving the water, they will only have a few weeks to feed before they have to search for hibernation quarters. In other words, if the average water temperature is colder than 20°C, the animals do not have enough time to complete their development.

If a pond is too shaded, the water temperature may easily drop by several degrees, and thus become too cold for development. Thus, studies of overgrown ponds may easily lead to the conclusion that low temperatures are critical. However, cool summers alone probably only poses a small problem. After the coldest summer in the 20th century in Denmark (viz. 1987), the first toadlets did not metamorphose until September 10th. Nevertheless, metamorphosed small toadlets were registered the following spring, i.e. they had survived hibernation despite their small size. This indicates that on the 55th parallel (Denmark), cold summers do not prevent development. On the other hand, dry and windy springs and early summers combined with dry summers (e.g. in 1992) results in very low breeding success. Thus, more Atlantic climate with cool and rainy weather like 1987 may be better than dry springs.

In conclusion, if ponds are kept open, e.g. by grazing, climatic limitations are hardly ever reached. However, overgrown ponds combined with cold climate may constitute a problem.

Yet another problem is winter temperatures. If winters are warmer, shallow ponds will freeze solid less frequently. Consequently, fish will rarely be extinguished in these ponds. More importantly, the eastern part of the range, where a permanent thick layer of snow during winter have resulted in moderate cooling of the ground, will experience problems. Milder winters mean increased snowmelt, thus resulting in thinner snow layers and occasionally black frost, i.e. frost without snow. The ground may become so cold that hibernating toads will die. This is considered a threat to the survival of *Bombina* in Latvia.

Over a longer period, global warming will raise the sea level. Some scientists argue that the level may rise be as much as c. 1 m in the 21st century. In addition, more storms will increase the risk of seawater floods along the coasts. Salty water floods are a major threat at some Danish localities today. In the long run, nearly all localities in Denmark, on Fehmarn, and at Mölle in Scania, are threatened because they are concentrated along the coast, and on small islands. Eventually, rising sea levels may threaten the species in most Danish localities.

4.3. Predators

4.3.1. Crayfish

In Scania, introduction of signal crayfish (*Pacifastacus leniusculus*) is a major problem. In some cases, the toads disappear altogether when crayfish are introduced. In other cases, coexistence is possible when crayfish are not too numerous. Nevertheless, improved conditions for the crayfish, e.g. placement of aeration aggregate in a pond, can result in a crashing *Bombina* population.

4.3.2. Fish

Natural events, such as floodings from ditches, rivers, lakes, or the sea, may introduce fish to the ponds. It is also increasingly more common


A pond at Stodthagen (Germany) is pumped dry to remove sticklebacks.

to actively introduce fish to ponds. Fish are naturally wiped out from ideal natural habitats because the ponds dry out, either yearly or more sporadically (e.g. every 10 years) or become frozen solid. In deeper ponds fish may survive decennia and thus systematically eat all eggs and larvae resulting in (local) extinction of *Bombina* populations.

If sticklebacks (*Gasterosteus* or *Pungitius*) enter a pond, *Bombina* will be forced out. A relatively small stickleback population will cause a gradual decline of *Bombina* over several years, but if the sticklebacks become numerous, *Bombina* will disappear quite fast.

Carps, eel and other fish prevent breeding success of *Bombina* completely. Not only do the tadpoles not survive; adults refrain from spawning the water contains fish. The toads will emigrate to other localities if possible.

Invasive fish species are serious threats. The pumpkinseed (*Lepomis gibbosus*), which


Sticklebacks from the pond above.

originates in North America, are popular with anglers. They have illegally been introduced in many European ponds. They live in shallow vegetated ponds and will eradicate many amphibian species in these ponds. Another example is the rotan (*Perccottus glenii*) which originates in easternmost Russia and northeast China. They were introduced to the St. Petersburg and Moscow regions and have spread through drainage systems. They now occur in large parts of Europe from Ural and Arkhangelsk to Poland and Italy. They are frequently used as live bait because they tolerate transportation over great distances. In this way, they are introduced to many ponds and drainage systems, and they spread efficiently between ponds. They live in bogs and almost stagnant shallow streams and tolerate poorly oxygenated water. Observations from Latvia indicate that they prey on tadpoles and newly metamorphosed specimens of *Bombina*, and may even eat adults.

Bombina tadpole with a severely damaged tail from a pond invaded by the rotan.

4.3.3. Other amphibians

Crested newts (*Triturus cristatus*) are often present in *Bombina* ponds and usually they do not interfere severely with *Bombina* breeding. However, if the density of large newts is very high, the survival of *Bombina* tadpoles may be impaired. Adult *Bombina* may also loose their motivation to breed.

Green frogs (*Rana esculenta*) have mixed effects on *Bombina bombina* populations. In most cases, the two species coexist without problems. Green frogs usually do not eat *Bombina*, but if they are inexperienced or hungry, they may do so. Sometimes green frogs chase every *Bombina* specimen they lay their eyes on, and thus completely prevent breeding since mating behaviour is impeded.

4.3.4. Birds

It is unclear if bird predation can threaten the survival of *Bombina* populations. However, experience strongly suggests that heron predation may eradicate *Bombina* populations. Heron predation is most intense early in the breeding season because low vegetation makes it easy to see the toads.

Duck rearing is a threat to *Bombina*. Experience from Denmark indicates that adult *Bombina* may stay in a pond which is used for rearing of a small number of ducks provided that the ducks do not change the vegetation. However, breeding success will be low or zero. If ducks become so numerous that they change the vegetation in the pond, *Bombina* will disappear.

4.4. Pesticides

An investigation on the effects of agricultural pesticides on growth and survival of *Bombina bombina* eggs and tadpoles (Briggs & Damm 2004) was carried out in eight previously dredged ponds on a small island south of Fyn and three ponds on the east coast of Fyn. Information concerning use of specific pesticides in fields surrounding the ponds was obtained from the farmers. Water samples for pesticide analysis were taken weekly.

Insecticides and fungicides were found in six ponds in the agricultural areas. Herbicides occurred in 10 out of the 11 ponds, including ponds outside cultivated areas. Herbicides were much more persistent than insecticides and fungicides. The concentrations of pesticides tended to decrease with increasing width of buffer zones (1-10 m). The height of the vegetation in the buffer zone (0-1 m) did not have an obvious effect. Not even a 10 m wide buffer zone was sufficient to prevent drift of pesticides in the ponds.

Pesticides applied to fields close to *Bombina bombina* areas result in poor water quality.

With one exception, eggs or tadpoles were not affected by pesticides. However, in a small pond with practically no buffer zone an effect was recorded. Concentrations of clopyralide and propyzamide were high, and tractor tracks in the field indicated that pesticides could have been sprayed directly across the pond. That year, herbicides eradicated the macrophytes (*Sparganium*) in the pond, and mats of filamentous algae took over. As a result, the epiphytic coverings of algae, protozoa, rotifers and bacteria almost disappeared. Since these coverings are vital to tadpole feeding, only one of 40 tadpoles survived to metamorphosis. Thus it appears that the effect was caused indirectly by disappearance of food substrate.

4.5. Collection and persecution by humans

Removal of specimens

The firebellied toad is relatively easy to catch, thus it has been the object of collection and relocation. Relocation of numerous specimens from localities with small populations to places where the animals cannot breed has been recorded. Relocations like this, have probably contributed to genetic impairment of populations already in danger of inbreeding. In a few cases collection of animals has probably led directly to eradication of the population.

On a few occasions in recent years LIFE project *Bombina* brood has been stolen from

Human persecution of *Bombina* can be a problem depending on intensity.

rearing cages. The most serious incident occurred in a locality where inhabitants in a holiday cottage nearby decided their sleep at night was disturbed by the calling males, and apparently succeeded in eliminating the species by collecting the brood.

Introduction of alien specimens
Release of alien specimens is also a threat to *Bombina* populations. *Bombina* specimens originating from the Pannonian region, most likely from eastern Austria, have been released in several localities in Scania and eastern Holstein. Apparently these specimens are relatively competitive, thus over time genetic material from Austria gradually replaces genetic material of local origin.

In a worst case scenario, specimens of *Bombina variegata* or *Bombina orientalis* could be released in *Bombina bombina* localities. The result will be some degree of hybridisation, and destruction of the original genetic composition. Luckily, apparently this has not happened thus far.

4.6. Reported causes of decline

A source of discussion in Denmark and Sweden is whether the decline of *Bombina* primarily is caused by climatic change or by deterioration of its habitats. In order to evaluate these different hypotheses, it has been important to investigate as many of the former localities as possible, and to interview persons familiar with changes in these localities.

In 1975, biology students investigated a series of former localities on Fyn and Langeland (Denmark) in order to determine whether extinction had verifiable local causes. None of the investigated ponds were still suitable for firebellied toads. In other words, no evidence indicated that climate change rather than habitat change was the crucial factor. Likewise, *Bombina* had been very numerous on Bornholm (Denmark) in 1916. It disappeared from the last localities on the island around 1930, and a local naturalist speculated that the reason might be a particularly cold winter. This speculation supported the ‘climate explanation’. However, a closer investigation in 1988, which included interviews with old people, revealed that each locality had specific causes for destruction (filling in with plant litter and acidification from a conifer plantation). In south Sweden, all former localities have changed drastically, except for the reservation at Mölle where the species persisted until 1960.

All ponds in Denmark (c. 45), where *Bombina* was present in 1970 and subsequently has declined, have been thoroughly surveyed. The data include ponds where the species became extinct as well as ponds where it almost disappeared, but was saved by remedial actions. Cases, where negative factors have played an important or crucial role for the

Eutrophication and overgrowing are some of the main reasons for extinction of local populations.

decline, are listed in Table 4.1. Sometimes, more than one factor is listed for one locality.

Table 4.1. Negative factors influencing the decline of *Bombina*.
In total: 61 causes, i.e. 1.35 causes per locality

Eutrophication from fields	10
Duck rearing	9
Shade from trees	7
Draining	6
Fish (introduction or immigration)	3
Cessation of grazing	3
Overgrowth (natural, not due to eutr.)	3
Flooding with seawater	3
Inflow of silage or dung water	3
Backfilling with soil	2
Illegal collection	2
Reduced grazing pressure	1
Cultivation of surroundings	1
Drying out	1
Unintended destruction of watertight bottom	1
Garbage	1
Dumping ground	1
Eutrophication from cattle	1
Road construction	1
Pesticide spraying from aeroplane	1
Cementation of banks	1

Outside Denmark, information on causes of decline is less precise. Prior to 1980 in Schleswig-Holstein, small ponds were destroyed to a great extent. After 1980, the ponds were left to deteriorate – i.e. eutrophication, shading, overgrowth and lack of vegetation management. Introduction of fish are also important.

In east Germany, some important negative factors are: drainage of wetlands, filling in of ponds, intensive cultivation, pesticide poisoning, eutrophication, transformation from grazed to cultivated areas and regulation of rivers to eliminate spring floods. Negative factors may be intensified by the increasing isolation of individual populations.

In order to gain arable land, many breeding ponds are drained.

If ponds are not managed, they will loose their function as breeding habitats due to overgrowing by trees.

Literature:
Briggs, L & **Damm**, N (2004) Effects of Pesticides on *Bombina bombina* in Natural Pond Ecosystems. Ministry of Environment, Danish Environmental Protection Agency, Pesticide Research no. 85. http://www.mst.dk/Publikationer/Publications/2004/10/87-7614-393-7.htm?wbc_purpose=basic%23%252

5. Management of Aquatic and Terrestrial Habitats

5.1. Habitat management strategy

5.1.1. Improvement of the entire habitat complex

Long term survival of a population requires a habitat complex in which all developmental stages can survive in sufficiently high numbers, even when disasters occur sporadically. If the population generally is healthy with respect to size and breeding success, and if the habitat is optimal, most hazards are soon overcome, and the population will recuperate when conditions improve again. For instance, if an important breeding pond is flooded and either fish enter or the salinity increases, the population will have alternative breeding sites available. On the other hand, in a poor habitat with only one or a few good ponds, a flooding may threaten the entire population. Therefore, conservation of *Bombina* must aim at protecting a varied habitat complex.

5.1.2. Permanent versus temporary ponds

A temporary pond filled with water in early spring has a much lower density of predators than permanent ponds. Such temporary water bodies are very attractive to firebellied toads early in the breeding season. The entire population of adult toads may actively seek out such floodings to spawn.

Migration between different types of water bodies in different years is an integrated part of the species biology. A good rule of thumb is to establish not one, but (at least) two ponds for *Bombina* in any given locality: a deep pond for breeding in dry and hot years, when shallow ponds dry out, and a shallow pond for breeding

A permanent pond with well developed vegetation on Birkholm.

in wet and cold years, when the deep pond is unsuitable. In this way at least one suitable pond will be available each year, thus the population is stabilised.

5.1.3. Overgrowing or silting of ponds

Natural succession results in slow gradual overgrowing of breeding or foraging ponds. Man-made surplus influx of plant nutrients to the ponds has greatly accelerated this natural process, and ponds grow over much faster. Open water also disappear because many ponds in the agricultural landscapes are subject to fast silting.

To reverse or reset ponds to prior optimal states, the bulk of plant nutrients must be removed. This is achievable in several ways. One is repeated removal of vegetation including the nutrient contents (exhaustion). Another method, which is often preferred, is to dredge the pond, i.e. to use a machine to remove the bottom sludge including its nutrient content.

Semi-natural habitats in riverine areas regularly managed by cutting sedge, grass or reed or by grazing will experience overgrowing with tall vegetation if management declines or changes. At first sedges or reed will take over the ponds, later bushes and trees (*Salix*, *Alnus*) will thrive. The introduction of machinery and the use of increasingly larger machines have resulted in decreased mowing of wet parts of the meadows. As a result, these important locations are overgrown fast.

Organic material and mud is a source of nutrients and should thus be removed from a pond.

Introduction of (summer) grazing can help improve the aquatic habitat. Trees and bushes must be cut; best during cold periods when hibernating frogs are protected by the frozen soil. In order to obtain a lasting effect, resprouting vegetation like *Salix,* reed or *Typha,* should be removed entirely.

5.1.4. Pond surroundings

The best results are obtained if the pond surroundings are moderately grazed. This creates a favourable habitat for migration of firebellied toads between ponds or between a pond and the hibernation site. Grazing with robust cattle all year or summer grazing with traditional cattle breeds are possible options. The first is probably preferable in most cases.

Often, the best option is to create ponds in existing cattle pens. Alternatively, the area can be fenced and stocked with cattle after the pond has been dug.

Survival of the first hibernation appears to be very important and frequently it is a restricting factor. Hibernation quarters should be available near the ponds. If suitable sites are not present, they can be constructed. In this way, long stretches through inhospitable habitats to reach the hibernation quarters are avoided. Furthermore, the animals can hibernate in places with good conditions. During the project, juveniles and many subadults have been observed at the newly installed hibernation sites, indicating use of these.

5.1.5. Avoiding predators

When planning and placing ponds, it is important to prevent fish immigration. Temporary ponds may now and then be colonised by sticklebacks or other fish. It is important that they dry out sufficiently often to prevent fish from surviving more than a few years at the most. Colonisation of fish must be completely prevented in permanent ponds. First of all, contact with streams should be avoided. Even drainage pipes may sometimes function as immigration routes for sticklebacks, and this is another reason for closing them. Furthermore, the highest water levels that may ever occur must be known. Local people often have knowledge about this. If, for instance, a short but intense period of heavy rain creates a temporary stream that for a few hours flows into a neighbouring pond, this short time span is sufficient for sticklebacks to enter the pond. Sticklebacks may also immigrate via melting water streams between snowdrifts. In other words, one must be extremely careful when trying to foresee possible immigration routes.

Crayfish may be a problem in certain cases. It is important to know that crayfish may disperse up to 40 m over land. No pond should be created closer than that to a water body with crayfish.

Other important predators may be birds such as ducks or herons. It may be important to avoid placing ponds in areas where such birds occur in unusual abundance.

In this pond some *Typha* was left to provide hiding places for adult amphibians against bird predation.

5.2. Planning

5.2.1. New ponds

A planned *Bombina* habitat must be analysed hydrologically and the following questions must be answered:

1. Can a temporary flooding be established, e.g. by blocking drainage pipes, stopping a pump or building a dam?
2. Can ponds be built on clay soil with good water quality in summer?
3. Can existing ponds be improved to become foraging or breeding ponds?
4. Are there any maps of the drainage system? If so, where is it optimal to place ponds relative to the drainage system?
5. Will the supply of rain water be sufficient to fill the pond?

Natural floodings often make the best *Bombina* ponds. A drained depression in the landscape can be restored to the original state by destroying drainage ditches or pipes. Usually, a deep layer of peat renders the site useless for *Bombina* ponds. Rather, it should be placed at sites with mineral bottom, preferably clay, or where the peat bottom is shallow and has a high clay content, e.g. meadows.

When new ponds are excavated, this is also done in clay soil, because a favourable water

The soil conditions at this site (pure clay) are good for a pond feeding only on precipitation.

quality and a water tight bottom are ensured. Test holes are made, by hand or with a machine, to find the best location for the pond. If test holes are made some days in advance, it is possible to see if ground water seeps into the holes, and in this way, the water level can be

A test hole is dug in order to examine the pedological conditions and to choose the right placement of a pond.

Blueish clay indicates an old waterbody. The sedimented or filled in top layers are removed to restore it.

found. Promising test holes are subsequently extended to become test crosses, i.e. two search profiles that cross each other at a right angle; the cross spans the pond outline.

Test crosses reveal possible pockets of sand, through which water may leak out. This method also allows location of drainage pipes even if these are placed below the future pond bottom; drainages hidden in the underground is often recognised from lines where clay and black top soil have been mixed when the drainages were covered with soil.

A pond on dense clay bottom, without inlets

A typical test cross revealing different pedological layers (from top to bottom): organic brown top layer, light brown clay layer, dark brown clay layer with organic material and white clay bottom layer.

from drainage systems, will have a water level determined by the balance between inflow and evaporation. As a rule of thumb, inflow is sufficient if the total surrounding catchment area is three times the area of the pond.

