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Effects of water acidification on the distribution pattern and the reproductive success of amphibians

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Summary. Nine amphibian species were encountered in poorly buffered waters of The Netherlands (alkalinity $\leqq 1$ meq $\cdot l^{-1}$). These soft water systems are highly sensitive to acidifying precipitation. The number of species as well as the percentage of waters which harbour amphibian populations are strongly reduced in the extremely acid pH-class ($\bar{p}H < 4.0$). The reproductive success of amphibians is negatively affected by low pH. The eggs become heavily infested with fungi (Saprolegniaceae). In acidifying systems many physico-chemical parameters are significantly correlated with the pH of the water. Strongly acidified waters are characterized by low alkalinity, conductivity and ionic content but high acidity and high concentrations of (heavy) metals and ammonium and a high relative sulphate concentration. Culture experiments with eggs and larvae of *Rana arvalis, Rana 'esculenta', Rana temporaria* and *Bufo bufo* show that apart from the pH, elevated aluminium, cadmium and ammonium contents may also affect the reproductive success of amphibians.

Key words. Acidification; pH; calcium; (heavy) metals; ammonium; amphibians; development of eggs; hatching percentage; mortality of larvae.

Introduction

During the last decades the deleterious effects of atmospheric pollution on aquatic ecosystems have become a growing environmental problem. In North America, Scandinavia and central Europe many poorly buffered water bodies have been very seriously acidified. Recent acidification of soft waters has been largely attributed to acidic precipitation resulting from anthropogenous emissions of SO_2, NO_x and NH_3[10,16,20,23,26].

Acidification of freshwater ecosystems is associated with a variety of physico-chemical and biological changes. The mechanisms of acidification appear to be very complex and, therefore, in many cases the effects cannot be ascribed to pH decrease alone. In spite of a growing interest in the acidity of the environment and its influences on commercially important components of ecosystems (i.e. fish, forest) amphibians have received little attention. It is evident, however, that amphibians add much to the ecological diversity, play an important role in food chains and are an important biological link between aquatic and terrestrial habitats[18,42]. All over the world amphibians are seriously threatened by the impact of human activity on their habitats and today many of them are suffering in small and isolated populations. Particularly during the last decades there has been a dramatic decline of amphibian populations. Acidification has been considered as a serious cause for this decline[5,15,16,18,24,29,42].

Amphibians exhibit complex life history patterns. (Sub)adults normally occupy terrestrial habitats whereas the reproductive phase of many amphibian species is completed in aquatic environments. Courtship, spawning, and the fertilization and development of eggs mainly occur in water, and the eggs always hatch into aquatic larvae. The larvae metamorphose into (sub)adults and these may return to terrestrial habitats. Many frogs, toads, salamanders and newts of the temperate zone breed in shallow waters of marshy lakes, ponds, streams and temporary pools. In areas with granitic bedrocks or poorly buffered soils many breeding sites of amphibians are acidifying[10,16,20,24,26,42]. Particularly temporary pools, which are filled solely with rain- or melt-water, become strongly acid. Therefore the aquatic life stages of amphibians are exposed to acidic circumstances in susceptible regions.

It has been suspected already for a long time that the natural distribution of amphibians may be limited by the acidity of surface waters[13]. In areas with acid bogs or pools many species are absent. Recently some field studies have established that acidification of breeding-sites leads to population reductions or restricted distribution patterns[14,15,17]. The reproductive success is negatively affected by low pH. Some laboratory studies already support these field observations[8,13,24,42]. The mechanisms which determine the distribution of amphibians and the underlying environmental factors, however, are not yet fully understood.

The present study was carried out in order to establish relationships between the distribution and reproductive success of amphibians in acidifying systems.

Material and methods

Field studies

96 soft water systems (alkalinity $\leqq 1$ meq $\cdot l^{-1}$) have been examined for possible effects of acidification on amphibians. In The Netherlands waters with low levels of alkalinity (i.e. moorland pools, some small lakes and dune pools) are restricted to the higher situated, poorly buffered, sandy soils in the southern and eastern part of the country, and the coastal dunes north of Bergen. The poorly-buffered water bodies are generally small (< 10 ha), shallow (mean depth 0.5 m), fully mixed and more or less isolated. The geographical position of the study areas is shown in figure 1. Nearly all the waters investigated are situated in nature reserves and protected against most human impacts, except for atmospheric pollution. Changes in physico-chemical parameters and aquatic biota indicate that many sampling sites have been recently acidified[9,23,33,34]. Today the selected waters reflect a good pH and alkalinity gradient, due to local differences in hydrology, buffering capacity of water and soil and acidifying deposition.

