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HIBERNATION SITES OF THE TOADS *BUFO BUFO* AND *BUFO CALAMITA* IN A RIVER FLOODPLAIN

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Closed drift fences, bow-nets and a telemetric system were used to study hibernation sites of two toad species in a floodplain. Both species hibernated terrestrially. *Bufo bufo* hibernated in meadows, thickets and woods/bushes on sand or clay in the higher as well as the lower parts of the floodplain. *Bufo calamita* clearly preferred sandy habitats in the higher parts where heaps of brick-debris were specially used. Both species selected their hibernation sites based on characteristics of vegetation and substrate, rather than in relation to the risk of flooding.

INTRODUCTION

There are only a few anecdotal reports of hibernation of amphibians in floodplains. These concern some adult specimens of frogs (*Rana esculenta* complex, *Rana temporaria*) and larval specimens of newts (*Triturus vulgaris*, *Triturus cristatus*) (in: Creemers, 1991). Hibernation of *Bufo bufo* and *Bufo calamita* has never been studied within a floodplain. Outside floodplains hibernation of both species was studied by Denton & Beebee (1993); that of *Bufo bufo* by Hagström (1982) and van Gelder, Olders, Bosch & Starmans (1986); and that of *Bufo calamita* by Sinsch (1989).

An important feature distinguishing floodplains from other ecosystems is the occurrence of flooding. Amphibians starting hibernation in a dry place, may be flooded during winter. This study reports on the selection of hibernation sites of *Bufo bufo* and *Bufo calamita* in a river floodplain in the Netherlands.

MATERIALS AND METHODS

THE STUDY AREA

All data were collected from 1991 to 1993 in the "Kekerdomse- en Millingerwaard", a floodplain of about 420 ha near the bifurcation point of the rivers Rhine and Waal (6°00' NB, 51°52' WL) in the Netherlands. Almost all floodplains in the Netherlands have been taken into intense agricultural use in the past. The floodplain studied is one of the less cultivated ones, having river dunes, oxbow lakes, small breakthrough lakes and, in the southern and western parts, also fragments of riverine willow woods. The northern and eastern parts are mainly in agricultural use. In one part of this area clay was recently excavated. In the past, two brick factories were situated on higher parts of the floodplain. From one of them the ruins of the old buildings are left. The other one is nowadays used as storage for debris, sand and pebbles.

All floodplains along the river Rhine in the Netherlands are separated from the populated hinterland by a high dyke protecting it against flooding. For agricultural purposes lower dykes have been built directly on most riverbanks to protect the floodplain itself against flooding during moderately high waters (mainly in summer), thereby decreasing the influence of the river upon floodplains. When the water overflows the lower dyke in our study area 93 % of the floodplain becomes inundated in a very short time. On average this happens once a year (calculated over the period 1901-1980), but it did not happen during our study. Sometimes also the higher, sandy parts of the floodplain become inundated. On average this happens once every ten years. Most floodings occur between December and March, during the hibernation period of most amphibian species (Bosman, 1994). Occasionally the floodplain is flooded during summer.

Seven terrestrial habitat types were distinguished within the area: "woods and bushes" (willow and poplar woods, and willow and alder bushes); "thickets" (high and dense-growing pioneer vegetations of, for example, *Urtica dioica* and *Rubus caesius*); "meadows" (all extensively grazed, lower herb vegetations); "pastures" (intensively grazed grassland); "arable fields" (predominantly maize cultures); "heaps of brick-debris" (the remainders of the brick factory); "sandy areas" (bare sand on river dunes, on the site of the former brick factory and at the bottom of recent clay pits). The waters present in the floodplain were divided into two habitat types: "permanent waters", which contain water througout the year and "temporary waters", which dry up every year. There are some houses with yards and gardens on the old brick factory site; these were not investigated in this study.

DRIFT FENCES

To locate terrestrial hibernation sites we used closed drift fences with pitfalls on the inner side (Gibbons & Semlitsch, 1982; Dodd, 1991). They were made of a plastic film strengthened with an iron mesh and enclosed an area of approximately 32 m². To prevent specimens from climbing in or out of the enclosed area, the top of the foil was folded 5 cm in and outwards at a height of 50 cm. To prevent specimens from burrowing in or out, the foil was dug 10 cm into the soil. In the enclosed area four pitfalls (10 litre buckets) were placed along the drift fence. The sampled heap of debris was about 40 m in diameter, so the drift fence could not be closed. To catch only amphibians wintering inside this heap, the two ends of the foil were placed as far as possible into the heap's centre. All drift fences were placed in position at the beginning of February and inspected daily after the breeding migration started in early March. From the end of April they were checked once a week only.