As to the size of the planned pond, each toad requires about 5 m^2 of pond area in the best breeding ponds (quality class I, see section 6.2.2.). If the pond is believed to become less than optimal (quality class II), 20 m^2 pond surface must be allowed per adult animal. In other words, a local breeding population of 100 adults requires more than 500 m^2 pond area, and probably as much as 2,000 m^2. In any case, newly dug ponds will grow over slowly, and it will take some years before they can function optimally. In addition, wetland areas that can function as foraging areas are necessary. This could be slightly more eutrophic ponds with some mud and many hiding places, e.g. with some overhanging hawthorn bushes, with dead branches, stone heaps, or bramble vegetation. A good *Bombina* pond must have a wide shallow zone with a maximum water depth of 40 to 50 cm. The centre of the pond should be deeper than 1.20 m to avoid heron predation. Optimally, the centre zone should be 30% to 50% of the total pond surface.

5.2.2. Enlarging and reshaping existing ponds

Instead of creating a new pond in an entirely new location, it may often be better to choose a site with a small pond; for instance a marl pit with steep sides, a pond overgrown with bushes or reeds, or any other type of pond which at present is without value to *Bombina*. It is possible to enlarge the pond while maintaining the same bottom level, to level out steep sides, to deepen a desiccating pond, or to fill in soil to make a deep pond shallower.

An old pond is cleaned and enlarged at one side.

5.2.3. Restoration of existing ponds

An existing unsuitable pond may become suitable without changing its shape and size. For instance, a eutrophicated pond may be dredged, i.e. the bottom sludge may be removed.

The first thing to consider is, if is possible to remedy the causes of the problems. Will the landowner allow cutting down shading trees? Can the inflow of polluting or eutrophic water be blocked? Can re-immigration of fish be prevented? Can renewed growth of reeds and bushes be avoided?

If the answer to these questions is yes, then a project can be formulated.

The best season to dredge and restore an existing pond is usually August or September, just after harvest. At that time, the soil is dry enough to carry the weight of machines and wagons, and the sludge from the pond bottom can be spread on adjoining fields. The water level in the pond is also at its lowest at this time of year. If the work is finished well in advance of the first frost nights, many seeds will sprout before winter, and there may be lush stands e.g. of *Batrachium* the following spring.

If the bottom sludge is a thin suspension of mud particles, it can only be removed once the water has been pumped out. Indeed, in most cases, it is practical to pump out the water just before the work starts.

If the sludge is polluted, e.g. if rubbish has been dumped in the pond, the sludge has to be transported to a facility where it can be properly treated. Thus, if old bicycles, car batteries, medicine bottles or pesticide barrels have been dumped there, heavy metals or other poisonous chemicals have leached into the sludge. On the other hand, if the pond is unpolluted, the sludge can be spread on an agricultural field.

Any doubts, whether the sludge is polluted or not, must result in an analysis. Samples from

The old pond above was enlarged with a shallow zone to provide the amphibians with water that warms up faster and will promote spawning. Shallow water also provides a good habitat for hunting. Furthermore, the shrubs surrounding the pond were removed to prevent shading, overgrowing and addition of nutrients. The result of the improvements is seen below.

a minimum of five different places in the mud are mixed in a clean jar covered with a lid that will not pollute the sample with metals or plastic. The contents are analysed by a firm specialised in sludge analyses.

5.2.4. Dealing with drainage systems

Existing drainage systems may often complicate the pond project. A worst case scenario is undiscovered drainage pipes just below the excavated bottom of the pond, or close to the edge. If that is the case, the pond will not hold water. Thus, it is a good investment to spend some time finding all relevant drainage pipes.

The situation is complicated further by the fact that water in drainage pipes usually has a considerable content of leached plant nutrients. If such water runs into the pond, it will become hyper-eutrophicated. To avoid this, it is necessary to block smaller drain pipes, and relocate larger pipes to prevent interference with the pond. Sometimes, this may be difficult or very expensive. If so, a second-priority solution is to create a small separate pond between the inlet and the main pond. The small pond functions as a kind of pre-sedimentary basin. For this solution to work, the small basin has to be dredged rather often, e.g. every second year.

In general, it is desirable that the surroundings are drained as little as possible. The

Pond eutrophicated by drainage water.

Drainages are found at almost all sites at different depths. In order to maintain water in the pond, these must be destroyed. The black line indicates the underlying drainage pipes.

An ancient stone drain has been found.

optimal habitat for *Bombina* is a pond which is already shallow in early summer, but which nevertheless holds water throughout the summer in most years. This is only possible if water in the surrounding soil only drains slowly during the summer, i.e. if the surroundings are not actively drained.

5.3. Negotiations and agreements

5.3.1. Negotiations with private landowners

Usually, the sites of interest are on private land and it is necessary to contact the landowners in order to arrange the necessary interventions for conservation or introduction of firebellied toads. In any case, it is important to respects the landowner's rights to his land and not do anything that will make the landowner oppose the project.

Unless the landowner is very interested in helping threatened animal species, any involvements will have to be costless for the landowner, or, if there are costs, these will have to be compensated.

When details are discussed with the landowner, problems may turn up. For instance, he may think that if a pond is dug on his land, he could rear ducks or attract wild ducks by excessive feeding. Or he may want to have crayfish. Or he may be unwilling to reduce the amount of fertilizer on the site where the pond is to be placed. Or it may be impossible to agree on an optimal grazing pressure. Or he may not accept that the grazing cattle are given access to the pond. Or he may be entirely against the idea of blocking drainage pipes.

It is thus important to discuss things thoroughly and make sure that the landowner knows what is being planned.

Landowner talk in Germany (above) and Latvia (below). Notice the *Bombina* van.

5.3.2. Surrounding habitats

When negotiating with landowners it is also essential to consider the management of the areas around the ponds. If the landowner is very interested, he may accept changing the management in order to benefit the firebellied toads. But generally, he will accept few or no changes.

In such cases, it must be considering if it is possible somehow to leave management and administration of the surroundings to public or semi-public authorities. Or it may be possible to enter agri-environmental contracts to compensate the landowner for the extra expenses.

Measures that may be possible if the land is purchased or expenses are compensated are for instance: regulation of grazing pressure, control of fertilization, change of the drainage regime and public access to the area.

5.3.3. Land purchase

If the landowner does not accept the necessary changes of the land management, an option may be to offer to buy the land.

Sometimes the landowner will accept this possibility immediately, thinking that a small valueless plot suddenly earns a decent prize. In other cases, it may take long to create mutual trust and convince the landowner that selling the land is a good idea. Trust can be created by frequent contact with the landowner. For instance, the landowner could be paid for carrying out certain services elsewhere, until a positive atmosphere has been created. It may in some cases take several years to obtain the necessary positive atmosphere.

In some cases where the landowner at present wants to keep the piece of land, it may be possible to make an agreement that if the plot is ever put up for sale, it will primarily be offered to people working with *Bombina* conservation.

This latter arrangement has been greatly elaborated in Schleswig-Holstein. Here, the semi-public institution Stiftung Naturschutz has been given the legal right to buy any land put up for sale within predefined regions with special nature values. Over the years, the Stiftung has been able to acquire coherent stretches of nature by purchasing series of small plots that were previously privately owned. This allows coordinated management over larger areas, for instance of water regimes and grazing pressure. The strategy is to secure the relevant areas for nature purposes, and subsequently initiate the required management.

Thus this type of organisation creates important new possibilities, and similar institutions in other countries are highly recommended.

5.3.4. Working on public land

Sometimes, the area of interest is already public property. This eases solutions in many ways, but disadvantages may also exist. One disadvantage is that the administrative system may not be geared to take rapid decisions, or perhaps special regards are required.

Experience with nature management on military training grounds has often been very positive. One major advantage in placing ponds on military areas is that these usually are unfertilized, and there may be several kilometres to the nearest fertilized land. This allows ponds with rather sparse vegetation and minimal overgrowth.

5.3.5. Agreements

Landowner negotiations aim at the signing of a written agreement between the landowner and the authority dealing with *Bombina* conservation. This agreement will define precisely what will happen, and which conditions must be observed by the landowner and by the authority. For instance, by signing the agreement the landowner acknowledges that he understands that he cannot plant shading trees along the southern banks of the pond.

Usually, a signature is considered sufficient evidence that both parties agree what is to be done and what is not accepted. One may also want to register a declaration in the land register, but the landowner is often reluctant to do this. If 95% of all landowners keep the promises in the written agreement, more is obtained to secure *Bombina* populations than if only 50% of all landowners are willing to enter the land register. Furthermore, registration is a slow and expensive process.

If the property is later sold to another landowner, it may be useful to have the conditions in the land register. However, in practice it is more important to make contact with the new landowner and to make him understand that it is necessary to protect the *Bombina* population.

5.3.6. Applications

Once an agreement has been signed, an application is sent to the obligatory authorities. For instance, in order to alter a protected pond, it may be required to get permission. If the drainage system is changed, the water authorities may have to give permission. The application must describe the plans exactly, e.g. what is going to happen to the excavated soil.

A certain degree of flexibility may be needed. For instance, the optimal placement of a pond may be unclear. It is therefore recommended to define this flexibility in the application. For instance, in Schleswig-Holstein, the common practice has been only to preliminarily define the size and position of the pond(s) in the application. When the excavator arrives, a representative from the nature protection authority is present during the digging of test holes. On basis of what the test holes show, this person accepts a definite plan for the exact position and size of the pond and the pond is subsequently dug.

5.4. Executing practical work

5.4.1. Contact to contractors

A contractor should be contacted well in advance of the planned work. Typically, a meeting is arranged with the head of the firm and the landowner at the planned site, and various details are coordinated. The choice of excavator is discussed, and it is decided if wagons to transport soil or mud away from the place are needed. A route by which the excavator can drive to the place is found.

5.4.2. Creating floodings

In order to create a pond by raising the water level, the first step is to detect if maps of the drainage pipes exist, usually by asking the landowner or a former landowner. If no maps can be located, it is necessary to search for eventual drainage pipes by digging ditches. Pipes are blocked by crushing them and replacing them with clay over a length of at least one meter.

One may also create floodings by building a dam across a depression in the landscape. When doing so, it is important to make sure no drainage pipes are present in the soil carrying water under the dam.

5.4.3. Digging new ponds

Usually, digging of a new pond starts with a test hole and a test cross, as described in section 5.2.1. The pond is then excavated.

If sandy strips are encountered during the excavation, these can be exchanged with clay in order to avoid water leakage through the sand.

Clay soils are often covered by nutrient rich, black topsoil. To avoid leakage of phosphorus into the pond water from the topsoil, the pond edges should be covered with a layer of clay to avoid direct contact. The clay should be wet and soft, and must be compressed during placement.

The destiny of the surplus soil resulting from the digging may differ. In some cases, the soil should be transported away from the site, in order not to cover valuable biotopes or vegetation. The landowner may often want to use the soil elsewhere e.g. for levelling a depression in a field, or for stabilizing a dirt road. In other cases, it is unproblematic to spread the soil in a thin layer around the pond, or the soil may be used to build a gently rounded dam to protect the pond from unwanted inflow of water. For instance it may be useful to place the soil between the pond and a stream.

Searching for drainage pipes, the sticks mark the pipes.

A dam is build to create a shallow flooding.

Sand dominates this site. A new pond would not retain water.

When soil is placed around the pond, it is important to make sure that inflow of rainwater from the surroundings is not blocked. On the contrary, it should be designed and modulated to maximise the area of gentle slopes toward the pond in the surroundings.

The maximum water level as well as the spot where surplus water leaves the pond will have to be defined. Often, an outlet well will be placed at the latter, especially if it is important to make sure no fish enter the pond via the outlet stream.

5.4.4. Restoring existing ponds

Before dredging an existing pond, some preparatory actions may be needed before the excavator arrives. Trees may have to be cut down to give access to the machine, and bushes and trees along the banks may have to be cut down. Often, a pump will have to be started one to several days in advance in order to empty the pond.

It may be advantageous to preserve parts of the existing vegetation. The machine can cut some turfs of favourable vegetation – for instance stands of *Eleocharis* or *Sparganium* – and set these aside. When restoration is completed, the turfs may be returned to the clean bottom layer of the pond. Often, however, this is not necessary. Small amounts of bottom mud will inevitably be left behind, and this mud contains many aquatic plant seeds which will soon sprout.

Next, the excavator starts digging. Willow bushes and alder trees are removed completely to prevent re-growth. Reeds are removed as well as possible, but the roots of common reeds (*Phragmites*) often grow too deep to be removed entirely. Next, the bottom sludge is removed. As a rule, the bottom sludge should be removed entirely exposing a blue clay bottom.

Before the mud is removed the pond is pumped dry by an excavator hydralic pump (12cm^3/min).

Rubbish in the sludge may be sorted out. Non-poisonous rubbish, e.g. wooden boards, glass or brick stones, often have to be sorted out before disposing the rest of the sludge. Metal objects can be sorted and sold to a metal dealer. Unpolluted sludge may preferable be spread on agricultural soil. Polluted sludge, on the other hand, must be transported to a facility for cleaning.

Some vegetation is saved for later use in the pond.

Very thin sludge can be removed with a slurry pump instead of an excavator. This is only advantageous if the bottom layer is completely free of stones and other objects that may block the suction tube.

5.4.5. Avoiding predators: Fish

It is important that fish are unable to enter the pond after creation or restoration. It is vital that the outlet does not connect with a ditch through which fish may enter when water levels are high. Usually, this means that a distinct outlet will have to pass through a small well. Since the outlet water falls down through the well fish cannot migrate upstream. See Figure 5.2 for illustration.

A problem may arise if fish are already in the pond or the water system above the pond. One solution is to use the strong poison rotenone, which kills all animals breathing with gills, including all fish. The poison must be used in the warm season. This method has been used successfully, but its use is banned in some countries.

Another option, electrofishing, is rarely efficient. Even if 90 or 99% of the fish are caught, the few remaining will breed and soon the fish population is back to normal. Electrofishing is only possible if water levels are low (< 1 m).

The most common method to eradicate fish is to empty the pond in a warm and dry period, preferably in July or August, but the method may also work in September or October if the weather is dry and sunny. The pond can also be emptied in mid winter, if there is a possibility that frost will kill the fish trying to survive in the bottom mud.

Different fish species tolerates drying out very differently. Most fish species will die

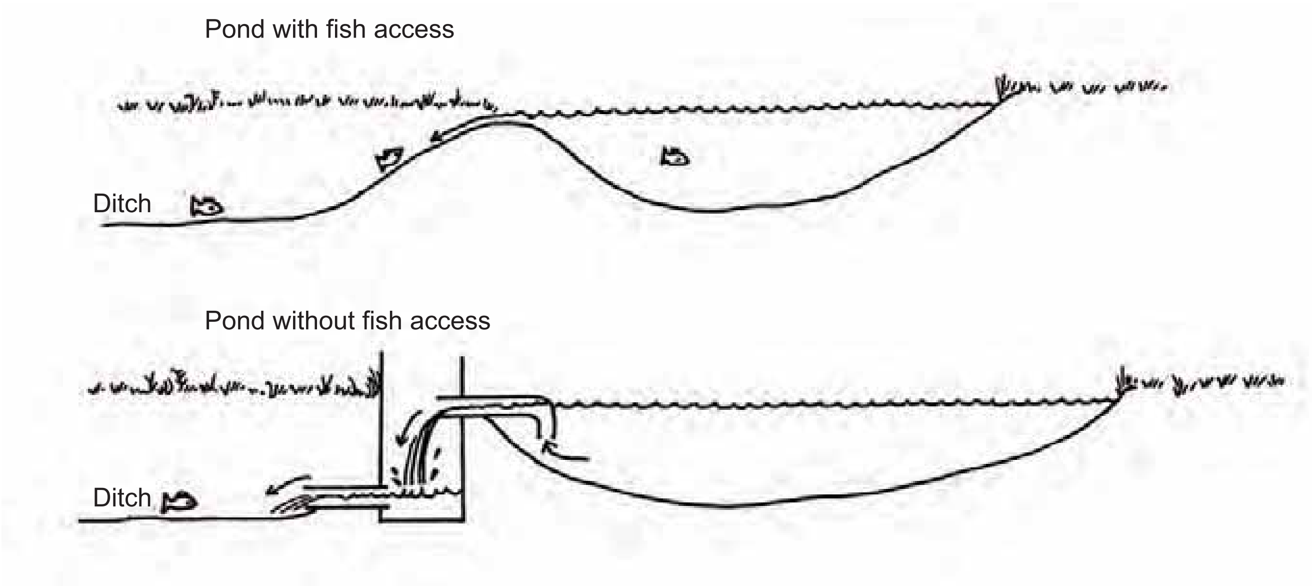


Figure 5.2. When the outlet from the pond falls down through a well, fish cannot migrate into the pond.

Fish barrier consisting of a 50 cm layer of gravel. This layer of gravel prevents fish migration between ponds, but permits percolation of water.

within two hours. Sticklebacks (*Gasterosteus, Pungitius*) may last a day or two. Eel (*Anguilla anguilla*) are also hardy. But two species, crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*), can survive out of water for about three weeks, if they are kept moist. To remove these species, it is necessary either to remove all mud leaving absolutely no hiding places anywhere in the pond, or to let the mud dry continuously through a period of three weeks.

Successful eradication of fish requires careful scrutiny of the desiccated pond bottom. It does not suffice ordering a firm to pump the pond dry without inspecting the result. Usually, the main problem is that small pools of water remain here and there on the bottom, e.g. in footprints. If the vegetation is dense, it is usually necessary the remove this vegetation (with an excavator), since the mud surface below the vegetation never dries out otherwise.

Heavy inflow of ground water to the pond can render emptying nearly impossible. In that case, a pumping hole may be excavated in the bottom, and some soil kept in form of a heap nearby. The pumping is halted temporarily, and eventual surviving fish comes out of the mud and enter the water phase again. Then pumping is resumed, e.g. the next day, and the fish will be sucked into the pumping hole. When a small amount of water remains in the pumping hole, the heap of soil is quickly returned to the hole to cover the last fish remaining in the water.