In 1983 each body of water was sampled at least three

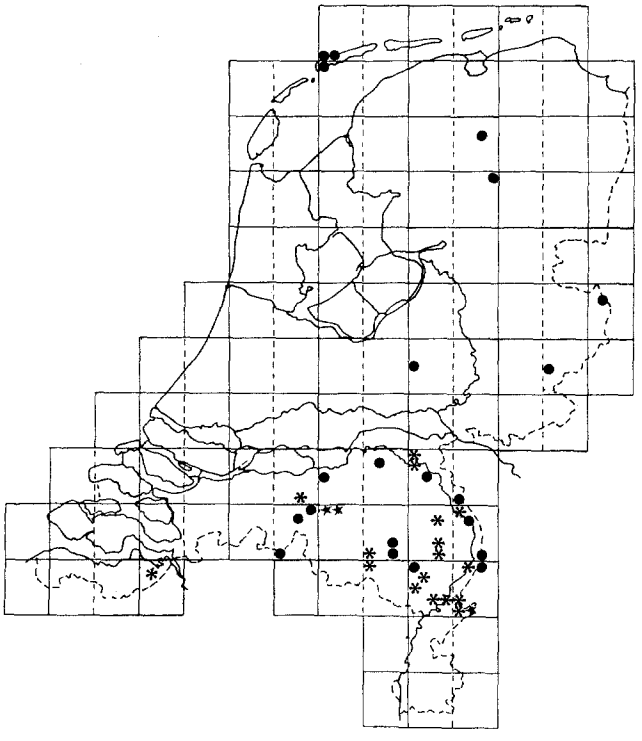

Figure 1. Geographical position of the study areas. ● Location with 1 investigated water; * Location with 2–5 investigated waters; ★ Location with more than 5 investigated waters.

times with dip-nets during the (post)spawning periods of several amphibian species (March–September). Additional data were obtained from visual and acoustical surveys and catches with seine-nets and fykes. A survey of the entire water body was made and the species presence and abundance determined. Also the abundance of egg masses, condition and developmental stage of the embryos and presence of larvae (indicating reproductive success) were investigated. In order to obtain correct determinations, some larvae and parts of egg masses were collected and raised in the laboratory until identification of the species was possible.

The pH-measurements were carried out with a Metrohm model E 488 pH-meter and a model EA 152 combined electrode. Alkalinity was estimated by titration of 100 ml of water with 0.01 M HCl down to pH 4.2 and the acidity by titration with 0.01 M NaOH up to pH 8.2. Electrical conductivity (EC) and temperature were measured with a portable conductivity meter (YSI-SCT meter, model 33). The measured conductivity data were always corrected for temperature and pH according to Vangenechten et al[44]. Oxygen measurements were carried out with a YSI model 57. Water samples were taken in two 100-ml iodated polyethylene bottles, immediatly passed through a Whatman GF/C filter and each fixed with 0.5 ml of a 200 mg·l^{-1} $HgCl_2$ solution. To one of the two samples some grains of citric acid were added in order to prevent precipitation of metals. All samples were transported to the laboratory in a refrigerated container. At the laboratory, the water samples were stored at −20 °C until analysis. For all samples, calcium, magnesium, manganese and iron were estimated with a Beckman model 1272 Atomic Absorption Spectrophotometer and aluminium, cadmium and lead with an IL Video AA:AE Spectrophotometer. Sodium, potassium and chloride were determined flame-photometrically and sulphate gravimetrically using a Technicon I Autoanalyzer. Orthophosphate, nitrate, ammonia and silicon were analysed colorimetrically with a Technicon II Autoanalyzer. The Dissolved Organic Carbon (DOC) measurements were carried out using an Oceanography International Model 05255 HR infrared carbon analyzer.

Experimental studies

In the laboratory fully-controlled culture experiments were conducted in order to study the effects of several chemical factors (pH, Al^{3+}, Cd^{2+}, NH_4^+, Ca^{2+}) on embryonic or larval mortality of amphibians. In each experiment, only one factor or a certain combination of factors was varied.

The technical equipment for the culture experiments is shown in figure 2. All experiments were conducted in glass containers (25 × 25 × 31 cm), which were placed in a stainless steel water-bath. The temperature was maintained at 15 °C or 20 °C by means of a Neslab-type Coolflow 75 cooling aggregate. The culture medium was continuously refreshed (1 l·h^{-1}) from black polyethylene 120-l stock containers by means of Colora-type 3610 multi-channel peristaltic pumps. Black silicone tubing was used in order to prevent algal growth.

The laboratory studies were performed with eggs and larvae of four amphibian species i.e. the frogs *Rana arvalis* (Nilsson), *Rana temporaria* L., *Rana 'esculenta'* L. and the toad *Bufo bufo* (L.). Only eggs in early stages of cleavage, young larvae and metamorphosing tadpoles were used for the bioassays. Freshly-laid egg masses and metamorphosing tadpoles were collected from the 'Roelofsven' near Nijmegen. The chemical composition of this moorland pool is given in table 1. During many years high population densities and normal development of embryos and larvae of several amphibian species were observed in this poorly buffered water. The young larvae were always obtained from artificially bred eggs. After hatching, these larvae were fed with a mixture of stinging powder and agar.