The number of drift fences in each habitat type was not proportional to the habitat extent, but depended on the variation within that habitat type, in both the higher and lower parts of the floodplain. For instance the habitat type "woods/bushes" consisted of poplar woods, willow woods, willow bushes or elder bushes whereas "arable fields" consisted of maize cultures only and therefore in the habitat type woods/bushes more drift fences were built (Table 1).

BOW-NETS

Our bow-nets consisted of a tetrahedal funnel whose small aperture led into a catching-box. An extension of the catching-box reached the water surface, allowing amphibians to breathe. The larger opening of the funnel measured about 70 cm along the base and it was about 30 cm high. Five temporary waters and five permanent waters were sampled. Each water body got one bow-net. They were installed at the end of December, checked weekly and removed at the start of the breeding migration in spring.

MICROTRANSMITTERS

With the aid of microtransmitters (van Nuland & Claus, 1981) implanted in the toads, the exact hibernation sites of six *Bufo bufo* and five *Bufo calamita* were determined. The animals were collected at night in autumn in habitats with an average flooding frequency of once a year (except one *Bufo calamita*, collected at a higher place in the floodplain). In the laboratory the transmitter was implanted surgically the day after capture. The evening of the same day the tagged animal was released at its site of capture. In the first year of study the presence of the animals in the hibernation sites was checked weekly with a hand-held receiver, in the second year the presence was monitored automatically every hour.

RESULTS

With the drift fences 15 *Bufo bufo* were caught. Together with six tagged *Bufo bufo* which hibernated terrestrially this gives 21 specimens hibernating terrestrially. Also all five tagged *Bufo calamita* hibernated terrestrially. Forty-two *Bufo calamita* were caught with the drift fences. So in total 47 *Bufo calamita* were found hibernating terrestrially. No toads were caught with the bow-nets.

Table 2 shows the flooding regimes and the substrate at the hibernation sites of both species. *Bufo bufo* hibernated equally at the higher and the lower parts of the floodplain. All six tagged specimens stayed in the lower parts where they were captured and released. *Bufo calamita* hibernated significantly more often in higher parts, i.e. parts which flood only once every ten years (Fisher's exact test, $P < 0.001$). Three out of four tagged specimens released in the lower parts migrated to the higher parts. The other one remained in the lower part in a sandy place. The fifth specimen, released at the higher part, migrated to the lower part and hibernated in a sandy place too. More, but not significantly more, *Bufo bufo* hibernated at sites with clay. *Bufo calamita* hibernated significantly more often in sandy soils (Fisher's exact test, $P < 0.001$).

Table 3 shows the results of the study with drift fences for each habitat type. Numbers were too low to make a statistical analysis. Comparing both species and taking into account the different number of drift fences per habitat type, it can be inferred that meadows were used equally by both species, woods/bushes were used more by *Bufo bufo,* and sandy areas and heaps of debris

TABLE 1. Number of drift fences placed in each habitat type per flooding frequency. - habitat not present.

Mean flooding frequency:	Every 10 years	Once a year
woods/bushes	3	4
thickets	2	2
meadows	3	3
pastures	-	1
arable fields	-	1
sandy areas	1	1
heaps of brick-debris	1	-
Total	10	12

TABLE 2. Flooding frequency and substrate of the hibernation sites of *Bufo bufo* (n=21) and *Bufo calamita* (n=47).

	Bufo bufo	*Bufo calamita*
Flooding frequency:		
every ten years	10	44
once a year	11	3
Substrate:		
sand	7	47
clay	14	0

TABLE 3. Number of *Bufo bufo* and *Bufo calamita* emerged from hibernation per habitat type.

Habitat type	No. of drift fences	*B. bufo* (n=15)	*B. calamita* (n=42)
woods/bushes	7	6	0
thickets	4	1	0
meadows	6	7	6
pastures	1	0	0
arable fields	1	0	0
sandy areas	2	0	2
brick-debris	1	1	34

more by *Bufo calamita*. No specimens were found in pastures and arable fields.