Another option is to add some base in the last remains of water. Grains of NaOH, or $Ca(OH)_2$ (slaked lime) can be used. Once the pH is above c. 11, fish cannot breathe. An additional effect of the latter compound is that Ca ions strongly bind phosphate, thus improving water quality in the following years. Use of NaOH or $Ca(OH)_2$ has no long term negative effects, because these bases react with CO_2 in the atmosphere to form $NaHCO_3$ or $Ca(HCO_3)_2$, respectively.

Other methods can include covering the pond during a long period with plastic, fill it in or let it become entirely overgrown for several years and thus create conditions in which the fish population cannot survive. After a long period, the pond can be dug out and improved. A risk is that the *Bombina* population might also look for alternative sites and when they are not available in the vicinity the risk of casualties during the migration is high.

Often it is argued that fish will enter somehow, because fish eggs may be transported e.g. on duck feet from pond to pond. In general, this is not true. Such spreading of fish eggs has never been documented, and it is unclear how long fish eggs can survive out of water. A possible exception is moderlieschen (*Leucaspius delineatus*), which sometimes appears mysteriously in hitherto fish free waters. How this happens is unknown, but eggs from this particular species may possibly tolerate transport via birds.

5.4.6. Avoiding predators: Herons

The main remedy against heron predation is to ensure that firebellied toads have places where the herons are unable to catch them. Thorny bushes extending from the bank over the water surface provides a hiding place for the toads. An alternative is to cut branches from thorny bushes and place them at the waters edge. The toads will be safe underneath these. They may also be safe between stones placed near the waters edge. Another strategy is to ensure that the pond have deep parts (> 1.2 m), e.g. with

Galloway grazing a pond with rearing cages.

attractive floating vegetation, where herons cannot go. Some ponds are constructed with a shallow zone along the banks, which soon are densely vegetated and other parts too deep for wading herons, but without zones of intermediate depth.

If such preventive measures are impossible or insufficient, various methods can keep herons away. An electronic scarecrow has been used with success, but the herons become accustomed to it after a few months, especially if the scarecrow remains in the same place. Another option is to stretch very thin, practically invisible fishing lines over the pond surface. When a heron hits a line it cannot see, it becomes scared and stays away. Lines may also be stretched tightly over the water surface along the banks or perpendicular to the bank perimeter. A wading heron will hit them with its legs, and this can also be relatively efficient. However, all these methods are aesthetically unsatisfactory, and/or they cannot be applied when cattle have access to the ponds, which is often the case with *Bombina* ponds.

5.5. Land use/land management in and around ponds

5.5.1. Grazing

Most *Bombina* ponds are grazed, and it may be difficult to maintain functioning ponds in the long term if they are not grazed. Every opportunity to continue or introduce grazing should thus be utilised. Grazing may be by cattle, sheep, horses or other animals. Cattle have the main advantage that they enter the ponds and keep vegetation in the entire shallow water zone down.

A disadvantage is that a high density of cattle may eutrophicate the pond heavily. On very warm days, when few drinking sites are available, cattle will spend a considerable part of the day in and at the water. This is especially true for large cattle races, such as Scottish Highlanders. This may result in complete destruction of the aquatic flora, causing turbidity and a high amount of faeces in the water.

One method to avoid this is to create at least two ponds in each fen, one primarily intended for amphibians, and another primarily intended for watering. For instance, if a pond is placed partly in shade near a row of sheltering trees, the cattle will often stay in this place. They will utilise the shade of the trees during hot summer days, they will use the pond for drinking, and they will urinate in it. The other pond, fully sun exposed in the middle of the pen, will be much less affected by the cattle; they will graze the banks, but not urinate here.

Horses may sometimes, and sometimes not, enter into the water. Individuals behave

Temporary flooding with low grazing pressure: 0.3 cattle per ha all year.

Temporary flooding without grazing.

differently in that respect. Sheep will remain on dry land, but they may graze the pond bottom if the water level sinks in late summer.

In order to obtain a sufficient degree of grazing at the pond edges, one animal unit (one adult cattle or horse) per 100 to 200 m of pond edge seems to be sufficient to control fast growing reeds like Typha species or Phragmites australis on clay soils. To facilitate reduction of tall Typha and Phragmites vegetation at existing ponds, one animal unit per 50 to 100 m pond edge should be allowed.

Grazing can be limited to the warm season, or may be all-year-round. The latter requires use of robust breeds of cattle, horse or sheep. An advantage with all-year-round grazing is that a smaller grazing pressure is sufficient. Plant species that may cause trouble if only summer-grazing is used, e.g. *Juncus* or *Urtica*, may be grazed during the winter months when the animals have little alternative food. Thus the need for a high grazing pressure in summer is omitted.

Overgrowth with bramble (*Rubus spp*.) or thorny bushes is not prevented by cattle grazing alone, regardless how high the grazing pressure is. There are two solutions to this: supplementary horse grazing or supplementary mechanical cutting of woody vegetation.

5.5.2. Hibernation facility

Sometimes obvious hibernation possibilities are located near the pond, e.g. stone fences. If this is not the case, such facilities can be made artificially.

The basement of a hibernation site should be above the maximum pond level, so that rain water will drain to the pond instead of flooding the hibernation site.

Below a series of pictures illustrate construction of a hibernation site.

Notice the shape of the stone pile: warmth cannot escape through the entrance due to the hume shape.

The work is finished.

When a new pond is dug, stones are often encountered. These stones may be placed in a heap near the pond. If no stones are present, a load of stones can be brought to the place. A widely used hibernation site model is to dig an oblong cavity from the pond's edge inward. The cavity is filled with stones and covered with soil, except for the end facing the pond. This end is left uncovered creating direct access from the pond to the cavity between the stones.

If it is impractical to build a hibernation facility at the pond edge, or if this is aesthetically unfavourable, a facility may be created at a suitable sun exposed site nearby. Stone piles or tree roots could be partially excavated and then covered with grass sods. The southern exposed side, preferably facing the pond, is left

A hibernation site one year after construction.

uncovered by sods giving access to the hibernation site.

Some rough structures between the hibernation cavity and the pond provide shelter against predation at the hibernation site.

Artificial hibernation facilities should be kept rather dry, or at least not suffer from flooding, and they should not be too overgrown with dense shading vegetation.

5.5.3. Management of ponds

After ponds have been (r)established, the state must be monitored. It is important to know if they hold water for shorter periods than intended, and if they become excessively eutrophicated. If such problems arise, the cause of the problem must be uncovered, e.g. undiscovered drainage pipes.

Problems with unwanted plant species may occur. Among these are *Elodea* and *Stratiotes*, both of which may spread and outcompete all other plant species. In general, it is very difficult to eradicate *Elodea*, and there are no safe-proof methods (but see section 5.5.4.). *Stratiotes* may be physically removed by pushing the floating plants to one edge of the pond, utilizing for instance a grate held in a vertical position. Here, a suitable machine can pick up the plants.

Excessive growth of reeds like *Phragmites* or *Typha* may be cut in September or October with a scythe. If the straws are cut under the water surface, no oxygen can enter the air channels in the straw, and the roots die from lack of oxygen. The cut straws should preferably be gathered and removed in order to get rid of the nutrients.

It may be difficult to oppress growth of *Fraxinus*, *Alnus* and *Salix* if the edges are not grazed. If they are oppressed by cutting, this should preferably be done in May, when the plants are most vulnerable. Trees (*Fraxinus* etc.) should be ringed (i.e. a ring of bark removed) some time before cutting. In this way, growth of new branches may be avoided.

Large water fluctuations can be optimal. If water levels are very high for extended periods (e.g. a month) willow bushes and other woody vegetation may drown. When the water level drops again, meadow plants rather than woody plants will dominate.

5.5.4. Canadian water weed control

Canadian water weed (*Elodea canadensis*) is an invasive species that entered Europe in about 1836. Here, it only spreads vegetatively via short stems. Often it spreads from garden ponds, but it can also be transferred by cattle or

This pond is invaded by *Elodea canadensis.*

machinery used for pond management. The plant survives freezing temperatures. The very fast growth enables it to take over the entire water body in a small pond. Such a pond is useless as breeding pond for firebellied toads, because hardly any water body is left. Furthermore, the plant consumes all nutrients, and the algae and bacteria cover that provides food for firebellied toad tadpoles almost disappears. The remaining water body heats up during summer days resulting in increased mass consumption of carbon dioxide which raises the pH to 9; conditions under which neither tadpoles nor fish can survive.

Therefore it is necessary to eradicate Canadian water weed in amphibian ponds. At least three methods are described in Great Britain: a mechanical, a chemical and a biological method. (http://www.pondconservation.org.uk/Resources/Pond%20Conservation/Documents/PDF/CEH%20Canadian%20pondweed.pdf)

The chemical method is not applicable at conservation sites at least not in Germany. The biological method suggests planting trees along the ponds in order to shade Canadian water weed. This method is not applicable for firebellied toad ponds because the toads need sun exposed water for breeding and leaf decomposition in the water inhibits breeding success.

The mechanical method was tested in one of the colonized ponds; the pond was cleared of all vegetation and the top 10 cm of mud. However, the following year the pond was almost overgrown with Canadian water weed again.

Instead, as a trial, it was decided to leave a shallow pond (2000 m^2) dry for one year. The pond was emptied via a ditch in October 2008. The ditch was left open, keeping the pond dry for several months. The pond area was grazed in order to maintain short vegetation, leaving the sun to dry up the entire clay bottom.

13 month later the ditch was filled again. Canadian water weed did not reappear the following year. The firebellied toad had very good breeding success and many aquatic plants recovered from the seed bank or survived with a land form, e.g. *Potamogeton natans*, *Potamogeton gramineus*, *Ranunculus aquatilis* and *Ceratophyllum emersum*. *Eleoda canadensis* does not have this option since the species cannot reproduce by seeds in Europe. Obviously, the species could not survive on land for such a long time. The costs for these activities were about 700 € for digging the ditch and closing it again one year later including transport of the excavator twice.

Ranunculus aquatilis.

This experiment tells us that it is possible to eradicate *Elodea canadensis* if a pond is kept dry for one year.

In cases where it is impossible to use a ditch for draining, a solar panel operated electric water pump placed in a solid layer below the bottom of the pond should be taken into account for management of Canadian water weed. Ponds also can be drained through pipes with a closing device (see Figure 5.4). In this way, the drying process is easily repeated.

5.5.5. Foraging aquatic habitats

Maintenance of foraging aquatic habitats is relatively unproblematic. It is vital that such habitats remain warm, i.e. that they are sun exposed. It is also important to secure presence of heaps of branches, stones, dense shrub of bramble or other microhabitats that allow the toads to hide.

5.5.6. Buffer zones

In agricultural landscapes and along roads, influence of agricultural chemicals and road salt should be reduced to a minimum. Esta-

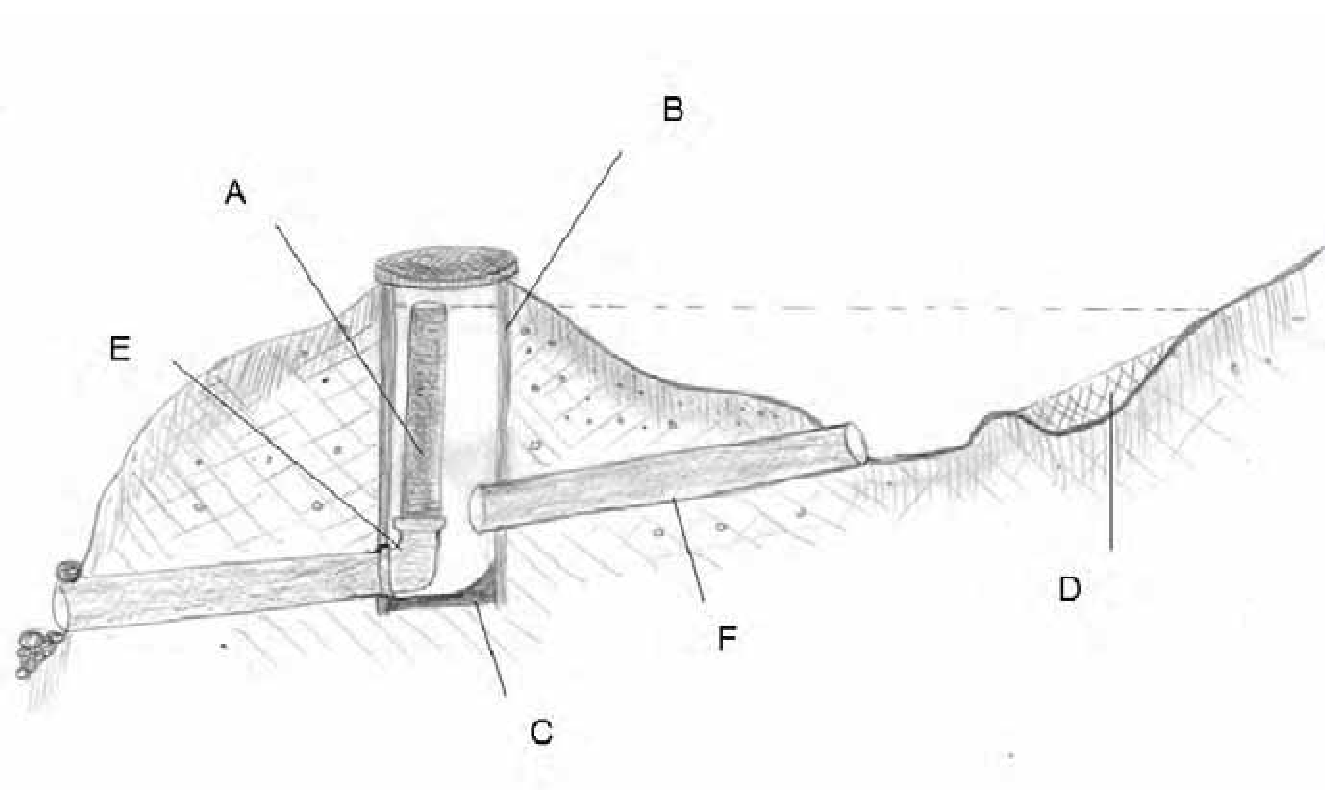


Figure 5.4. Pond barrage. A: removable pipe KG DN 300 (diam. 30 cm), B: concrete hopper with concrete lid (diam. 100 cm), C: concrete waterproof bottom to facilitate complete drying up, D: depression in pond bottom filled with clay to prevent remaining water, E: removable bent pipe KG DN 300 (diam. 30 cm), F: KG DN 300 (diam. 30 cm).

blishment of buffer zones is an important tool in this respect. Such zones may typically be 5-10 meters wide; the wider the zone, the better the protection. Bushes or large stones in the outer fringe of the zone prevent agricultural vehicles from driving into the zone, or spraying booms from sweeping the zone. On the other hand, bushes may expand and start shading the water surface.

The main problem with buffer zones is management. If the farmer is unwilling to manage the zone, the nature authorities are obliged maintain low vegetation in the future. Once the buffer zone is invaded by willow or alder trees, this can be a time consuming task.

5.6. Contact to local people

In order to manage the population properly, it is important that local people are aware of the species. In this way they can help to safeguard a population – by avoiding mistakes caused by ignorance and by actively supporting management and supportive activities. Management tools are education via schools, leaflets, excursions, information panels, etc. Especially informative activities for farmers and hunters can have a positive influence on the pond quality.

6. Population Management

6.1. Genetic aspects

6.1.1. Inbreeding and population size

If a population becomes very small, eventually the remaining individuals will be closely related, and the population will be inbred. How small can a population be before inbreeding reduces fitness markedly? Theoretical considerations imply that this depends on the 'effective population size', designated by the symbol N_e. This is roughly the number of animals contributing to the next generation (see below for more thorough explanation). N_e does not relate to each pond, but to the system of communicating ponds. Two ponds may be considered to belong to the same population if at least one migrant per generation successfully transfers genes from one pond to the other.

According to population genetic theory, the loss of genetic variation per generation is $1/(2 N_e)$. Thus, for $N_e = 5$, the loss is 10% of the variation per generation, and for $N_e = 500$, it is 0.1% per generation. Theoretical considerations imply such losses can be tolerated for a number of generations equalling N_e. In other words, with $N_e = 5$, the population will only survive 5 generations before crashing.

In general, a population size of at least $N_e = 50$ on a short term, and at least $N_e = 500$ on a longer term is recommended.

If $N_e < 50$, neutral or even favourable alleles will be lost so fast that natural selection is unable to counteract this. Many alleles will be lost over the generations, and whether the lost genes are detrimental or favourable is random. When natural selection practically has been disabled, the conservation status certainly is unfavourable. Therefore, N_e must always exceed 50, which means a time perspective longer than 50 generations. With a 2 years generation time for *Bombina*, this equals 100 years.

The reasoning behind the $N_e = 500$ criterion is the allegation that loss of alleles is compensated by accumulation of new mutations, offering natural selection a constant amount of variation to act upon. Although perhaps arbitrary, this gives a time perspective of 1,000 years. This seems sufficient for all practical purposes, because nobody can foresee what the world will look like after that.

6.1.2. Effective population size: the time perspective

Consider a population that is fairly large over many generations. The population size, N, is the number of adults. Then a catastrophe strikes and N is very small for a few generations, but soon the population recovers to the original size. In other words, the population has passed through a bottleneck.

The effect of a bottleneck on the amount of genetic variation passed on through generations may be comprehensible from calculating the effective population size over time. This is calculated as the harmonic mean of the sizes at each generation. The harmonic mean is calculated by inverting each population size (i.e. changing N to 1/N), then taking the average of all the inverted values, and finally inverting this average.

As an example, take the situation on the Danish island Hjortø. For simplicity, we only consider the number of animals, and assume that in each generation, all animals contribute equally to the next generation. After 1990, the number of adults (counted every other year) declined approximately as follows: 65 - 70 - 50 - 20 - 6 - 4. The average population size is 36. But the effective population size calculated as the harmonic mean, is $N_e = 12$. This means that the loss of genetic variation equals a population of 12 animals. Thus, at this level, 12 generations would be sufficient to extinguish the

Bombina bombina from Hjortø have a very distinct belly pattern.

population. As six generations have already passed, only six generations are left to save the population. Within that time span, the population would have to grow drastically to exit the danger zone.