Egg masses or larvae were always divided into several groups (20–50 eggs or larvae) and placed in the aquaria

Table 1. The chemical composition of the 'Roelofsven' near Nijmegen (Average values 1983/1984)

pH	5.0	NH_4^+ (μmol·l^{-1})	59	Mg^{2+} (μmol·l^{-1})	58
Alkalinity (meq·l^{-1})	0.081	PO_4^{3-} (μmol·l^{-1})	0.52	Fe^{2+} (μmol·l^{-1})	7.2
Acidity (meq·l^{-1})	0.230	SO_4^{2-} (μmol·l^{-1})	165	Mn^+ (μmol·l^{-1})	4.4
$EC_{18°C}^{H^+}$ (μS·cm^{-1})	71	Na^+ (μmol·l^{-1})	210	Al^{3+} (μmol·l^{-1})	9.2
Cl^- (μmol·l^{-1})	275	K^+ (μmol·l^{-1})	73	Cd^{2+} (nmol·l^{-1})	1.1
NO_3^- (μmol·l^{-1})	5.0	Ca^{2+} (μmol·l^{-1})	73	Pb (nmol·l^{-1})	15

Table 2. The amphibian species, media and gradients which were used in the different culture experiments

Experiment	Species	Eggs	Young larvae	Metamorphosing larvae	Media	Gradients
1	*R. temporaria*	×	–	–	Habitat water	pH/alkalinity
	R. arvalis	×	–	–		
	R. 'esculenta'	×	–	–		
	B. bufo	×	–	–		
2	*R. temporaria*	×	×	–	Basic culture medium	pH/aluminium
	R. arvalis	–	–	×		
	R. 'esculenta'	–	–	×		
	B. bufo	–	–	×		
3	*R. temporaria*	×	×	–	Basic culture medium	pH/cadmium
4	*R. temporaria*	×	×	–	Basic culture medium	pH/ammonium
	R. arvalis	–	–	×		
	R. 'esculenta'	–	–	×		
	B. bufo	–	–	×		
5	*R. temporaria*	×	×	–	Low calcium medium	pH/calcium

with different culture media. Because the test objects were limited, most bioassays were performed using 1–4 replicates. All tested eggs remained within the jelly mass. The eggs and larvae were examined daily to observe embryonic or larval development and to remove dead animals. The experiments with embryos were terminated when all eggs had either hatched or died. Bioassays with larval stages always lasted three weeks. Identification of developmental stages was carried out with the tables of Witschi[45]. At the end of each experiment the cumulative mortality percentage of embryos or larvae was calculated. Abnormalities were determined by rough morhological examination.

In this paper five series of experiments are described (table 2). For all bioassays (exept experiment 1) a synthetic culture medium was used (table 3), based on the addition of certain chemicals to twice-demineralized water, in order to simulate the hydrochemistry of an undisturbed poorly buffered water in The Netherlands[23,33,34]. In experiment 1 an eight-step pH/alkalinity gradient was created by adding H_2SO_4 or NaOH to habitat water. Three-step pH gradients were used in all other experiments. In experiment 2 the basic culture media were enriched with 0, 7.4, 92.5, or 185.0 $\mu mol \cdot l^{-1}$ aluminium by adding $AlCl_3$. The media used in experiment 3 were enriched with 0, 2.5, 17.5, and 35.0 $nmol \cdot l^{-1}$ cadmium by adding $Cd(NO_3)_2$ to the basic culture medium. The effects of high ammonium concentrations (1000 $\mu mol \cdot l^{-1}$) at several pH-levels of the basic culture medium were studied in experiment 4. Experiment 5 was performed in order to study the effects of low calcium media on the reproductive success of amphibians. In the containers with stock media the pH was measured daily and if necessary adjusted with H_2SO_4 or NaOH.

Table 3. The chemical composition of basic culture media with normal and low calcium content

Major components ($\mu mol \cdot l^{-1}$)		Other components ($\mu mol \cdot l^{-1}$)	
Na^+	660	Sr	0.210
K^+	14	F	0.100
Mg^{2+}	75	Br	1.380
Ca^{2+}	525 or 65	I	0.005
Cl^-	1795 or 875	P	0.001
SO_4^{2-}	40	+ Trace elements	
HCO_3^-	3		