All six tagged *Bufo bufo* hibernated in woods/bushes where they were originally captured and released. Three tagged *Bufo calamita* hibernated in a heap of debris after a long migration to this habitat, the other two stayed the winter in meadows.

DISCUSSION

In this study we sampled all habitat types in one floodplain using different methods. Houses with yards were not sampled (trespassing was not allowed), but they only covered less than 1% of the floodplain. After the date of placing the drift fences the tagged animals remained at the same locations, so the results of the telemetric research confirm that the timing of placement of the drift fences was well-chosen.

Although toads generally hibernate terrestrially, observations exist (Hagström, 1982) of aquatic hibernation. Indeed, in floodplains, where terrestrial habitats often become flooded during winter, aquatic hibernation could be more common. To check for this the bow-nets were used. In winter-time amphibians are not very mobile. Nevertheless it is possible to catch them with bow-nets then, especially when the water becomes covered with ice during long frost periods. Then oxygen depletion may eventually occur (Bradford, 1983). When bow-nets are checked frequently and thereby are kept free from ice, they form places where the animals can escape from oxygen stress. In another study (unpublished) we caught a number of amphibians under such circumstances. Also in this study we captured small numbers of *Rana temporaria* and *Triturus vulgaris* in the bow-nets placed in temporary waters. As the winter of 1992-1993 was a mild one and therefore the waters were covered with ice only for a short period, some doubt is thrown on the notion of a purely terrestrial mode of hibernation in both *Bufo* species. Nevertheless aquatic hibernation will surely be exceptional. Comparing the results of the drift fences and bow-nets with those of the telemetric research shows that they are in agreement.

One special purpose of the use of microtransmitters was to study the effect of flooding during terrestrial hibernation. Due to the absence of flooding the effect of this feature remains unknown. Schmid (1965) studied the effect of hydration stress on nine American amphibian species. One, *Bufo americanus*, which is ecologically comparable with *Bufo bufo* and *Bufo calamita*, survived hydration stress for only a few days.

Of all drift fences the highest number of animals was caught in the habitat heaps of brick-debris (Table 3). Besides the species studied, frogs of the *Rana esculenta*-complex and some specimens of *Triturus vulgaris* were also caught there. The heap of debris studied, however, was not enclosed totally (see methods). So the high yield may have been caused by specimens coming from the inner part of the heap and thus enlarging the sampled area. However, in spring 1993 a totally enclosed smaller heap yielded five *Bufo bufo* and 194 *Bufo calamita*. This drift fence enclosed 224 m^2. Correction for the average surface of the drift fences of this study gives a single *Bufo bufo* and 28 *Bufo calamita*. This result strongly supports the suggested value of our heaps of debris.

As described the heaps of debris are the remnants of human activity in the area. They can be compared with stone heaps on riverbanks, as can be found locally upstream along the river Rhine. As the lower Rhine predominantly transports sand and clay this habitat is absent in the Netherlands.

In our floodplain the most important hibernation sites of *Bufo calamita* (heaps of brick-debris) are situated in a sandy area that seldom becomes flooded. The central part of this area is predominantly bare sand with some young trees of *Populus nigra* and an open pioneer vegetation. At the edges there are some older poplar woods. In a gravel pit Sinsch (1989) found hibernating *Bufo calamita* for the greater part in "Sandy slopes without vegetation". Indeed, the central part of the sandy area, where the heap of brick-debris was situated, seems to correspond with that habitat.

The results of this study show that *Bufo calamita* clearly prefers sandy places for hibernation. In floodplains these places are situated mainly on the higher parts, which seldom become flooded. But they can also be used when present in the lower parts as demonstrated by two of the tagged specimens. These hibernated there in a vegetation of *Calamagrostis epigeios*, which is typical of the higher sand dunes in a floodplain (Bosman, Bekhuis & Helmer, 1993). This suggests that the choice of a hibernation site is determined by the direct suitability of a habitat and not by its flooding frequency. The drift fence study showed that *Bufo bufo* has no preference for the different areas. It appeared to use the same habitat types in the higher and the lower parts of the floodplain (Bosman, 1994). So both *Bufo* species seem to select their hibernation sites based on the direct suitability of the habitat in the sense of vegetation and substrate, and not by its flooding frequency. Whether specimens that hibernate in the lower parts of the floodplain survive flooding during winter is not known.

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