This kind of calculations can be performed to measure the effect of catastrophes as well as the effect of more normal variations from year to year. Obviously, large fluctuations over time, minimises N_e relative to N.

6.1.3. Effective population size: effects within a generation

In the preceding section, we assumed for simplicity that all adults contribute equally to the next generation. If that is not the case, N_e will be further reduced. For instance, if eight males and three females are present and only two matings occur, six of the males and one of the females have no offspring and are not part of the effective population. If the remaining four animals contribute equally, the effective population size is four. But if they do not contribute equally – if one mating produces many surviving offspring and the other few surviving offspring – the effective population size is further reduced.

An existing data set may allow computation of N_e for a *Bombina bombina* population during one generation. This data set registered survival of reared animals in the small population at Enø in Denmark. Only 13 adult individuals were present during the years 1988-1990. The only offspring was produced by artificial mating (the females were kept in captivity during the mating season). The reared offspring was individually marked and when the offspring was released into nature, a marked difference in offspring surviving to sexual maturity was registered for different parents. This was partially due to differences in number of eggs produced during each mating, and partially to varying survival of offspring, especially after release into nature.

Of 13 potential parent animals, only nine produced offspring. The effective population size for these nine parents has been calculated by a formula not given here. The result is an effective parent population of 3.27-3.71. If this is divided by the census population size of N = 13, we obtain N_e/N = 0.25-0.29. This represents the reduction of N_e due to differential contribution to the next generation. It must be noted that the conditions in this study are special, with matings forced by the experimenter in order to allow as many individuals as possible to become parents, and with the offspring spending the first part of their life in captivity. Still, the differences surviving offspring are impressive and probably not much smaller than they would be in a fully natural population.

6.1.4. Effective population size: general considerations

Often, the census population size N of a *Bombina* population is known with fairly good or very good precision, but how much smaller N_e is unknown. Biological literature have estimates of the ratio N_e/N ranging from 0.01 to 0.9 in the few studies dealing with anurans; in other words, a range from almost 0 to almost 1. That is not very informative. A general survey of many animal species reports a median of $N_e/N = 0.08$, i.e. N_e is generally much lower than N. However, this was mainly because populations fluctuate widely from year to year. When the effect of variation over time was excluded, the result within a generation was a median of $N_e/N = 0.38$.

In most cases, *Bombina* populations have been monitored over many years, and it is thus possible to calculate the reduction in N_e due to variation over time directly. This makes up the larger part of the reduction and knowing that reduces the overall uncertainty of N_e/N. The remaining effect due to differences in number of offspring between individuals within a generation is unknown. Considering that the median value for many animals is 0.38, and that the value from Enø is 0.25-0.29, it may be fair to assume a general value of at least 0.25 for *Bombina bombina*.

This means that in order to fulfil the criterion $N_e = 500$, the census population size in one generation must be at least 500/0.25 = 2,000. This must also be the harmonic mean over many generations.

Returning to the example from Hjortø (section 6.1.2), the harmonic mean over the generations was 12. Multiplying this with $N_e/N = 0.25$, equals $N_e = 3$. According to this, the population would become unhealthy after three generations rather than 12. Indeed, signs of severe inbreeding were registered in the offspring reared in captivity.

6.1.5. Measuring genetic variation: sampling

When investigating the genetic composition of a population, it is usually considered appropriate with a sample size of 30 individuals, but smaller samples, down to about a dozen individuals, may also be valuable. It is important that the sample is random, and that sampling of closely related individuals is avoided. For instance, sampling of newly hatched larvae originating from one or a few egg clutches should be avoided. All life stages, except newly spawned eggs, may be used.

When sampling tadpoles, 30 freely swimming tadpoles are caught and the tail tips are cut off with a pair of scissors (the tadpole tolerates this without problems). Each tail tip is placed in a sample vial labelled with place, date and individual number.

When sampling adult animals, the most lenient method is to take buccal swabs, i.e. mucus samples from the mouth cavity. The mouth of the toad is forced open, and a clean cotton swab is inserted in the mouth. This is most easily done using small cotton swabs with a small diameter. The swab is moved around in the mouth until sufficient amounts of mucus adhere to it. The stick is kept as a sample. This is unpleasant to the animal, but not damaging. In some cases it may be difficult to get enough material.

An alternative method, which ensures sufficient organic material, is to cut the tip of a toe. This has the disadvantage that the animal is hurt, and although usually no lasting damage is afflicted to the animal, the wound may become infected. To avoid that, hygiene must be as good as possible in the field. The scissors should be disinfected with alcohol at short intervals.

The sample is placed in a vial containing a preserving liquid. This could be ethanol (95%) or a mixture of water and dimethyl sulfoxide. The samples are stored cool (e.g. in refrigerator) until used.

Taking genetic samples.

6.1.6. Measuring genetic variation: results

The most informative part of the DNA for studying population size effects is microsatellites. This nuclear DNA has a high mutation rate and hence much variation. Microsatellites are believed to be unimportant to the fitness of the animal, i.e. they are selectively neutral. However, when the genetic variation in microsatellites declines, the genetic variation in fitness-related genes is also expected to decline.

The degree of variation is usually expressed as 'expected heterozygosity', abbreviated H_e. This is an average calculated from the frequency of alleles at all studied microsatellite loci.

As expected, the analyses of *Bombina bombina* performed by the Potsdam group (section 2.3.2) showed that the east European populations which are part of a large, coherent distribution (eastern Germany, eastern Poland and Ukraine) had the highest microsatellite heterozygosity. Here H_e values were around 0.7. Lower values were expected in the smaller populations near the distribution border. However, the population in Lauenburg (Schleswig-Holstein) had a similar value, indicating that it may be in contact with populations further east in Mecklenburg. Most populations in Schleswig-Holstein had slightly reduced values (in the range 0.5-0.6). Most populations in and around Sjælland (Denmark) had values around 0.5, except for the population on the island Nekselø which had a value of only 0.35. Populations in the Fyn region had values in the range 0.3-0.45. The small populations in Latvia at the extreme northern distribution border had small values as well (around 0.45). Thus, compared with more intact populations in eastern Europe and Lauenburg, populations near the distribution limit have lost up to half of the genetic variation.

If inbreeding is severe, the loss is even larger. The former extremely small population on Hjortø has a heterozygosity of only 0.25. That is, compared with other weakened populations in the Fyn region, the Hjortø population has lost nearly half its variation.

6.1.7. The relations between genetic variation and fitness

Populations with reduced genetic variation often have reduced fitness even though this is not always the case. Fitness has not been recorded directly in any *Bombina* population. Reduced fitness may appear as less intense breeding activity, smaller clutch size, or slightly reduced survival rates in any life stages. Such effects may slow down population growth. For instance, if an artificial population is created by introducing equal number of animals from two different populations, the animals originating from the population with high genetic diversity may proliferate more than the others and thus contribute more to the future genetic composition of the mixed population.

Direct competition

In Scania all populations are created artificially by introduction from more than one source population. In most cases, the genetic composition of a mixed population is much skewed, with much greater contributions from some source populations than others. Two of the analysed populations, Mölle and Fredriksberg, are affected by illegal introduction of specimens from the Pannonic region (Austria). The frequency of alleles of Pannonic origin is surprisingly high, much higher than expected from the assumed original number of illegally introduced specimens. It seems that animals with Pannonic alleles have a higher fitness than those with Danish alleles. This is understandable, considering that Pannonic specimens have higher genetic variation. There is no evidence that Danish specimens have any adaptive advantages to the local climate.

At Skogshuset in Scania, equal numbers of specimens have been introduced from three populations on Sjælland. Almost all alleles in the present population originate from one population, viz. that at Nekselø.

At Bäckhalladalen in eastern Scania, specimens from three populations in the Fyn region have been released. Microsatellite analyses indicate clearly that the dominating genetic origin seems to be Avernakø, with only a small contribution from Tårup Strand.

Thus, evidence from Scania suggests that if different genotypes compete directly, some are more competitive than others.

Danish introduced populations

Attempts have been made to establish at least one mirror population for all Danish populations by introducing reared offspring to a new site. The results have been very varied, and the variations may be related to differences in fitness.

The best results included offspring from Nekselø, which were released at Røsnæs. The first cohorts released at the new site had an astonishingly high survival rate over the first two years (nearly 100% survival), and later on, the new population has demonstrated an unusual ability to disperse to new localities. This suggests that the Nekselø population has a high overall fitness.

Results from other mirror populations are less positive. A few have failed altogether. However, this was probably due to bird predation rather than low fitness, so conclusions are not easily drawn.

On Ærø, offspring have been released from at least two sources: Hjortø and Avernakø. The introductions were made before the Hjortø population became severely inbred. But even so, the genetic composition of the present mixed population indicates a higher proportion of Avernakø alleles. This suggests higher competitive ability of Avernakø animals compared with Hjortø animals.

Survival of very small populations
Some *Bombina* populations were diminutive in numbers before they were rescued.

The Hjortø population represents the worst case. In 2000 only four animals, two of each sex, remained. Offspring was obtained from all four individuals, and reared in captivity for several generations. When the first captive generation started breeding severe problems occurred. In some clutches, nearly 100% of the eggs or tadpoles died. This is very likely due to inbreeding. Animals with detrimental alleles died, and those surviving must have had sounder alleles. This sorting process was repeated over several generations and the detrimental alleles were gradually 'purged' from the population, eventually resulting in rather healthy animals. The population was thus not doomed by genetic causes, but genetic effects were very severe at a certain stage. The present generations are doing fairly well in captivity and apparently also in nature.

The population on Enø, originating from only seven to nine parent animals, is the second smallest. One of these animals had a rare chromosome number aberration and produced very few viable offspring. Survival of released animals from other parents has not been particularly high either. The population has grown slowly during 15 years, but then declined drastically, and breeding activity has been low. Establishment of a mirror population has failed completely. No definite conclusions can be drawn, but fitness seems to be low.

The population on Fehmarn originates from approximately 10 parent animals. Rearing has been unproblematic, with nearly 100% survival during the tadpole stage, but when released into nature, the reared animals had a low survival rate, maybe partially because of suboptimal habitats. Survival of later generations seems to have improved.

The population on Avernakø counted about 13 animals at its lowest, but in addition some individuals from this genetic group survived at Korshavn nearby and they have later been released on Avernakø. There are no particular indicators of weakness in the population. Survival of released toadlets has been low at some sites, but fair at other sites. As stated above, those released at Bäckhalladalen in Sweden have faired better than animals from other localities released at the same site.

Overall trend
The expected relationships between genetic bottlenecks and fitness are not very clear. The population from Hjortø did have a very severe inbreeding depression in the first captive generations before detrimental alleles were purged out of the population. Other small populations have shown few or no signs of inbreeding depression during captive rearing, but in most cases they have not done very well when released into nature.

No clear relationship between genetic variation (microsatellite heterozygosity), population size and fitness exist. For instance, the genetic variation in the Enø population is as high as in any Danish population, but the population has been very small, and fitness appears to be low. On the other hand, even though genetic variation is relatively low in the Nekselø population this population has remained fairly large and fitness seems to be high; reared toads from this population perform surprisingly well when released in nature.

Thus, in general, no close link between population size, genetic variation and fitness exist. However, there is a weak general tendency toward a relationship; in particular it seems to be difficult to make a population thrive after it has been very small.

6.1.8. The relationship between MHC genes and fitness

The Potsdam group has studied genetic variation in both neutral genetic markers and in the MHC genes (Major Histocompatibility Complex). The MHC genes code immunity system proteins and thus reflect part of the ability to fight pathogens, i.e. potentially, the genes are related to the animal's chance of survival. High genetic variation in MHC should indicate ability to fight a wide spectrum of pathogens.

Including MHC in the genetic analyses is important, because the results for genes of adaptive value can be different from results for neutral genes. The Danish and Latvian populations with reduced variation in neutral markers (microsatellites) have little or no reduction in variability of MHC genes compared with the large connected populations in east-central Europe. This implies that a selection pressure acts against loss of rare MHC alleles and this again suggests that these genes are invaluable to the animal that carries them. In cases where a population suddenly crashes within a few generations (like on Hjortø), selection cannot preserve all MHC alleles. However, if selection has sufficient time, e.g. if a population decreases slowly over several generations, considerable MHC variation is preserved.

On the other hand, no relationship has been found between recorded MHC variation and fitness. The Danish population at Nekselø has very little MHC genetic variation; the level is as low as in the inbred Hjortø population even though Nekselø is the most successful Danish population in recent years. The Danish population at Enø has an unexpectedly high MHC genetic variation; the level is comparable to the largest populations in Germany and Poland even though this population has been unsuccessful.

6.1.9. Do different clutches have different fitness?

Artificial rearing of offspring from Enø has yielded interesting information about different clutches that do not survive equally well. Individuals were kept in captivity for one year and released as 1-year-olds. When the animals were captured later on, they could be identified individually. Number of recapture of different individuals after one or two hibernations in nature was registered.

Survival was analysed in relation to all factors of interest, but the only important factor turned out to be ancestry. Animals with different parents differed in their survival rate. The differences were significant after the first and highly significant after the second year.

The longest surviving clutches had mothers or fathers who were known to live for a long time (c. 10 years). It seems likely that they had passed on some genes contributing to longevity. However, these parents had a less active breeding behaviour. On the other hand, one particularly active male produced the least viable offspring. Thus a high survival rate is not necessarily a good measure of fitness, because there may be a trade-off between survival and breeding activity.

6.1.10. The importance of local mixing

The Enø population was saved by artificial rearing from only seven or nine parent animals. Despite this low number of ancestors, the genetic variation in the population was high when sampled approximately 15 years after the first rearing. The microsatellite heterozygosity was as high as any Danish population, and the variation in MHC alleles was as high as in large coherent populations in east-central Europe. Preservation of so much genetic variation must be explained by the fact that the ancestors originated from three widely separated ponds. Genetic drift may have caused different alleles to survive in each pond and when the gene pools were combined much of the former genetic variation must have been restored.

The present population on Ærø is a mixed population originating from at least two sources; Avernakø and Hjortø before the latter population became severely inbred. The microsatellite heterozygosity on Ærø is as high as any population from the Fyn region and a few alleles that otherwise went extinct probably survived here. The total MHC allele variation is considerably higher than expected merely from mixing the source populations. Genetic variation lost in other populations, has survived to some extent in the current mixed Ærø population. This could mean that individuals from Ærø with a high MHC allele variation have had a better survival rate and have contributed more offspring. These alleles must have originated somewhere, and the source must be the gene pool on Hjortø that existed prior to loss by inbreeding. In other words, transfer of genetic material from Hjortø to Ærø has had some importance. The result is better than if the population had been founded by material from Avernakø only.

6.1.11. Conclusions

- It is important that population sizes are high. Populations passing through bottlenecks lose considerable genetic variation. Populations should never decline to less than 200 adults (of approximately equal sex ratio), and on a long term should consist of at least 2,000 adults.

• If a population becomes small, it is important to know that subpopulations in widely separated ponds may have preserved different alleles. The original genetic variation is best preserved by obtaining offspring from each of the isolated ponds, rather than getting offspring from just the largest or most easily accessible pond. If no artificial rearing is carried out, it is important that gene flow barriers between separate ponds are reduced enabling all subpopulations to contribute genetically to the future population.

• Individuals within a population may differ in genes that influence survival.

• Selection pressure seems to maintain a high variation in the MHC genes compared with neutral genetic variation, implying that individuals with high MHC variation may have higher fitness. However, no clear relationship between MHC variation and our measures of how well the population fares can be documented.

6.2. Population size

6.2.1. The number of males as an indicator of population size

In small populations it is possible to estimate the population size with a reasonable degree of precision by the capture-recapture method. However, when dealing with populations approaching the required size, e.g. populations of 1,000 animals or more, it is necessary to use a more superficial method. Usually, the calling males are counted.

The counting of calling males gives fairly reliable results when the number of adults in a pond does not exceed 200. This corresponds to about 30 calling males in the pond. If more than 200 adults are gathered in one single water body, the actual proportion of calling males is unknown. In cases like that, the number of calling males only gives us a very imprecise indication of population size.

6.2.2. The pond area necessary to maintain large populations

The density of firebellied toads in breeding ponds is important. Rather precise capture-recapture population estimates have been made in some good breeding ponds in Denmark. In each pond, the observed maximal density of adult toads varied from 3.3 to 13 m^2/individual. The average density for small breeding ponds under optimal conditions may be set to about 5 m^2 per individual.

High densities may also exist in larger areas. The largest *Bombina* population in Denmark inhabits a pond of 5900 m^2. The estimated population is about 500 adults; this yields 12 m^2/adult.

Data from Scania indicate 50-60 calling males in ponds of 1000-3500 m^2, > 100 calling males in a pond/lake of 6000 m^2, and > 300 calling males in a lake of 20,000 m^2.

In these cases, only the number of calling males is known, and the true number of adults can only be inferred indirectly. Of course these are the maximal densities in Scania. Most of the approximately 100 breeding ponds have lower or much lower densities.

Suboptimal conditions will lower the density. One example is a large brackish swamp on Knudshoved, Denmark, with a population density of about 25 m^2/adult.

Out of breeding season many animals will migrate to other nearby ponds to feed, and the population may be distributed over a larger area. On the other hand, they may also concentrate in small, eutrophic foraging ponds at extreme densities, sometimes up to a density of 1 m^2/adult.

It may be more relevant to study how much area juveniles need than how much adults need. This point can be illustrated by data from a pond on Knudshoved in Denmark. According to capture-recapture studies approximately 160 one-year-olds were present in 2000. In subsequent years, the rate of mortality was low, but growth rate was slow. Even though firebellied toads usually are sexually mature after two years, these were not mature until the age of three. This may indicate that the density was too high, and that food was limiting. Half of these animals stayed in the breeding pond, while the rest gradually migrated to other nearby localities. The breeding pond now had a density of 5 m^2 per juvenile, and including those migrating to larger neighbouring ponds the average density was about 10 m^2 per juvenile. The conclusion is that this area is too small to allow optimal growth. The necessary area may arbitrarily be set at five times the area required for breeding adults, i.e. at 25 m^2 per animal.