Results

Field observations

During the field surveys nine amphibian species were encountered in the poorly buffered waters of the Netherlands. Table 4 illustrates that populations of *Rana arvalis, Rana 'esculenta', Rana temporaria, Bufo bufo* and *Triturus helveticus* occur frequently in the soft waters, while populations of *Bufo calamita, Triturus vulgaris, Triturus aljpestris* and *Triturus cristatus* are sparse. *Pelobatus fuscus* also occurs in low alkaline waters of The Netherlands[22,41], but this species has not been noticed in the studied sites. The adults of most frogs, toads and newts occur in aquatic habitats with a rather wide range of pH and alkalinity. However, in strongly acidified systems the percentage of waters which harbour amphibian populations is much lower than in moderately acid and alkaline waters. The percentage of waters with *Rana arvalis, Triturus helveticus* and *Triturus alpestris* populations is highest for the moderately acid waters, whereas

Table 4. The relation between the pH and the percentage of soft water ecosystems which harbour amphibian populations

Species	Circum neutral and alkaline waters $\bar{p}H \geqq 5$ (n = 37) %	Moderately acid waters $4 \leqq \bar{p}H < 5$ (n = 31) %	Extremely acid waters $\bar{p}H < 4$ (n = 28) %
1 *Bufo bufo*	84.4	57.5	11.8
2 *Bufo calamita*	13.3	3.0	0.0
3 *Rana arvalis*	21.9	62.5	35.3
4 *Rana 'esculenta'*	76.6	76.9	63.3
5 *Rana temporaria*	68.8	55.0	17.7
6 *Triturus alpestris*	9.1	9.7	0.0
7 *Triturus cristatus*	8.1	6.5	0.0
8 *Triturus helveticus*	21.6	32.3	10.7
9 *Triturus vulgaris*	18.9	6.5	0.0

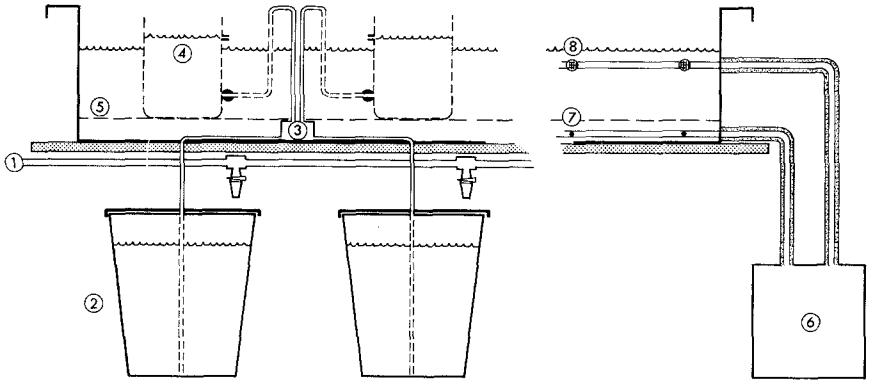

Figure 2. Experimental set-up for culture experiments (according to Roelofs et al.[34]), with: (1) demi-water supply; (2) polyethylene stock containers; (3) peristaltic pumps; (4) glass aquaria; (5) waterbath; (6) cooling and heating aggregate; (7) in-flow and (8) out-flow openings.

for the other amphibian species this percentage is highest at a pH above 5. As most waters were visited only a few times, it is difficult to estimate the exact population density of adult amphibians. Well developed populations of amphibians were only found in the slightly acid and alkaline waters.

Detailed results of field observations of *Rana arvalis* are shown in figure 3. Most populations of this frog occur in soft waters with a pH between 3.5 and 6.0 (fig. 3a). Spawning of *Rana arvalis* is observed over a wide pH-range; however, the eggs are mainly deposited in waters with a pH between 3.5 and 5.5 (fig. 3b). More than 50% of the egg masses have been found in waters with a pH between 4.0 and 4.5. Figure 3c shows that the average number of egg masses per body of water decreased with decreasing pH of the spawning sites. Because each frog normally deposits only one clump of spawn this may indicate that the population size also decreases with increasing acidity. Unfortunately the range in the observed number of egg masses is rather wide. The mortality rate of the eggs rises steeply to 95% when the pH of the water drops from 5.0 to 3.5. Application of SAS probit analysis shows that 50% of the eggs will die in waters with a pH of 4.2 (LC_{50}). In waters with a pH of 3.2 (LC_{100}) the mortality may amount to 100%. In acidic waters the egg masses of *Rana arvalis* become heavily infested with fungi (Saprolegniaceae). The same phenomenon is observed for egg masses of *Rana 'esculenta', Rana temporaria, Bufo bufo* and *Bufo calamita*. As newts deposit their eggs solitary, these are difficult to find; consequently too few data have been obtained for reliable analysis.

At many spawning sites of *Rana arvalis, Rana 'esculenta', Rana temporaria* and *Bufo bufo* no tadpoles were encountered if the pH of the water was below 5.0 (table 5). This also indicates that the reproductive success must be considerably reduced in acid waters.