A complete evaluation of a pond system's carrying capacity may be done as follows. All ponds are classified as belonging to one of five possible groups, group I, II, III, IV and V, respectively. Evidently optimal breeding ponds are classified as type I. Fairly good breeding ponds, but not completely optimal, are in group II. Suboptimal ponds are in group III, and ponds that may occasionally produce a few metamorphs are in group IV. Ponds without breeding success, e.g. ponds with fish, belong in group V. A similar classification is made for each pond according to its value as foraging habitat. Here, fish ponds may get higher ranks.

Next, the carrying capacity for each type of pond is defined. The capacity for optimal ponds is based on evidence, such as presented above. The capacity for the poorer categories is a certain fraction of that for optimal categories, and this fraction may be chosen arbitrarily (in agreement with the definition of each group). A set of possible values is given in Table 6.1.

Consider the situation in and around the pond referred to above on Knudshoved. This and the neighbouring ponds are placed in relevant quality classes, and their areas are measured. According to this, the total carrying capacity for *Bombina bombina* in this pond system is calculated. See Table 6.2.

Table 6.1. Carrying capacity for ponds in five different quality classes with number of adults or animals per m^2.

Quality class	Breeding ponds	Foraging ponds
I	0.2	0.05
II	0.05	0.02
III	0.01	0.005
IV	0.002	0.001
V	0	0

If these calculations are realistic, a pond system may on average accommodate 138 adults over the years if the breeding site capacity is considered or 55 adults if foraging capacity is taken into account. According to this, the foraging capacity is the limiting factor for the population, and the population can only grow if the foraging area is improved or enlarged. Improvement of breeding ponds, on the other hand, is not expected to increase population.

A habitat restoration may be planned in the same way. The planned ponds are classified according to presumed future values for breeding and foraging. The planned pond areas are calculated as above and the numbers will indicate the realistic *Bombina* population size sustained by the planned habitat.

6.2.3. Other minimum habitat requirements

The firebellied toads do not stay in ponds all year, thus the surroundings must also be considered. The surroundings serve several functions:

Table 6.2. Calculation of the carrying capacity of a system of ponds at Knudshoved in Denmark. Two capacities are given; one for breeding activity and one for foraging.

Pond no.	Area m^2	Foraging class	Breeding class	Carrying capacity for foraging	Carrying capacity for breeding	Recorded no. of adults over the years
1	300	IV	V	0.3	0	
2	600	II	V	12	0	
4		I	I	22.5	90	
5a	650	II	II	13.2	35	
5b	1500	III	III	27.5	15	
Total for that pond system				55.5	138	8-180

- The animals may feed there, especially in late summer.
- They hibernate there.
- The animals pass through the surroundings during their yearly migrations.
- They pass through them when they move from one pond to another.
- They pass through them when they disperse from one pond system to another.

Certain types of landscape – especially large cultivated fields – pose relatively high risks for migrating animals. The animals are exposed to less risks if the time spent migrating in these landscapes can be minimised. Therefore, the position of the different centres of activity is important. For instance, ponds positioned closely together means that animals only have to spend a short time migrating through the landscape, and hibernation sites positioned close to the breeding pond will minimise the yearly migrations. If the hibernation site is situated right next to the pond edge, the character of the surrounding landscape may be virtually unimportant to a large fraction of a population migrating only between hibernation and breeding sites.

However, whenever a population counts less than the required size of 1,000-2,000 adults, it must be possible for at least a few roving individuals to disperse to and from neighbouring populations. Thus, dispersal corridors are essential in a suitable landscape.

Habitat requirements are fulfilled if the animals can make the necessary movements – migrations and dispersal – without having to cross unsuitable habitats.

6.3. Population management strategies

6.3.1. Evaluation of the situation

When evaluating a population's management needs, the first question is: Is the population part of a larger coherent metapopulation extending over a large area, or is it an isolated population?

If the population in question is part of a large population, the risk of local catastrophes is less important because new individuals will immigrate from outside. Management will concentrate on *improvements of the habitat*, in order to offer immigrating individuals good conditions and create a strong population by mixing surviving individuals from the local, threatened populations with the immigrants.

If the population in question is isolated – i.e. if immigration from other populations is unlikely – a local catastrophe cannot be tolerated. The next question is: How do we prevent this isolated population from dying out? This depends on the situation.

A population with effective population size N_e above 500, i.e. the census population size N is above 2,000, is sufficiently big. However, since the population is isolated, there is a risk of being hit by a catastrophe in the future, and if that happens, the species will not be able to re-immigrate. To prevent this scenario, a *mirror population* may be created in a separate locality. To be exact, a good habitat is provided elsewhere and reared offspring from the original population is transferred to the new site in sufficient numbers to secure practically all genetic variation. If a catastrophe, e.g. a contagious disease, hits the original population, the genetic material has survived in the mirror population. The original population can be restored by transferring offspring from the mirror population back to the original site.

A population with effective population size N_e below 500, i.e. the census population size N is below 2,000, is too small. It is necessary to *enlarge or improve the habitat* to increase the carrying capacity. If space in the actual locality is insufficient, it is possible to expand by creating a mirror population as described above.

A population with effective population size N_e below 50, i.e. the census population size N is below 200, is smaller than can be tolerated even on a short term. The population must be enlarged as fast as possible. If relevant *improvement and enlargement of the habitat* can be carried out immediately, this may be sufficient to remedy the situation. However, if the present habitat quality is low and cannot be improved fast, breeding success will be low or absent. Insufficient breeding success may be sustained by *supportive breeding in-situ*. This only makes sense if survival possibilities are sufficient. *Artificial rearing, i.e. supportive breeding ex-situ* may be initiated if breeding success is absent in order to produce new generations until the habitats can be improved. If the prospects on the site are very bad and suitable conditions cannot be achieved within a few years, *captive breeding* may be necessary. The surviving animals are caught and bred in captivity, possibly for several generations until suitable habitats have been provided.

If a population has experienced a bottleneck in the past and is genetically impoverished, the possibility of *mixing animals from two or more populations* in order to increase the genetic variation may be considered. However, it is important to realise that such mixing may do more harm than good and it should be considered very carefully before it is implemented.

6.3.2. Principles of artificial rearing

Supportive breeding can be done in-situ or ex-situ.

In-situ breeding usually takes place in breeding cages (see section 6.4.1.3) placed directly in the ponds. Eggs are provided as described below and placed in the cages. The eggs can develop with a low predation pressure and hence a high survival rate. When the tadpoles metamorphose, they are released.

The eggs can be transported to a rearing station and reared *ex-situ* if the water quality in

Net cages for mating placed in the shallow parts of a pond.

the pond is less than optimal. After metamorphosis, the brood is returned to the ponds. Given an experienced staff, good rearing conditions and sufficient water quality, ex-situ is usually a less risky procedure.

It is not necessary to release the brood immediately after metamorphosis. Sometimes, release of some or all of the large tadpoles can be successful and sometimes, metamorphosed toadlets may be kept for several weeks or months and fed with insects to increase their physical condition and survival possibilities.

In *captive rearing*, adults are caught and kept in captivity for the rest of their lives or until the habitat has been improved. The animals are coerced to breed in captivity. This requires much experience and success is not guaranteed.

A *mirror population* is created by ex-situ rearing of offspring from the original locality and release of the resulting toadlets in the new locality. Offspring should be obtained from as many different parent animals as possible. The number of released toadlets must be at least several hundred and preferably several thousand, and the process (rearing and release) should be carried out over several years.

If a population is or has been small and has reduced genetic variation, the question arises if it should be attempted to increase the fitness by *mixing*, i.e. introducing animals from other localities. This is not generally recommended. If the small, inbred population has low fitness and the introduced animals have a higher fitness, animals descending from the inbred population will likely be outcompeted and almost all genetic material from that population will disappear. The net result will be loss of genetic variation. Mixing may be considered useful if the original population is large (after restoration) and only few foreign animals are introduced. Another situation where mixing can be useful, is when two populations are small and inbred. These may mutually increase each other's fitness by mixing the gene pools. A few generations after mixing, fitness may have increased somewhat. Then, it is possible to introduce a few foreign specimens from a population with relatively high fitness. In any case, the choice will be a trade-off between preserving rare genes and boosting fitness to a level where the population will actually survive on a long term.

6.4. Population management techniques

6.4.1. Providing eggs

6.4.1.1. Initial remarks

Collection of eggs in nature does not necessarily result in lowering the natural breeding success in the source population as long as more than 10% of the collected eggs are released in the original pond at later stages. During the LIFE project we observed that many egg clutches perished in the original pond, whereas eggs brought to the breeding station developed healthily.

6.4.1.2. Gathering eggs in nature

Eggs may be gathered directly in the ponds. In large populations, eggs can be abundant and many eggs can be collected.

Collected *Bombina* eggs.

Time

Eggs are usually spawned from early May until late June; sometimes even later (mid or late July). The peak period is often the last 10 days in May. Since the eggs may hatch after just 3-4 days in warm weather, the ponds should be searched twice a week.

Searching for eggs in a pond.

A lot of trouble may be avoided by omitting search when the ponds are brimming with *Rana esculenta* eggs. These are often spawned during the last five days in May, so the optimal period for collecting *Bombina* eggs is often May 20th-25th.

Weather

Spawning usually occurs in periods with warm, sunny weather following a period with precipitation. If periods with dry sunny weather are prolonged, especially if it is windy, spawning will be sparse or absent. Eggs may be spawned in overcast, rainy weather, provided that the pond has already been heated during a preceding period with sunny weather.

Weather during searching

Eggs are best discovered when the sun is shining at low angle into the pond. During rain or when the skies are grey it is extremely difficult to discover eggs below the reflecting water surface. It may be necessary to use a hydroscope or to shade the water surface.

Where to search

It is important to know the microhabitats where eggs usually are deposited. The following parameters are important:

- Water depth more than 20 cm.
- Presence of vertical, clean structures where the female can attach the clutch (e.g. stems of *Potamogeton spp*., *Juncus spp.*, *Eleocharis spp.*).
- Not too dense vegetation around the egg-clutch.
- Oxygen-rich water.
- Sun exposure.

However, the places where *Bombina* lays their eggs vary from pond to pond and from time to time. In some cases, eggs have been collected at water-depths approaching one meter; in others, in very shallow water close to the pond edge. Some have been collected in very dense vegetation or in mats of horizontal straws of *Batrachium spp*.

Recognizing the eggs

Rana esculenta eggs are extremely variable from clutch to clutch and may often be very similar to *Bombina* eggs. See section 2.2.5.

How to gather eggs

- Carry a bucket or plastic bag for the eggs.
- Walk slowly and carefully along the favourable spawning microhabitats, not stirring up sediment and mud (in ponds with muddy or clayish bottom a boat is useful).
- Look for eggs below the water surface on the clean and fresh plant stems and sprouts.
- Be very observant in microhabitats with calling males.
- If the vegetation is dense, suitable patches with open water can be created prior to the egg-laying period. Some female toads will choose these artificial sites for depositing their eggs.
- Later in the year the eggs may be laid in very deep water (e.g. on a floating island in the pond centre). This can be reached by boat.
- Once an egg clump has been located, search for additional clumps nearby, because one clutch usually consists of several clumps deposited within a few meters.
- Cut off the stem/plant to which the eggs are attached to avoid turbidity; the clump will probably float to the surface where it can be collected.

Transport

For safe transport of eggs:

- Use as much fresh pond-water per egg as possible (ideally one clump per two litre and no less than 10 litres per 500 eggs).
- Add vegetation, such as moss or *Batrachium*, to the bucket to prevent destruction of the clumps.
- Fill bags or buckets completely so eggs are not banged against the walls of the bucket during transport. Buckets are closed with lids.
- Maintain high levels of dissolved oxygen

Lars Briggs is catching *Bombina.*

and medium temperature during transport. 50 eggs per litre can be maintained for 3h at 20°C without additional oxygen if the water is clear.

- If the weather is hot and transport is long, it may be better to place the eggs in plastic bags halfway filled with air, in order to secure oxygen supply. The plastic bags are packed in something soft to prevent the eggs from being shaken.

6.4.1.3. Mating techniques

An efficient way to obtain the maximum amount of eggs from small populations or from inaccessible sites is to let the animals mate under controlled conditions.

When to catch animals
Females should be caught as soon as they arrive in the breeding pond, or shortly after. Early in the season females usually stay at the pond's edge or in shallow areas with high water temperature and abundant food. When they are ready to spawn, they move toward the centre of the pond, which makes them more difficult to catch. Once a female is ready for spawning, it is usually rather easy to catch a male – males are visible when calling.

Which animals to match
Basically, the female should be fat and in good condition – fatness indicates likelihood for mature eggs ready to be spawned.

The water surface may be scrutinized around the strongest calling males; a passive female dwelling within 1-2 meters from the male strongly indicates that she is willing to spawn. Males can be small or large – what is important is that he is calling eagerly.

Mating in plastic buckets
Animals can be mated in a plastic bucket, typically a 10 L bucket with c. 5 cm water at the bottom and nothing else. The female is placed in the bucket. An eagerly calling male is selected and caught while calling. It is placed directly in the bucket and the lid is closed. The low water depth means that the female cannot escape the male, and the animals will usually have gone into amplexus within 10-15 minutes. If the male is rather inactive, the bucket can be shaken gently bringing the animals in closer contact with each other.

Adult *Bombina* waiting to be placed in breeding cages.

Mating in net cages
In situ breeding in net cages placed in ponds has been used successfully during the LIFE project. The cages are made from water-resistant plastic with a fine mesh, avoiding intrusion by large swimming predators. The dimensions of the cages are 1.4 m × 1.4 m bottom area and 1.0 m height. All cages must be covered with a net at all times to prevent bird predation.

Cages can be used either as foraging cages or breeding cages.

Foraging cages contain animals which at present are not ready for mating. They should be kept captive since it may be very hard to catch them again later. Foraging cages are equipped so that plenty of food is attracted to the cage giving the amphibians the opportunity to feed and grow.

Breeding cages should be placed in 40-60 cm deep water. The cages should be provided with vertical structures where the female can attach the eggs. Fresh sprouts and stems of plants are functional; especially stems of *Juncus* have proved very useful because the pointed tips easily fit through the mesh in the side of the cage. Floating vegetation should be present inside the cage to provide the animals with resting and hiding places and to attract food. One or two males can be placed together with several females in one cage, while some eagerly calling males remain free outside the cage; these will stimulate the male(s) inside the cage to call. The animals can easily be left in the cage for up to one week, sometimes longer. If weather conditions are right during that time,

A male approaches a female inside a net-cage, trying to enter amplexus.

mating and spawning is likely to occur.

Females likely to spawn soon must not be kept alone in a cage since there is a risk they might spawn alone without fertilization.

After some days, captive males are typically swapped with free males. In this way, males in mood for mating are always present in the cages.

Cages are checked for eggs twice a week by inspecting the water plants.

If weather conditions are unsuitable for spawning or males loose their interest in mating, they should be released.

Treatment of pairs in amplexus

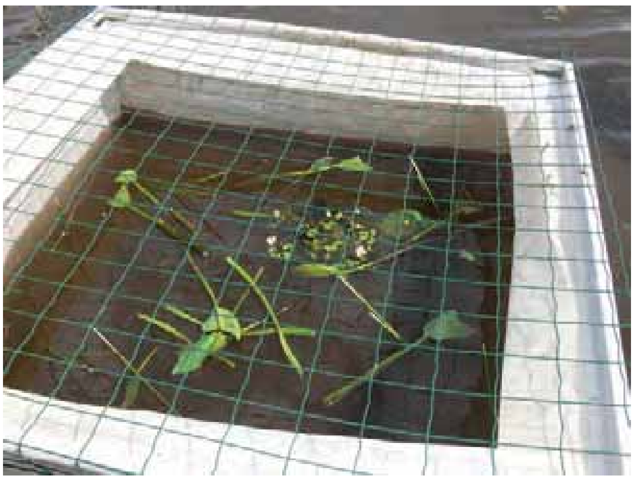
A newly formed amplexus-pair will usually produce eggs during the first night, usually right after midnight. If they remain together after the first night, they may spawn again the following night.

If an amplexus-pair is observed swimming in the pond, they may be caught with a dipnet. A bucket is held under the net, so the animals remain swimming. The net and bucket are carefully carried to the net cage, where the pair is released and can spawn under controlled conditions.

If a pair has been formed by placing the animals in a plastic bucket, they may gently be placed in a net cage.

Another option is to place the pair in an indoor aquarium with heating. 25 L aquariums have been used with success, but larger aquariums are better. The aquarium is filled 80 to

A 1000 litre container for tadpoles equipped with oxygen supply, UV and water filter.



The cages are equipped with a cover to prevent bird predation and vegetation for the toads.

90% with water. Suitable aquatic plants are fixed to the bottom with stones. A heating body set at about 25°C is placed in the aquarium. The amplexus-pair is placed very carefully in the aquarium taking care that the male does not loosen its grasp. The warm water will stimulate the female to start laying eggs. The aquarium most be covered with a tightly fitted lid, because the animals, especially the male, will try to leave the aquarium after spawning.

6.4.1.4. Handling the eggs

Egg clumps, attached to a plant stem, are transported as described above to the rearing station. Here aquariums or tanks meant for receiving the eggs are ready. Tap water (without chlorine), pond water, or a mixture of the two can be used. The water temperature must be

Juncus straws are fixed inside the cage to provide suitable substrates for egg deposition.

approximately the same as the eggs, since eggs do not tolerate fast temperature changes. Often, a tank is filled with cold tap water a day in advance temperate it. This may cause many small air bubbles to attach to the sides of the tank. Small air bubbles may be a danger to newly hatched larvae if they swallow them. The bubbles must therefore be removed before the larvae hatch.

Often, it is advantageous to introduce many different organisms from the pond, so it is recommended to bring extra buckets of water from the pond.

The eggs can neither rest on the bottom nor float in the surface; in an outdoor tank floating eggs may be damaged by UV radiation.

The water is aerated with an aquarium pump, tube and air stone. The flow of air must be slow and gentle.

The water temperature should preferably be between 20 and 25°C and at least not below 18°C. The water may be heated by heating bodies controlled by thermostats.

Dead eggs, especially is they are infected by moulds, are removed as soon as possible to minimise the risk of infecting other eggs or larvae.

When the eggs hatch, the larvae may remain in the same tank or they may be transferred to another tank. The latter option has two advantages: 1) the larvae can be counted; 2) it is possible to make sure that no predators, such as small dytiscid larvae, are in the same tank as the larvae. Larvae are transferred by sucking them into a plastic tube.

The tadpoles may be reared either in an indoor rearing station or in net cages in the pond.

6.4.1.5. Rearing tadpoles in rearing stations

Volume and temperature

The rearing tanks must contain at least 1 L of water per tadpole; when the tadpoles grow 2 litres per tadpole should be provided. If some larvae from the same clutch grow faster than others, the density of tadpoles per water volume has to be reduced.

Fresh tap water should never be used. Water must stand at least 24h in order to get rid of air-bubbles and to temperate it. Pond water is often preferred, because it contains algae and other microorganisms that will grow on the tank sides. The tadpoles can feed on these.

Additional heating can accelerate the larval

The water intake of the filter must be tadpole proof.

development. Temperatures should never exceed 32°C and water temperatures should fluctuate.

Distribution of tadpoles in many small tanks is preferable to a few large tanks since this will minimise the risks, e.g. a contagious disease that infects all tadpoles.

The tanks are aerated by pumping a slow stream of air bubbles through the water.

Feeding

Newly hatched larvae do not feed; they attach to a surface and hang there quietly. If food is given at this stage, it will not be eaten but rather rot. After some days the larvae start to move with small jerky movements, searching different surfaces. This indicates that they have started to feed, and food must be provided. They can be fed with commercially available food for aquarium fish which is relatively rich in protein, for instance TetraRubin®, Tetra TabiMin® and/or *Spirulina*. The food is crushed or ground to a fine powder and distributed evenly. If food is concentrated in one place, the larvae may fail to find it. Growth of algae on the sides of the tank and/or on water plants in the tank will guarantee a steady food supply.

Small amounts of food should be provided often to avoid accumulation of uneaten food that potentially can be infected with mould.

Cleaning

Various methods deal with faeces and surplus food and preventing turbid and rotten water.

Material can be sucked up from the bottom of the container using a fine silicon-hose (9 mm Ø) carefully removing the water. The removed water has to be replaced, but only approximately ⅔ of the water should be replaced at once. Filamentous algae growing in the container must be removed since they are indigestible to the tadpoles. It is useful to keep daphnia in the containers because they are good at keeping the water clean.

Another method is to install a gravel filter which may be bought in aquarium shops. Water is sucked through a layer of fine, washed gravel, organic matter are trapped here and decomposed by bacteria. Usually, good water quality can be maintained for more than a month without having to change the water. This method is therefore less time consuming and suitable if the aquariums cannot be checked every single day. A drawback is that zooplankton (*Daphnia* or *Ostracoda*) may become so numerous that they damage the tadpoles by eating from their tail fins. If this is the case, water must be replaced more frequently, or *Triturus* larvae can be introduced to control the amount of zooplankton.

The water can also be cleaned by pumping it through some kind of filter outside of the tank. The inlet tube must be covered by a fine-meshed net to prevent tadpoles from being sucked into the filter. The water passes up through a tower containing a filtrating material, e.g. glass wool or glass wool mixed with charcoal. An alternative is to sterilize the water using UV radiation. Afterward, the water is pumped back into the tank in a jet whereby air is stirred into the water. The advantage of such an external filter is that the tadpoles' mutual growth inhibition is removed enabling the tadpoles to be kept at a higher density than otherwise possible.

Irrespective of method, signs of turbidity must be taken seriously. If the water tends to become grey and turbid, it must be replaced at once or within a couple of hours, because this indicates conditions that may suddenly kill all tadpoles.

Predators and diseases

It is important to avoid predators in the tanks. Dytiscid beetles often lay eggs in the same plant stems toad eggs are attached to. Very small beetle larvae will hatch at the same time as the *Bombina* larvae. The small dytiscid larvae are easily overlooked. The tanks must therefore be scrutinised frequently to detect these or any other predators.

One or several tanks may become infected with parasites or diseases. This is usually recognized when dead tadpoles are seen on the bottom. Once this is discovered, it is important to act very fast because diseases spread fast and may kill all tadpoles in one to two days. First, a new tank with fresh water must be prepared and healthily looking tadpoles must be transferred to this tank. Slightly weakened, but not seriously ill, tadpoles should be placed in separate tanks. They are already infected and will infect others if they are not kept separate. Preferably, each weakened tadpole is kept in a small, separate container. Some will recover and may later be returned to the others, but most will die.

The next step is to treat the water in the new tank with antibiotic. Aquarium dealers have chemicals used to combat fish diseases. The kind of disease must be guessed at, for instance by inspecting weakened tadpoles with magnifying glass to detect growth of fungal threads already before death. If so, a fungicide is appropriate. A remedy against bacterial diseases or parasites is employed, if this is thought to be the cause. Usually, the water has to be treated at least twice as described on the package.

Nonetheless, sometimes tadpoles surviving a disease and treatment with antibiotics are damaged. They may be malformed after metamorphosis, for instance with abnormally small legs.

The time around metamorphosis
When the tadpoles have large, thick hind legs, it is time to carefully look for appearance of the front legs. As soon as the tadpoles have entered the four leg stage, they must be able to rest with their head above water, for instance on a floating piece of wood or bark. Soon after, it must be possible enter land; otherwise they will drown. A land part may be created in the tank or the animals may be transferred to a sloping tank where the upper half is dry. Equip the dry area with pieces of bark, moss or other possibilities for the animals to hide.

When the animals have metamorphosed and entered land, they may be released at once, or kept for some time. They are able to feed on insects when approximately half the tail has been resorbed. A good food source during the first few days is collemboles. In some ponds, dense mats of philamentous green algae along the edges have large numbers of collemboles feeding on them in late summer. Some pieces of the algal mats including their population of collemboles may be gathered and given to the newly metamorphosed toadlets. After feeding on these for a few days, they are ready for larger food, first e.g. fruit flies (*Drosophila*) and later very small earth worms cut in pieces. Colonies of tubificid worms can be found on the banks of muddy ponds.

Metamorphosed animals need terrestrial habitat to prevent them from drowning.

6.4.1.6. Rearing tadpoles in net cages in ponds

Preparation
Bombina brood may be reared in net cages placed directly in a pond. The cages should be placed in the water several weeks before the larvae/tadpoles are reared. This period allows development of a film of algae and other microorganisms on the plastic net. The cages must be exposed to sun and placed rather deep in the water, although not so deep that flooding after heavy rain is a risk. Extra weight (e.g. flat stones) should be added to the cages to prevent them from being blown away by strong winds. These stones may also serve to fasten the roots of aquatic plants placed in the cage to offer hiding places and extra foraging surfaces. The aquatic plants must be rinsed thoroughly to


200 litre containers for rearing of fewer animals.

remove as many predators as possible before they are added to a cage. Avoid rearing unless the water quality in the pond has been demonstrated to allow fast growth and healthy development. If the local conditions are unknown, growth has to be monitored in order to act in time and avoid massive losses.

Introduction of Bombina brood
Bombina brood may be introduced as eggs, small larvae, or tadpoles.

Eggs will need extra protection from predators, such as small leeches which find ways to enter the cage even if the mesh is very fine. For protection, the clutches can be kept in specially designed egg-bags with the finest mesh possible. These egg bags can be attached to a corner in the cage. Once the larvae have hatched and grown to a certain size, they can be released in the bigger cage.

Introduction of brood as very small larvae is not recommended, because they may escape out through the net.

Net cages work well for rearing tadpoles. Capacity may be insufficient for all tadpoles at the rearing station. However, when the tadpoles are intermediate in size and demand much space, the surplus can be placed in net cages in the field. Each net cage (described in section 6.4.1.3) may be stocked with 50 to 100 tadpoles, depending on the nutritious value of the microorganism films on the sides.

In contrast to rearing in an artificial station, neither extra food nor oxygen has to be provided, and hygienic problems are avoided.

One of the main problems is predation. It is almost impossible to prevent predators from entering the cages. Therefore, it is recommended that the cages are checked every day.

At almost every new field location where in-situ breeding is applied, unexpected problems occur. Overall the losses per clutch are therefore somewhat higher than in indoor tanks.

Metamorphosis
As soon as the animals start metamorphosing, the cages can be moved from deep water to the pond shoreline. The animals will need more terrestrial habitats as metamorphosis advance. Plants and shelter in the cages attract food and provide the toadlets with refuge and resting places.

6.4.2. Captive populations
Sometimes, the natural habitat may be very unfavourable for the survival of an isolated *Bombina* population. It may be preferable to catch the last survivors and keep them in captivity. The main challenge is to make them breed. However, this is possible. Several extremely endangered populations have survived because the last survivors were kept in captivity and produced offspring there.

One of the institutions serving to keep and breed captive populations is Copenhagen Zoo. The majority of the animals are kept indoors in tanks of various sizes and in aquariums. During some months each winter, temperature is lowered to around 5°C to simulate hibernation.

In order to instigate breeding, it is essential to change the living conditions. For some time, the animals are kept at mildly unpleasant conditions, including very low food supplies, low water level, low temperatures, or no access to the other sex. When conditions suddenly change, for instance when food suddenly is unlimited, the water level rises, the temperature is increased, or the sexes are brought together, the males may start calling. If calling is sufficiently strong and persistent, the females are brought into spawning mood.

The procedure applied by Copenhagen Zoo is typically as follows:

In late March or early April, the toads are removed from the hibernation container where they have spent about four months at 5°C. At first they are placed in aquariums measuring 30 × 60 × 40 cm, and during the following weeks, they are fed intensively. The temperature is increased gradually from 15°C to about 22°C.

Approximately one month after leaving hibernation, the breeding tanks are readied. Each tank is a plastic container of 60 × 120 × 60 cm. The land part consists of a wooden plate sloping toward the water surface. The toads can enter land via plants bending into the water. Water covers 60 × 120 × 35 cm.

As substrates for egg deposition, straws of *Eleocharis* are provided. Blocks of oasis are placed at the bottom of the container and *Eleocharis* straws are easily prodded into these. When the straws get too old, they are simply exchanged.

Early May, the toads are separated; males into one breeding container and females into another. Each container holds five to six individuals. The sudden change from a small aquarium to a much larger tank stimulates the males, and they soon start calling. The keeper refrains from interfering with that behaviour and maintains a high feeding level. In addition, the tanks are frequently sprinkled to simulate rain.

After a while, the sexes are brought together, i.e. half of the males are shifted to the female tank and vice versa. Usually, amplexus will occur within 24 hours and eggs are spawned within a few days.

The eggs are removed from the tanks. This is done easily be cutting the *Eleocharis* straws just below the egg clumps. The egg clumps can be lifted out of the tank without leaving the water by holding a jar below them. They are placed in an aquarium where algae growth on the sides has been initiated several weeks earlier. The tadpoles will feed on these algae after hatching. After an additional couple of days, a supply of air bubbles is installed and extra food is supplied. Every day, one fifth of the water is changed to maintain a good water quality. When the tadpoles are approximately three weeks old, they are moved to 80 litre barrels to increase the water volume per tadpole. More than one litre per tadpole is required. Still, one fifth of the water is changed every day.

Adult toads, which have remained in relatively small aquariums, are now gradually placed in the breeding tanks. For instance, a relatively skinny female in the tanks is replaced with a relatively thick female from the aquariums. The males notice the new female at once and mating will usually occur producing new egg clutches. Sometimes, a male becomes somewhat dull or exhausted, thus he is exchanged with another male. Males can also be stimulated by being swapped from one breeding tank to another.

The mood of the animals can also be influenced by air pressure (i.e. the outside weather) and the sound of a firebellied toad chorus from a recording. From experience, a combination of rising atmospheric pressure, an exchange of a considerable part of the tank water at a single occasion, and the opportunity for toads to meet new individuals, is a good guarantee for spawning.

A private home in Denmark houses another facility for captive populations. A small garden pond is equipped with the ordinary type of plastic net rearing cages. Each cage is provided with air tanks which guarantee that a suddenly rise in water level due to heavy rain will not flood the cages, since the cages rise as well. If the rim of the cages were flooded the captive animals would swim out. The cages are equipped with a piece of floating land or a mat of floating vegetation.

The animals are treated approximately the same way as toads in the zoo. They are removed from hibernation, fed well for some weeks, and then suddenly placed in the open air cages, preferable in a period with warm, sunny weather. This may result in breeding activity, although it may also fail. The animals are then kept for some weeks under poorer conditions, e.g. during a period with cooler weather. A change in weather – a rainy period leading to a perceptible rise of the water level in the cages followed by some days with rising atmospheric temperature – may induce calling and subsequently spawning. If that fails, the animals can be transferred to other cages placed in large circular plastic basins usually used for kids. The basins are filled with cold tap water and the cages placed inside. The animals are placed in the cages in cold water and allowed to experience the gradual warming during the following days. This change may stimulate them to call provided that the weather is sunny and warm or with warm showers.

When eggs are deposited on straws in the cages, they are treated like the eggs in the zoo.

Success is very dependent on weather conditions. A spring with long periods of dry, sunny and windy weather may create problems stimulating the toads to breed that year. If it is vital to get offspring from old animals that probably will not survive another year, a last resort is to inject them with hormones (chorion gonadotropin). The hormone solution is injected with a sterilized syringe into the subcutaneous cavity, for instance under the skin of the back. A suitable dose is typically c. 600 international units. Males can usually be brought to call by injection and once they call they will do so persistently for at least a couple of days. This may stimulate the females. If not, the females may also be injected with the same hormone in similar doses. This procedure often results in successful mating and spawning, but success is not guaranteed.

6.4.3. Release methods

In general, more developed animals have a greater chance of survival after release. Release of tadpoles can be successful; experiences with strong and healthy looking tadpoles (metal-bluish colour, round belly, strong reactions to disturbances) which are longer than 3.5 cm are good. Metamorphosed animals are not predated by water insects, but they are often eaten by birds, especially herons in their preferred dwelling places close to the pond-edge. It is thus rewarding to equip the shore with plenty of good hiding places, such as plants, branches, algae mats etc. In terms of survival, it is

Animals that metamorphosed in the net cages are released in the pond.

Newly released young *Bombinas*.

advantageous to postpone the release at least a few weeks. Rather good results have been obtained releasing 1-year-old the animals.

The weather conditions on the day of release are important. Animals should not be released at the beginning of a cold, grey period.

How the animals are released is not important. Studies indicate that survival is not affected by release method.

6.5. Managing parasites

6.5.1. Chytrid fungus

The organism *Batrachochytrium dendrobatidis* is a so-called chytrid fungus. It is not a fungus in the strict sense of the word, but a multicellular organism growing on amphibian skin. This organism can be fatal.

Recently, chytrid related problems in Europe have become more obvious and the potential risk has increased. The methods for captive and supportive breeding programs must therefore be adjusted to lower the risk of infection. In practice, it is impossible to avoid spreading of chytrids and other pathogens, but people working with aquatic habitats and organisms can lower risks to a level where rare populations not easily are infected.

Development of strong, large and vital populations is possibly the best tool to avoid extinction of (local) populations due to pathogens.

Complete separation of populations must be arranged in artificial rearing, and establishment of separate mirror populations should be stimulated. If a population is infected, all persons and materials have to be completely disinfected; even the car, its carpets and other apparently irrelevant equipment should be disinfected. However, if persons, who are active in conservation management on a large scale, do not dedicate the necessary time on disinfection, threatened populations may have gone extinct before the desired breeding materials can be collected.

Portable breeding station

Copenhagen Zoo has developed a portable field breeding station with a net cage placed in a portable tank and with a pump system filtering water from the pond. By applying this system, transportation of brood from the field to a central rearing station, where it might be infected, is avoided.

Disinfection in captivity

Experience from Riga Zoo, where the amphibian department was infected with chytrids, tells us that disinfection with commercial antibiotics is expensive and very difficult. This is because extreme care must be applied in disinfecting every single item and every small crevice in every cage. However, a low-cost solution was found: the chytrids are killed by the substances in garlic and if garlic is planted in the cages, the chytrids will disappear without any harm to the amphibians.

7. Monitoring Firebellied Toads

7.1. Monitoring purposes

Many *Bombina* populations are or have been very small, and a constant risk of inbreeding or even extinction exists. The main purpose of monitoring is to make sure populations are safe. Large populations should remain sufficiently large in a favourable habitat. Small populations should be increasing, protected from of acute danger, and have a habitat that is improving. If monitoring shows development in the wrong direction, failing breeding success, or deteriorating habitats, action must be taken to reverse the negative trend.

Back patterns form early and can be used to identify even juvenile *Bombina.*

7.2. Measuring population size

7.2.1. Individual recognition

An important tool in the assessment of population size is individual recognition. Each individual has a unique pattern. If caught individuals are photographed and kept on file, recaptured individual may be recognized checking this file.

The most characteristic spots are on the belly. In order to photograph the belly, the animal is placed in a box with a transparent bottom, a so-called bombinograph or photo-aquarium. The box is made by Plexiglas® and it is subdivided into two or four departments, enabling photography of two or four animals at a time. A little water must be added to the box before use. The box is closed with a lid to prevent the animals from jumping out. The box is held by hand or placed on a stand and photographed from below. A piece of cardboard or a small plastic board is placed on top of the box to prevent excessive light.

The spots on the back are also unique. Photos of the back are taken by placing the animals in the bombinograph and covering them completely with water leaving no air bubbles below the lid.

The back pattern forms early. It has practically formed when the animal metamorphoses, and the pattern is almost unchanged for the rest of the animal's life. Photos of back patterns can thus be used to monitor juveniles. The belly pattern does not reach its final shape for several months. Belly photographs are therefore only useful for half grown and adult animals.