During the field surveys several water samples were taken at the spawning sites of amphibians and analysed in the laboratory. Statistical analysis of the physico-chemical parameters of these sites shows that most parameters were significantly correlated with pH (table 6). Many factors i.e. alkalinity, conductivity, total phosphorus, dissolved organic carbon and several anions and cations exhibit significant positive Spearman rank correlations with the pH, which means that these parameters decrease with decreasing pH. The acidity, the sulphate/chloride ratio and the ammonium, manganese, aluminium and cadmium content of the waters show significant negative correlations with the pH. So, the spawning sites with low pH are characterized by low ionic content and a low level

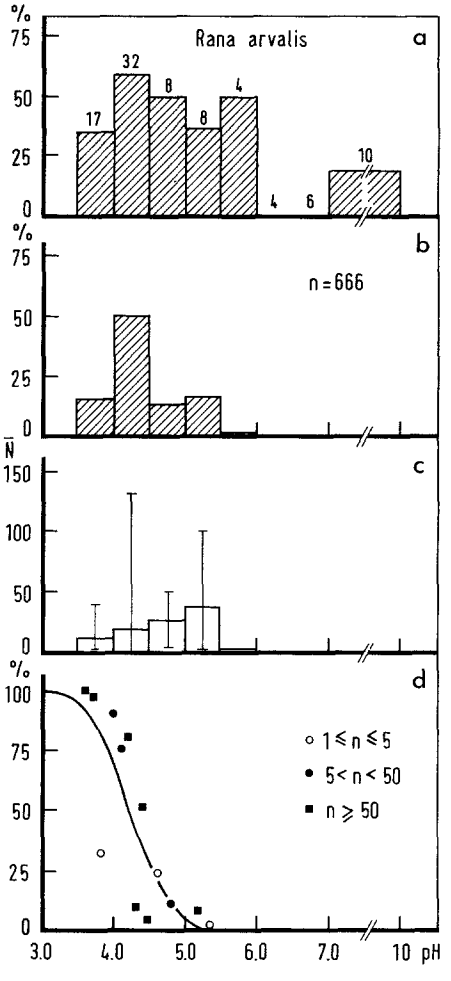

Figure 3. Field observations of *Rana arvalis* populations in poorly buffered waters. *a* The relation between the pH and percentage of soft water ecosystems in which adult specimens were caught. The numbers above the bars indicate the total number of waters which were examined in a given pH-range; *b* The relative percentage of egg masses in a given pH-class; *c* The mean number of egg masses in several pH-classes. The lines within the bars indicate the range of observations of egg masses; *d* The mean mortality rate of eggs in relation to the pH of the spawning site. The theoretical curve is calculated with SAS probit analysis and the different symbols indicate the numbers of clumps of spawn (n).

of dissolved organic carbon and by high relative concentrations of sulphate, and high contents of ammonia and (heavy) metals. Thus, apart from pH, some other environmental factors may be involved in the reduction of the reproductive success of amphibians under acid circumstances.

Experimental studies

In order to determine the importance of some pH-correlated factors (aluminium, cadmium, ammonium and calcium) for the reproductive success of amphibians, several bioassays have been conducted. The results of experiment 1 show that the egg mortality of four amphibian species rises steeply when the pH of the habitat water decreases below 4.5 (fig. 4). In media with a pH below 3.5 all eggs died. There are only small differences in the responses of the four species to acidified media. The mortality rate of *Rana 'esculenta'* eggs show remarkable fluctuations in media with a pH between 4.5 and 7.0. These fluctuations have also been observed in field studies.

Detailed studies with freshly laid eggs of *Rana temporaria* illustrate that in the basic culture medium, with a pH of 4.0, 75.9% of the eggs died in stages 7–12 (fig. 5). Only 3.4% of the embryos hatched, and most hatchlings were deformed. At a pH of 4.5 or 5.0 most eggs hatched normally, only a few hatchlings were deformed and the egg mortality manly occurred at older developmental stages. The eggs did not swell and consequently the body movements of the embryos were reduced, resulting in crooked vertebral columns in the embryos.

In experiment 2 the mortality of *Rana temporaria* eggs was studied in relation to the aluminium content and pH of the basic culture medium (fig. 6). The mean mortality percentages increased significantly (Student's t-test, $p < 0.05$) if the aluminium content of the culture media was raised to 92.5 or 185 $\mu mol \cdot l^{-1}$. Particularly in the media with a pH of 4.5 or 5.0 the mortality rate rose steeply when the aluminium concentration was elevated from 7.4 to 92.5 $\mu mol \cdot l^{-1}$. In media with a pH of 4.0 and high aluminium concentrations all eggs died. In the less acid media with a high aluminium content a low percentage of the eggs hatched, but all larvae showed abnormalities. With SAS probit analysis it has been calculated that in media with a pH of 4.5 and 5.0 the LC_{50} values may be expected at aluminium concentrations of 38.0 and 87.4 $\mu mol \cdot l^{-1}$ respectively.