Belly pattens can only be used to identify adult *Bombina.*

7.2.2. Capture-recapture

Purpose

Capture-recapture is a relatively precise method for estimating populations. For instance, this method is indispensible if an estimate of a small population is needed in relation to a genetic analysis and the risk of inbreeding. A drawback is that it is very time consuming, not only in terms of field work, but also in terms of comparing photographs afterward.

Capture period
The recommended period for capturing is from the beginning of the season, at least mid May, until early July. After July 10th, many adults hide and will not be captured, thus the population estimates becomes too low.

Estimating N
The principle is as follows: Imagine that 50 animals are caught in a pond at one occasion. One week later 60 animals are caught. Of these, 20 are recaptures, whereas the rest are caught for the first time. Statistically, 20/60 = 1/3 of the animals were captured the first time and since the total catch was 50, equalling one third of the population, the total population size N must be 3 × 50 = 150.
More generally, the population size N is calculated as

$$\frac{n1 \times n2}{n12}$$

where n1 is the number caught the first time, n2 the number caught the second, and n12 the number caught both times, i.e. the number of recaptures.

In case of three catching events, N may be calculated as

$$\frac{(n1 \times n2) + (n1 \times n3) + (n2 \times n3)}{n12 + n13 + n23}$$

When adding catching events, the formula is extended by the same principle.

Confidence interval
It is important to be familiar with both the population estimate and the uncertainty. The number of animals captured the first and second time is given, but the number of recaptures is the result of random selection. In cases with two catching events, the uncertainty on the number of recaptures is calculated by the following formula, which gives the standard error:

$$SE = \sqrt{\frac{n12\ (N - n1)\ (N - n2)}{N\ (N - 1)}}$$

For three catching events, the formula is

$$SE = \sqrt{\frac{x}{N\ (N - 1)}}$$

where x = n12 (N-n1) (N-n2) + n13 (N-n1) (N-n3) + n23 (N-n2) (N-n3)

In each case the SE is multiplied by 1.96 to get the 95% confidence interval.

If only few animals are recaptured, the uncertainty becomes very large. A demand that the difference between the lowest and the highest end of the confidence interval is maximally a factor of 2, requires at least one catch of more than half of all individuals in the population. This is usually possible with a concentrated effort.

The required number of caught animals is illustrated in table 7.1. The table deals with a hypothetical population of 100 individuals. An equal number of individuals are caught in each of two catching events. The table illustrates the uncertainty on population estimate as a function of the number of animals caught. Clearly, it is too little by far to catch only 10 or 20 animals every time, but catching 40 provides a fairly good estimate and catching 80 gives an excellent estimate.

A and B animals
Some individuals may become accustomed to the presence of humans, and do not dive and hide when approached. Such individuals, which are caught almost every time, are called A animals. Other individuals are much shyer and hardly ever caught; they are called B animals. During three catching events more animals than expected will be caught every time and more than expected will be caught only once, whereas fewer than expected will be captured twice. As a consequence, the number of recaptures will be artificially high and the population estimate will be too low, sometimes much too low.

Methods for calculating the number of A

Table 7.1. Required number of caught animals.

Animals caught per event	Recaptures	No. of different individuals caught at least once	Population estimate (95% confidence interval)
10	1	19	27-∞
20	4	36	56-472
30	9	51	69-185
40	16	64	77-142
50	25	75	84-125
60	36	84	88-115
70	49	91	92-109
80	64	96	96-105
90	81	99	99-102
100	100	100	100-100

animals exist, thus the population estimates can be corrected accordingly.

Migrations
Many individuals migrate between neighbouring ponds during the season. Statistical methods compensating for individuals moving in and out of the pond exist. However, when dealing with *Bombina*, it is preferable to make captures in all neighbouring ponds and make a total population estimate for the entire pond group. This also provides valuable information on when and how often the toads migrate between ponds.

7.2.3. Calling males count

Population size may be estimated from the number of calling males. There is generally a 1:1 sex ratio in *Bombina* populations, making it possible to multiply the number of calling males by two to get total adult population size. However, not all males call at once. In larger populations, the proportion of calling males at any given time is lower. The relationship has been studied in a number of Danish populations, and the results are given in Figure 7.1 and Table 7.2.

For this method to apply, calling activity must be optimal. The maximum number of callers per visit is usually obtained after rainfall when conditions are warm and calm. In the beginning of the season, calling may be most intensive in mid afternoon when temperatures

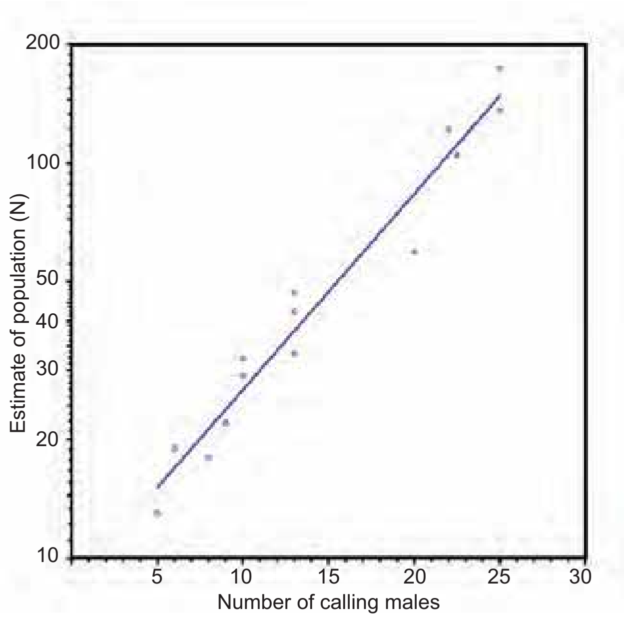


Figure 7.1. The number of calling males is not equal to the number of males in the pond.

are highest. Later in the season, calling will not start until the last half of the afternoon. In very warm weather, calling will start around 18 o'clock and at least continue to midnight. Every season has several peaks of calling separated by periods without calling. Each peak period typically last about a week. Periods of calling may differ from pond to pond. To find the maximum number of males calling, the pond must be visited during optimal weather conditions two to three times per season. The

Table 7.2. Calling males vs. estimated number of males.

Counted no. of calling males (NCount)	Estimated no. of males
1	1.5
2	4
3	7
4	9
5-25	8.526 x potencia (1.121;NCount)
> 25	8.526 x potencia (1.121; NCount) + 2(NCount - 25)

highest count obtained is used, provided that calling males are not suspected of having swapped ponds between visits.

It is important to take some precautions when counting. In order not to disturb the calling males, bend over and approach the site carefully. However, after some habituation the males may not be disturbed by walking at the pond's edge. If possible, each calling male is located, for instance using binoculars. Often, only few males can be seen directly. Instead it is important to listen to the 'melody' of the chorus and decipher the number of 'notes' constituting the melody. If the males are distributed along the banks, it is usually possible to make precise individual counts for up to 25 animals. When more animals are calling, or when the animals

Filling in a *Bombina* pond form.

are concentrated in the middle of the pond, it is difficult to distinguish between individuals.

7.2.4. Fencing and traps for total capture of population

If an entire population needs to be caught (e.g. because of relocation of the population or for scientific purposes) the breeding habitat must be fenced in early spring before migration to the pond starts. The fence must prevent the animals from climbing over. Additionally, traps should be installed to catch the toads while trying to pass the fence. The animals can either be photographed and set free inside the fence or removed from the pond. To make sure that no animals have been missed, the pond should remain fenced until hibernation starts. This is necessary because animals might be migrating to the pond during the season, or animals may have hibernated inside the fence and thus have been missed.

7.2.5. Searching in hiding places

Hiding places can be checked for animals. All types of holes close to the pond are appropriate sites; under dead wood or loose stones, under garbage (such as foil, plastic bags etc.), under branches, in cattle or horse tracks, or under loose soil.

This method may be useful if it is necessary to catch the animals and transfer them to some other place, but it can not be recommended as a monitoring method. *Bombina* reacts very negatively to being found in its hiding places. Once it has been uncovered, it will not return to its hiding place. Instead, it will migrate and this may expose it to dangers.

7.3. Measuring breeding success

Breeding success can be investigated by detection of tadpoles or metamorphs.

Counting tadpoles

Detecting larvae can be used to verify whether a site is being used as a breeding pond. The pond must be dip-netted in order to count larvae because it is extremely hard to spot *Bombina* larvae in the water. This method requires some familiarity with the whereabouts of the larvae. The site should be visited, preferably in sunny weather, from late May to mid July when the tadpoles have reached larger sizes. If older tadpoles are caught, it is very likely that the pond is a successful breeding habitat even if only a low number is caught. On the other hand, even though no tadpoles are caught, it can not be concluded that successful reproduction is absent, because tadpoles might go undetected. It is almost impossible to quantify the amount of tadpoles in a pond.

Counting metamorphs

To count metamorphs, the pond is visited when most larvae are thought to have completed their metamorphosis. This will be happen between the latter half of July and early September depending on the summer climate. Good weather conditions (warm and calm) are essential for a successful count. The metamorphs usually stay close to the pond edge, both on the land and in the water.

A semi-quantitative estimate of the breeding success is obtained by observing while slowly walking along the pond's edge. Often relatively few metamorphs are seen during the first walk, but by repeating the walk after c. 15 minutes more individuals are usually observed. Tufts of dense vegetation at the water's edge are stirred with a stick chasing metamorphs out of hiding. The number seen on the second walk, or the combined number, may be noted as the result.

Observation of newly metamorphosed animals with remains of the tail gives definite proof of natural breeding success. Larger metamorphs may in principle have immigrated from another place, for instance a nearby pond that has just dried out.

7.4. Monitoring frequency

How frequently populations should be monitored depends on the situation.

Small, threatened populations should be monitored every year with regards to adult population size and breeding success – especially if the population is concentrated in one

Pond complex at Geltinger Birk. Both temporary and permanent ponds are visible in this photo.

pond which could be exposed to a catastrophe. A population on Enø in Denmark which unexpectedly went from 120 to zero in just three years may illustrate the point. The populations must be monitored every year to avoid a suddenly crash.

Frequent monitoring may also be necessary to get an impression of variations in breeding success. This may vary enormously from year to year and if recordings are not made every year, misleading results may be obtained.

Managers of *Bombina* populations should at all times know if there is (annual) reproduction success. If only the adult population is monitored, necessary actions in case of a population crash will be taken too late or it will become too costly to reach the targeted results.

The larger the population size and the larger the number of breeding ponds, the less frequent may the monitoring be. In the EU monitoring system, species are generally monitored every six years. However, only well secured *Bombina* populations can safely be monitored at such long intervals. The EU monitoring scheme in Germany generally includes one visit per six years; in Denmark it is two visits per six years. Populations that might potentially collapse due to a short series of poor seasons need more monitoring.

Table 7.3. Important parameters when monitoring ponds.

Parameter	Germany		Denmark	
Area	+	m^2	+	m^2
Shade	+	0%; 1-50%; > 50%		
Extent of shallow parts	+	percent		
Emergent vegetation	+	structure and percentage cover	+	percentage cover
Submersed vegetation	+			
Grazing			+	yes/no
Duck rearing			+	yes/no
Fish	+		+	yes/no
Fishery utilization	+			
Pollution (nutrient biocides)	+		+	yes/no
Disturbed water balance	(+)	(facultative)		

7.5. Recording the state of the habitat

The conditions for a *Bombina* population may also be monitored by checking the state of the habitat. The state may be evaluated with binary parameters (answering yes or no) or with quantitative indicators (how many percent, how large an area etc.).

Parameters which are registered monitoring ponds according to the EU habitats directive in Germany and Denmark are shown in Table 7.3.

In Germany, each locality is defined as a *local management unit,* i.e. a single population or group of populations (combining neighbouring populations up to 500m apart) and the surrounding area (up to 100m from the ponds). Parameters recorded here incude:

- Size and numbers of ponds in the management unit.
- Structure of the land habitat in the immediate surrounding.
- Hiding and hibernating sites in the land habitat.
- Distance to the closest neighbouring population.
- Endangerment by use of heavy machinery in land habitat (agriculture and forestry).
- Traffic infrastructure in the immediate land habitat.
- Isolation of management units by intervening monotonous agricultural landscape or human settlements.

7.6. Details on monitoring methods in Germany

The population size is estimated through three inspections from April to August. One inspection must be carried out during the main calling period (April/May). A significant indicator is reproductive success – or lack thereof – within the management unit. The reproduction success (in form of tadpoles or metamorphs) is recorded qualitatively during the survey trips (May–August). An overview of the classification system is shown in table 7.4.

Table 7.4. Overview of the German classification system.

Criteria	A	B	C
Population estimation • size • structure; breeding success	**Excellent** • > 100 calling males • reproduction recorded	**Good** • 50-100 calling males • reproduction recorded	**Medium-bad** • < 50 calling males • no reproduction recorded
Habitat quality	**Excellent**	**Good**	**Medium-bad**
Water quality • no. and size of ponds belonging to local population (no. ponds, estimated covered area m² • extension of shallow areas (< 0.4 m); percentage of shallow areas in total pond complex (estimation) • submerged and emergent vegetation • sun exposure (percentage of shaded water surface)	• complex of ponds (> 15) • extended shallow areas in pond system, majority of all ponds shallow (> 70%) • extended submerged and emergent vegetation (> 50% cover) • full sun exposure	• complex of ponds (5-15) or single large pond (> 0.5 ha) • shallow areas in segments of ponds; half of all ponds shallow (30-70%) • moderately extended submerged and emergent vegetation (10-50% cover) • moderately shaded (1-50% shaded surface)	• complex of ponds (< 5) or single small pond (< 0.5 ha) • few or no shallow areas; few ponds shallow (< 30%) • little vegetation in pond complex (< 10% cover) • majority of water surface shaded (> 50%)
Land habitat • specification of land habitat in immediate pond surroundings (100 m)	• complex structured, extensively used grassland, fallow land or forest; high density of hiding places, e.g. holes, hedges, stone fences (>50%)	• structured agricultural land; medium density of hiding places (10-50%)	• poorly structured agricultural land; few hiding places (< 10%)
Biotope connectivity • distance to closest population (m); (available data only)	• < 1,000 m	• 1,000-2,000 m	• > 2,000 m
Derogation	none to low	moderate	heavy
Water habitat • occurrence of fish and fishery utilisation (expert vote w. statement) • obvious influence of contaminants (expert vote w. statement) • water balance (expert vote w. statement)	• no fish • no influence of contaminants • undisturbed	• fish detectable; marginal fishery utilisation of ponds • influence of contaminants or eutrophication visible indirectly • marginally disturbed	• evidence of extensive fishery utilisation of ponds • influence of contaminants visible directly • heavily disturbed
Land habitat • endangerment by use of heavy machinery (agriculture and forestry) (bufferzones: yes/no; width)	• no use of heavy machinery; no ploughing in surroundings (100 m radius)	• some use of heavy machinery; no ploughing in immediate surroundings (10 m buffer zone)	• intensive use of heavy machinery in the surroundings (no buffer zones)
Isolation • infrastructure (roads) in surroundings (100 m radius) • isolation by monotonous agricultural landscape and settlements (perimeter percentage specified)	• non-existent • non-existent	• existent, rarely frequented (< 20 vehicles/night) • partially present (< 50% of migration corridor blocked)	• existent, frequented moderately to heavily • present (> 50% of migration corridor blocked)

8. Financing Amphibian Conservation

8.1. EU-Funds

Different options should be taken into account when searching for funds to finance amphibian protection. Different EU programs offer several possibilities. Important is the LIFE+ program where EU co-finances conservation projects within NATURA 2000 sites directly. The project must improve the conservation status of the target species in a designated NATURA 2000 site. For further information:
http://ec.europa.eu/environment/life/funding/lifeplus.htm

This website includes a project data base where it is possible to search for projects on a specific species. It is highly recommended to make contact with running projects and participate in knowledge exchanges if you plan a new project.

Previous experiences with amphibians and reptiles can be found in a LIFE Focus brochure: http://ec.europa.eu/environment/life/publications/lifepublications/lifefocus/nat.htm#amph_reptiles.

The LIFE+ program has a transparent selection procedure and it is possible to have best practise projects financed during several years.

However, Annex IV amphibian species are often found outside the NATURA 2000 network, thus LIFE+ cannot be used for these populations. If this is the case you have to look for other funds. One possibility is the Common Agricultural Program (CAP). Projects focusing on NATURA 2000 habitats and species can be funded through the second pillar, as e.g. in Northern Germany, Schleswig-Holstein (http://www.schleswig-holstein.de/UmweltLandwirtschaft/DE/LandFischRaum/11_ZPLR/ein_node.html). Actions co-funded by EU within the German ZPLR program include:

- Land purchase
- Habitat management such as pond digging, hibernations sites, planting hedges and instalment of extensive grazing regimes
- Population management

Whether this source of money is available for nature conservation purposes depends on how the national implementation of the program is managed in your country. Notice that CAP programs only finance 50% of the project costs. The remaining 50% must be financed nationally. In Schleswig-Holstein, the Ministry for Agriculture, Environment and Rural Areas contributes to this through its nature conservation budget.

Since the project has to approved of both EU and the national agricultural authorities it might be challenging to get funds for a nature conservation project. Up to 100% of the costs can be funded (50% EU and 50% national budgets), but frequently, the funds are granted for shorter periods according to national budget plans.

Another possibility is the INTERREG Program (http://en.wikipedia.org/wiki/Interreg). INTERREG is designed to stimulate cooperation between member states of the European Union on different levels. One of its main targets is to facilitate cross border cooperation among member states under different topics such as economy, tourism, fishery, social affairs, environment and implementation of NATURA 2000. This program also co-finances projects.

INTEREG offices can provide more information on how to apply. Our experiences are that it is hard to get a nature conservation project accepted. The reason is that a board of politicians decides which proposals are funded. Usually, other topics such as economy, tourism, etc. are deemed more important by the board. Compared with the other programs, it is more difficult to run a nature conservation project as an INTEREG project, because the yearly budget plan must be fulfilled. Adjustment of project implementation is less flexible compared with e.g. a LIFE+ project.