Experiment 3 shows that elevation of the cadmium content (2.5–35 $nmol \cdot l^{-1}$) in the culture media did not significantly affect the mortality rate of *Rana temporaria* eggs (fig. 7). However, the standard deviations in the data are large. A strong enrichment of the culture media with ammonium (experiment 4) or reduction of the calcium content (experiment 5) did not result in higher mortality rates for the eggs either (table 7).

Performance of the same bioassays with young larvae of *Rana temporaria* showed that high aluminium, cadmium and ammonium concentrations in the culture media caused an increase of the mortality percentages (fig. 8). The toxicity of aluminium and of cadmium seem to increase with increasing pH, whereas ammonium seems to be more toxic at low pH. Low calcium content of the media did not effect larval mortality.

The results of the bioassays with metamorphosing tadpoles of *Rana arvalis, Rana 'esculenta'* and *Bufo bufo* are summarized in table 8. The mortality rates of these larvae may also increase if aluminium or ammonium is added to the culture media. Particularly the metamorphosing tadpoles of *Rana 'esculenta'* seem to be highly sensitive to aluminium concentrations. For *Rana arvalis* and *Bufo bufo* the toxicity of aluminium increased with increasing pH, as has already been described for young larvae of *Rana temporaria.*

The laboratory studies indicate that apart from pH also the aluminium, cadmium and ammonium contents of the spawning sites may be involved in the reduction of the reproductive success under acidic circumstances.

Discussion

It has been established during several field surveys that the distribution and abundance of amphibian species may be limited by the acidity of the surface waters[1,4,6,13–15,21–25,31,36,41,42]. This study has also shown that

Table 5. The number (n) of spawning sites with a pH below 5.0 and the percentage of sites where larvae were observed in 1983

Species	n	%
Rana arvalis	27	48.2
Rana 'esculenta'	25	50.0
Rana temporaria	27	52.0
Bufo bufo	28	64.0

Table 6. The Spearman rank correlation coefficients between the yearly averages of several physico-chemical parameters and the pH of poorly buffered waters in The Netherlands

Alk.	0.939*	SO_4^{2-}	0.265*	Mn^+	−0.285*
Acid.	−0.695*	Cl^-	0.682*	Al^{3+}	−0.585*
$EC_{18°C}^{H+}$	0.758*	Na^+	0.511*	Cd^{2+}	−0.574*
PO_4^{3-}	0.429*	K^+	0.638*	Pb	−0.001
t-P	0.241*	Ca^{2+}	0.585*	O_2	0.156
NO_3^-	0.003	Mg^{2+}	0.538*	DOC	0.422*
NH_4^+	−0.265*	Si^{4+}	0.525*	SO_4^{2-}/Cl^-	−0.328*
t-N	0.025	Fe^{2+}	−0.065		

* significantly correlated ($p < 0.05$)

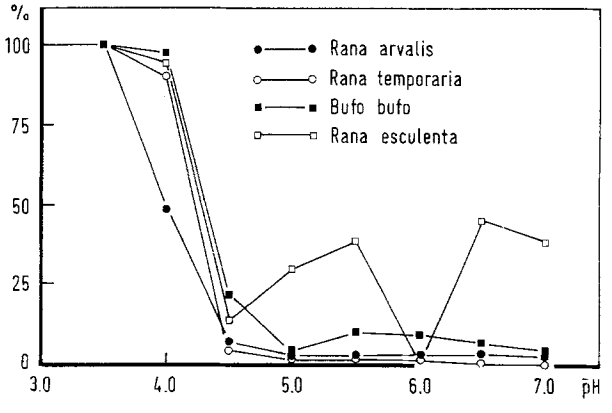

Figure 4. The egg mortality rates (%) of four amphibian species in relation to the pH of the culture medium.

Table 7. The mortality rate (mean percentage ± SD) of *Rana temporaria* eggs in different culture media

pH	n	Basic culture medium	Medium 4 $[NH_4^+]$ 1000 µmol · l^{-1}	Medium 5 $[Ca^{2+}]$ 65 µmol · l^{-1}
4.0	4	96.6 ± 2.8	98.0 ± 2.0	95.3 ± 7.0
4.5	4	16.0 ± 5.8	11.0 ± 7.6	8.2 ± 9.9
5.0	4	8.0 ± 8.5	10.5 ± 10.4	4.2 ± 2.6

the presence of amphibian populations in poorly-buffered systems depends very heavily on the pH of the water. The percentage of extremely acid waters ($\bar{p}H < 4.0$) which harbour amphibian populations is small, and the number of species in such waters is reduced in comparison with less acid habitats. It is pointed out that the egg mortality increases with decreasing pH of the spawning site. The eggs become heavily infested with fungi (Saprolegniaceae). At many acid spawning sites ($\bar{p}H < 5.0$) tadpoles have not been encountered. Several authors record similar observations[4,13–15,22–25,41,42].