8.2. National instruments

8.2.1. Examples from Germany, Schleswig-Holstein

In Germany it is possible to find other funding for amphibian conservation. The German impact mitigation law prescribes that impact to nature (biotopes according to the national nature conservation law, woodland according to the woodland law) and to ecosystem services (groundwater renewal, destruction of migration routes, etc.) must be compensated. Additionally, specific rules for impacts to NATURA 2000 sites and species exist.

One of the many ponds within Stiftung Naturschutz's properties.

A developer who plans impacts on a site must organise mitigation measures on a compensation site. The idea is basic, but sometimes hard to execute e.g. due to the urban surrounding and lack of suitable compensation sites. Some years ago, an additional possibility was established. Compensation can be organised as a bigger conservation project and before an impact is even planned. Someone has to invest money in land purchase, a restoration plan and implementation of conservation actions. The quality of the conservation measures is converted to so called 'eco-points'. When a developer wants to initiate a project, he can present eco-points instead of a compensation site. This rule has established a free market of eco-points. The Stiftung Naturschutz Schleswig-Holstein owns a company, the Ausgleichsagentur (www.ausgleichsagentur.de), which is responsible for more than 40 compensations projects in Northern Germany. Amphibians are one of the target groups of the conservation measures within these project areas.

Cattle grazing a Stiftung Naturschutz area.

9. Conservation strategies

9.1. Legal protection

By now, the EU habitats directive applies to all member states. Article 12 in this directive protects *Bombina bombina* by prohibiting killing, collection of eggs, etc. See section 9.3.1.

9.2. Conservation strategies in different member states

9.2.1. Denmark

Concern about *Bombina* arose particularly in the 1940s. Considerable effort was put into mapping occurrence of *Bombina*. The first pond, situated on Lolland, was protected by declaration in 1946. Many ponds on the islands south of Fyn were protected in 1949, 1951 and 1956, and one pond on Fyn was protected in 1969.

Sometimes even the surrounding landscape was protected. Three of the most important localities were protected by declaration in 1951, 1952, and 1953.

However, as early as the 1950s some of these actions made no sense. For instance, some ponds were protected a few years after *Bombina* had last been heard there. The hope was that the animal would return, which, of course, it did not.

The number of protected ponds where *Bombina* survived at least up to the mid 1980s can be seen in Table 9.1.

As shown in the table, it is not efficient to preserve *Bombina* populations by protecting ponds by declaration. The firebellied toads have in almost every case disappeared when ponds alone were protected, and they have disappeared in more than half the cases when both pond and surroundings were protected.

Table 9.1. Number of protected ponds in Denmark where *Bombina* has survived from the 1940s to the mid 1980s.

	***Bombina* has**	
	not survived	survived
Ponds protected	21	2
Ponds and surrounding area protected	26	15

In the mid 1970s, the surviving populations of *Bombina* in Denmark were mapped. This effort resulted in popular articles on the need to protect the species, and it also motivated legal protection toward collection. Surviving populations were carefully monitored from the early 1980s, but a continued decline was demonstrated. Of 15 populations recorded in 1974, seven had already disappeared by 1987 and it became increasingly evident that something needed to be done. The first artificial pond was established in 1981 on a private initiative and in 1985 additional ponds were created using public money and money from a donation. The first attempt to eradicate fish from a protected pond was made in 1982. The first artificial rearing was initiated in 1982, and rearing from two additional populations was initiated in 1986. After 1988, all remaining Danish populations were supported. A few populations were only monitored carefully, but the majority was tended by active biotope creation and artificial rearing.

9.2.2. Germany, Schleswig-Holstein

A first attempt to improve the conservation status of the firebellied toad was initiated in 1985 in Schleswig-Holstein through an action plan called 'Artenhilfsprogramm Rotbauchunke' (Dierking-Westphal 1985). In connection with this action plan, all remaining ponds with calling males were mapped. A total of 128 ponds were investigated, but only 28 had more than 20 calling males. On the island of Fehmarn, which was formerly known for large firebellied toad populations, only a few sites on the western half of the island remained.

This action plan pinpointed the most important target regions for conservation actions. Focus was placed on pond creation and the following recommendations were applied: ponds larger than 500 m^2, depth: 0-50 cm in 4/5 of the pond while the remaining parts are deeper, full sun exposure, no plantings and cattle grazing on the edges.

The program was implemented using agri-environmental contracts. The contracts were made for full land plots. On the contracted plots the land use must be extensified (no use of fertilisers, reduced grazing, conversion of arable land into grassland) and on 1-2% of the plots pond habitats could be created.

After nine years the program was evaluated in the mid 1990s (Dierking 1996). The result was rather disappointing because firebellied toad populations were still declining. Dierking identified the following reasons:

- The program was dependent on private land owners offering their land for conservation measures. Participation, especially with good soil areas, was too small to be effective.
- Only 1-2% of the contracted area was used for pond creation. Due to the limited land owner response, this was insufficient additional pond habitat.
- In many areas the remaining populations obviously were too small to retain their ability to colonise new ponds more than 1 km away.

This experience let Dierking to conclude the following:

- Conservation on landscape level is impossible when combined with the intensive land use. Conservation must be concentrated on the remaining populations with the aim re-establish larger populations.
- Within the core sites at least 50% of the area must be dedicated to habitats for firebellied toads. Therefore, private land had to be purchased by public entities.
- Land use must be carried out without machinery and fertiliser. Extensive grazing is the recommended land use for keeping the areas and ponds open.
- Ponds should preferably be created by closing drainages in the purchased plots.

Experience exchange during the LIFE-*Bombina* project in a pond-digging work-shop.

The land purchase was one of the most important activities in conservation policy and work in Schleswig-Holstein in the 1990s. However, another 10 years went by before the Landesamt für Natur und Umwelt initiated the targeted actions for the firebellied toad in 2003. The activities were increased during the LIFE-*Bombina* project. At this point the purchased land and the Danish experience from targeted habitats and population management could be perfectly combined.

During the LIFE-*Bombina* project, four management units of firebellied toad populations were defined based on the genetic survey, which was conducted by Prof. Ralph Tiedemann from University of Potsdam. The After-LIFE strategy aims on re-establishment of a metapopulation structure on landscape level in order to re-connect all the remaining populations within each management unit. Separated from the four original populations, a fifth mixed population originating from several Danish and one German population was established at the project site Geltinger Birk. A separate metapopulation is planned for this population in the landscape of 'Angeln' (East of the town of Flensburg) next to the Danish border.

The implementation is carried out by the Stiftung Naturschutz and other organisations such as NaBu Eutin, Froschland-Projekt and the Ausgleichsagentur Schleswig-Holstein, a company dealing with compensation projects.

9.2.3. Latvia

Although *Bombina* had been observed in several localities during the 1970s, by 1980 Islice south of Bauska, near the Lithuanian border housed the only stable occurrence of the species. It has been known at Islice since 1929. In addition, the species was observed near a biological field station at Ilgas southeast of Daugavpils. In the 1980s, the localities at Islice and Ilgas were appointed nature reserves.

Some individuals from both localities were brought to Riga Zoo where they founded a captive population. This population still exists.

In 1992 active measures at the protected sites were discussed, but no measures were taken until the LIFE *Bombina* projects after 2000. Without these measures, *Bombina* would probably have disappeared. For instance, the protection at Ilgas did not prevent overgrowth of ponds no longer utilised for grazing and hay harvesting. Furthermore, it did not prevent immigration of predatory fish to the most important breeding pond.

Learning about the refinements in pond-digging.

9.2.4. Sweden

The last original Swedish *Bombina* location was recorded in 1925. Subsequently, the population was handled inappropriately by naturalists; e.g. the locality was not protected until 1951 when it became a nature reserve. The creation of the nature reserve did not prevent the species from dying out in 1960.

Two illegal reintroductions were made in 1974 and 1977. Later, it was officially decided to reintroduce *Bombina bombina* in Sweden using financing from WWF and the Swedish Environmental Protection Agency. The first animals were released in 1983 and the last in 2002. However, during the early years, the reintroduction program and the local authorities in Scania administering the landscape did not cooperate. As a consequence, the habitats were not actively improved prior to release of the animals. In 1993, the western Scania authorities financially supported the establishment of a number of new ponds. These were primarily created for *Hyla arborea*, but they were situated in an area where *Bombina* had been introduced successfully. Some of these ponds were colonised immediately, others after a few years, and this markedly consolidated the *Bombina* population. Subsequently, a few of the most important original ponds were ruined by release of crayfish.

Since then, cooperation between authorities and biologists has improved and an activity plan (åtgärdsprogram) for the years 2010-2014 has been produced for *Bombina* in Sweden. According to this plan, the four areas housing *Bombina* populations will be improved to support viable populations (> 500 adults). This implies (r)establishment of more ponds. In the area north of Ystad where *Bombina* is numerous and widespread, ponds will be created to allow dispersal to valuable nature areas in northwest.

The number of ponds in the protected areas in Scania can be seen in Table 9.2.

Table 9.2. Protection status in Scania 2007. Data for the region around Baldringe and the total numbers are not precise. Source: Stenberg & Nyström, 2007.

Area	No. calling males	No. ponds	% protected ponds
Mölle	135	10	90
Lunkaberg	22	2	0
Bäckhalladalen	56	7	71
Region around Baldringe	7300	290	15
Total	**7513**	**309**	**19**

Most of the ponds near Mölle and Bäckhalladalen are situated within protected nature reserves. The Lunkaberg locality is not protected. A number of nature reserves at Skoghuset, Skoghejdan and Fredriksberg in the region around Baldringe include some core areas for the species, but other core areas in the neighbourhood are situated outside the reserves.

9.3. *Favourable conservation status*

9.3.1. The Habitats Directive

Management of *Bombina bombina* populations must be carried out in accordance with the EU Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). According to article 2 in this directive, 'Measures taken pursuant to this Directive shall be designed to maintain or restore, at favourable conservation status, natural habitats and species of wild fauna and flora of Community interest'. *Bombina bombina* is a species of community interest and is listed in Annexes II and IV.

The directive has two tools for preservation of *Bombina bombina*. The first tool applies to the species in Annex II. According to article 3, a coherent European ecological network of special areas of conservation shall be set up under the title Natura 2000. This network shall enable the species' habitats to be maintained or, where appropriate, restored at a favourable conservation status in their natural range. Each member state shall contribute to the creation of Natura 2000 in proportion to the representation within its territory of the species. Member states shall take appropriate steps to avoid, in the special areas of conservation, the deterioration of the habitats of the species as well as disturbance of the species.

The second tool applies to the species in Annex IV. Protection of these species is defined in article 12. This article declares that member states shall establish a system of strict protection for the animal species in their natural range, prohibiting:

a) all forms of deliberate capture or killing;
b) deliberate disturbance of these species;
c) deliberate destruction or taking of eggs;
d) deterioration or destruction of breeding sites or resting places.

What are the implications of these provisions?

First, the provisions apply to the species in its natural range, and they shall secure that the range does not shrink. Each member state where the species occurs shall contribute to the protection of the species.

Second, the habitats shall be maintained or restored.

Third, deterioration or destruction of breeding sites or resting places is prohibited, wherever the species occurs.

Fourth, measures shall provide a favour-


Analysing water levels from test holes during experience exchange workshop.

able conservation status.

9.3.2. What is meant by favourable conservation status?

The term 'favourable conservation status' is defined in article 1 of the directive:

'Conservation status of a species means the sum of the influences acting on the species concerned that may affect the long-term distribution and abundance of its populations within the territory referred to in Article 2.

The conservation status will be taken as 'favourable' when:

- population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats, and
- the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and
- there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis'.

What are the implications of this?

First, the natural range cannot be reduced. This implies the necessity to preserve populations at their distribution border.

Second, the paragraph on the species range mentions the term 'the foreseeable future'. How far into future can we see? This is particularly relevant in relation to climate change. At present, the intergovernmental panel on climate change (IPCC) declares a more than a

90% certainty that the ongoing manmade global warming will result in rising sea levels. A large fraction of all *Bombina* localities in Denmark are situated on small islands or close to the sea and these will be affected greatly if sea levels rise by 1 to 2 metres. Thus, many populations at the border of the species' range are in danger of being extinguished. If this catastrophe can be foreseen – and according to IPCC it can – the directive demands that the change is compensated for; e.g. by creating or replacing habitats further inland before the coastal habitats are flooded.

Third, the directive demands 'a sufficiently large habitat to maintain its populations on a long-term basis'. This assumes a relationship between the size of the habitat and the duration of the population. And indeed this is very likely so. The larger the habitat and the greater the number of ponds, the greater is the chance that at least some individuals survive a negative stochastic event. In addition, a larger habitat will house a larger population. The larger the population, the slower is the process of genetic erosion leading to inbreeding and hence loss of fitness.

A nature reserve with a sufficiently large carrying capacity to ensure that the population will not suffer from inbreeding depression for a hundred years must nevertheless be enlarged before 100 years have passed. However, if the reserve created to protect the species is very small and all kinds of infrastructural development are allowed outside the borders of the reserve, the new infrastructure may block all potential to enlarge the reserve later on. It follows, that the population must be viable at least as far into the future as the infrastructure outside of the borders. Presumably, this means a time perspective of more than 100 years. Therefore, it seems that 'long term' means more than 100 years. 1,000 years may be the upper limit for a reasonable time perspective. That is, a reserve must be sufficiently large to prevent inbreeding a thousand years ahead.

9.3.3. How large is large enough?

Unfortunately, it is impossible to define exactly how large a population must be in order to survive and remain healthy for 100 years or 1,000 years. However, population genetics provides us with some rules of thumb. Debilitating inbreeding depression is avoided for a number of generations equalling the effective population size. If the generation time of *Bombina* is set to two years, an effective population size N_e of 50 animals will last for 50 generations; i.e. 100 years. Thus, after 100 years the population may no longer be healthy. If N_e = 500, the population will stay healthy for 1,000 years. This target is reasonable for another reason: if N_e = 500 the loss of alleles is compensated by accumulation of new mutations, providing natural selection with a constant amount of variation to act upon.

The next question is what the relationship between N (the census population size) and N_e is? How small is the ratio N_e/N? This was dealt with in section 6.1.4, and, with our present fraGmentary knowledge, the answer is that N_e/N probably equals about 0.25 if one generation is considered. An effect of the fluctuating population size from generation to generation will also influence the result. The reduction of N_e due to such fluctuations is calculated using the harmonic mean of the N values, as described in section 6.1.2.

In conclusion, a population is large enough if N_e = 500. As a consequence, the harmonic mean of the census population size of adults N is 500/0.25 = 2,000 over the years. Again, in most years, N must be greater than 2,000 because small downward deflections in N values in a few generations equal upward deflections in most generations.

9.3.4. The pond area necessary to maintain large populations

How large does a nature reserve have to be to house a carrying capacity corresponding to a harmonic mean of an adult population size of 2,000 individuals?

This question may be answered by the calculations outlined in section 6.2.2. Here, each pond is classified in one of five quality classes with respect to value as a breeding site and as a foraging site. Knowing the maximal possible density of adult toads in each ponds class, it is possible to calculate the carrying capacity. If either the capacity for breeding animals, or the capacity for foraging animals, is less than 2,000 (N_e = 500) the habitat is too small or too poor to sustain a viable and healthy population for a long time. If the carrying capacity is less than 200 (N_e = 50) the habitat is too small or too poor to prevent continuous genetic degradation. The population will barely remain viable and healthy for 100 years.

9.4. The needed minimum habitat requirements to secure favourable conservation status

9.4.1. Isolated versus coherent populations

Two main types of firebellied toad population structure exist:

- Isolated populations which inhabit groups of ponds without contact to the general range of the species.
- Sub-populations which are in contact with the general range and thus may constantly be renewed by immigrations from much larger populations.

The criteria for assessing the favourable conservation status of firebellied toads differ depending on population structure.

9.4.2. Isolated populations

A population is isolated if the distance to other populations is larger than the distance travelled by firebellied toads during dispersal. The dispersal range depends on the breeding success and the population density in the ponds concerned. 1.5 km is average, but if conditions are favourable, dispersal can be larger; in rare instances up to c. 5 km. If conditions are unfavourable it could be smaller, possibly down to 500 m.

In order to avoid inbreeding in a long term, an isolated population must have sufficient available habitat to allow a harmonic mean size of at least 200 adults (medium time perspective) or 2,000 adults (long time perspective), as explained in the preceding sections.

An isolated population must be robust concerning short time calamities, i.e. they must be able to survive unusual weather situations, because no re-immigration is possible if the original population dies out. Thus, even during seasons with unusual weather conditions, the pond system must have at least one functional pond. Presence of at least one semi-permanent pond which dries out in dry years and one permanent pond that never dries out is vital. Occurrence of at least one temporary pond or shallow flooding is an advantage since this type of water body often produces the highest breeding success if it holds water sufficiently long.

Furthermore, more than one pond is essential simply because possible threats tend to strike one or two ponds, but rarely three ponds at once.

Preferably, several pond systems should be connected by rare dispersal events. If a contagious disease hits one of the subpopulations, the other subpopulations will probably remain unaffected, and they may later re-colonise the stricken area.

Firebellied toads do not stay in the ponds all year and the surroundings must also be considered, as described in section 6.2.3.

9.4.3. Sub-populations in contact with the main range

A pond system connected to larger populations via corridors shorter than approximately 1.5 km, has different criterions for favourable conservation systems than the systems described above. In this case, possible problems can be compensated by re-immigration from neighbouring populations.

As an example consider the *Bombina* population at the Ilgas reserve southeast of Daugavpils in Latvia. The population is at present very small, but it is not threatened because the distance to a large population (across the border of Belorussia) is only 1.4 km, and the population is constantly renewed by immigration. Calling males at Ilgas will attract dispersing animals. The local population must be robust concerning short term calamities, i.e. the same conditions must be fulfilled as for pond systems housing isolated populations. One to several breeding ponds must be present and at least one breeding pond must be functional every year, irrespective of various weather conditions. Furthermore, the landscape traversed by dispersing animals must remain hospitable to *Bombina*.

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