So, the restricted distribution of amphibians in poorly buffered systems may be the result of the reproductive failure under acidic circumstances. Alternatively it may be that amphibians survive by choosing the less acidic waters to breed in. A study of the habitat selection of amphibians during their aquatic phase indicates that some anurans avoid oligotrophic and acid waters[41].

Recently many poorly buffered water bodies and temporary pools have been affected by the deleterious impacts of acidifying precipitation. It is evident that acidification of breeding sites leads to population reductions and the local disappearance of some species[14,15,17,18,32]. Particularly in areas with isolated water bodies amphibians are not able to escape by means of habitat selection. For instance, acidification of lake Tranevatten in Sweden (pH 4.0–4.5) resulted within a few years in the complete loss of *Rana temporaria* and an aging population of *Bufo bufo*[14,15]. In New Hampshire (USA) the artificial acidification (pH 4.0) of a section of the Hubbard Brook caused the disappearance of salamanders from the study site[17]. Acidification of breeding sites may be an important factor contributing to the recent decline in British and German frog populations[4,18,32].

During the complex life cycle of amphibians several stages may be exposed to acidic circumstances. Many adult amphibians utilize aquatic habitats for foraging and breeding. During the reproductive phase the courtship, spawning, fertilization and the development of embryos and larvae mainly occur in aquatic environments. Several laboratory studies have been performed in order to study the susceptibility of different life stages of amphibians to low pH[3,7,8,11–13,16,23,24,29–31,34–39,42,43].

The mechanism by which adult amphibians are affected by acid stress may be similar to those for fish[16]. Exposure to acid decreases the sodium influx in isolated frog skin and thereby reduces the active sodium transport. However, no significant change has been found in osmotic permeability of intact frogs *(Rana pipiens)* exposed to low pH. So, at present there is no evidence that the restricted distribution of amphibians in poorly buffered waters may be determined by the acid tolerance of adults. Moreover in some extremely acid waters adult amphibians were still encountered[4,7,21–24,41] (tables 4 and 5).

On the contrary it is evident from several laboratory studies that, in particular, the reproductive phase is strongly affected by acid stress[7,8,11–15,23,24,29–31,36–39,42]. The eggs of frogs and toads are mainly fertilized during external amplexus. Because the sperm and the eggs are shed directly into the water, they are exposed to the ambient pH before and during gamete fusion. When the pH is lower than about 6.3 *Rana temporaria* sperm is not motile[12]. The sperm motility of *Rana pipiens* also decreases with decreasing pH[39]. For this species there is no sperm motility below pH 4.5. The fertilization of *Rana pipiens* is more susceptible to low pH than activation or cleavage of the eggs[39]. Probably the eggs and sperm of salamanders are less affected because they are fertilized internally.

The experiments with fertilized *Rana temporaria* eggs show that in media with extremely low pH most eggs die in early stages of development (fig. 5). At moderate pH the mortality mainly occurs in older embryos. Eggs of *Xenopus laevis, Ambystoma jeffersonianum* and *Ambystoma maculatum* have been exposed in several developmental stages to acid stress[42]. The results of those shock experiments indicate that the mortality increases significantly during all stages of development[42]. Indices of mortality and abnormalities among embryos exposed later in their development are slightly higher than among those exposed in their initial stages. Therefore it has been suggested that the lethal effects of acid media are concen-

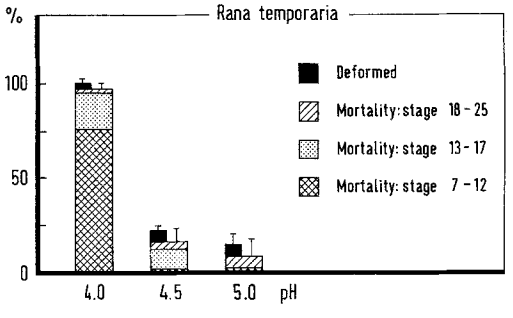

Figure 5. The mean egg mortality rate and percentage of embryos which hatched as deformed larvae (±SD) in relation to the pH of the basic culture medium (n = 4).

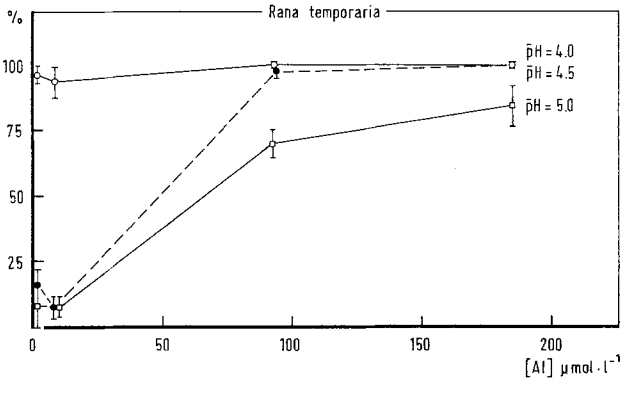

Figure 6. The mean mortality rate (±SD) of *Rana temporaria* eggs in relation to the aluminium concentration and pH of the culture medium (n = 4).

Table 8. The mortality rate (%) of metamorphosing larvae (stage 29–33)

Species	pH	Mortality percentage Basic culture medium	Medium 2 $[Al^{3+}]$ =185 µmol·l^{-1}	Medium 4 $[NH_4^+]$ =1000 µmol·l^{-1}
Rana arvalis	4.0	0.0	0.0	8.3
	4.5	0.0	75.0	8.3
	5.0	0.0	83.3	0.0
Rana 'esculenta'	4.0	6.3	100.0	33.0
	4.5	6.7	100.0	0.0
	5.0	0.0	100.0	0.0
Bufo bufo	4.0	23.8	14.3	8.3
	4.5	0.0	20.6	50.0
	5.0	0.0	47.6	16.7

trated within the superficial tissues[42]. Eggs of salamanders and frogs from ponds with a pH between 4.5 and 5.0 failed to retract yolk plugs[42]. In ponds with a pH of 5.5 the eggs developed normally, but subsequently the embryos developed stunted gills and swellings of the body wall near the heart[42]. One feature characteristic for anuran and urodelan embryos, exposed to moderate acid stress, is the failure of the perivitelline space to absorb normal quantities of water[11,13,30,37,42] (this study). This leads to a tight coiling of the enclosed embryo and the total or partial failure of hatching. This process apparently does not involve physiological changes detrimental to embryos since normal development occurred after removal of the membranes and jelly coats[11]. It can be concluded that embryos exposed to media with sublethal pH are subject to a specific blockage of hatching[11]. The process of hatching in amphibians is supposed to involve several phases[3,27]. An increase in volume and perivitelline fluid ruptures certain layers of the jelly coat. Then the secretion of a hatching enzyme by the embryos weakens the structure of the surrounding membranes. In concert with embryonic movements this process results in hatching. In vitro, a complete inhibition of the hatching enzyme of *Xenopus laevis* occurs in media with a pH near 4.0[43]. It is possible that the inhibition of hatching in acid media is related to a blockage in the functioning of the hatching enzyme, or a related enzyme critical for the hatching process[11]. In fishes, the normal enzymatic breakdown of the chorion is also disrupted at low pH[28,35,40]. Alternatively the lower activity levels of the embryos at low pH may result in failure to rupture the outer capsule membrane[28]. Acid stress may also induce some changes in the physical structure of the outer membrane, making it more difficult to break[28].

In addition, young and metamorphosing larvae of amphibians seem to be sensitive to acid stress (fig. 8, table 8). However, ontogenetic changes may occur in acid tolerance. Young larvae of *Rana sylvatica* were more acid tolerant than embryos and this tolerance appeared to increase during larval development[29]. In the present study the same phenomenon has been observed for *Rana arvalis, Rana 'esculenta', Rana temporaria* and *Bufo bufo*. Caution must be exercised, however, in drawing conclusions from data on field-collected metamorphosing larvae[29]. If natural selection had previously eliminated larvae with little acid tolerance, then the survivers that were collected might display a higher tolerance than the initial unselected population[29].

The acid tolerance of amphibians seems to be more or less species specific[13,42]. The tolerance of amphibian embryos for acid media is summarized in table 9. Although a variety of methods was used to determine acid tolerance, the variations in the data are small. There appear to be only slight differences in the sensitivity between several families and species. In most instances a pH between 4.0 and 5.0 is critical (about 50% mortality) and a pH below 4.0 lethal (complete mortality). Some amphibian species appear to be able to evolve a certain degree of tolerance to acid media (table 9). Probably long-term acid tolerance may have evolved in populations of salamanders[25,42]. However, the mechanisms of genetically controlled acid tolerance have not yet been fully examined.

Acidification of poorly buffered waters is associated with a variety of physico-chemical and biological changes[10,20,23,26,33,34]. Table 1 shows that many physico-chemical parameters of spawning sites are significantly correlated with the pH of the water. It should be clear that not the pH per se but also the changes in other environmental factors may be involved in the reproductive success or distribution pattern of amphibians. Culture experiments, with *Rana temporaria, Rana arvalis, Rana 'esculenta'* and *Bufo bufo* have been performed in order to elucidate the importance of some other pH-correlated environmental factors (table 2). These experiments have shown that apart from the pH, the aluminium content of the spawning site may also determine the mortality rate of amphibian eggs. The larval mortality was

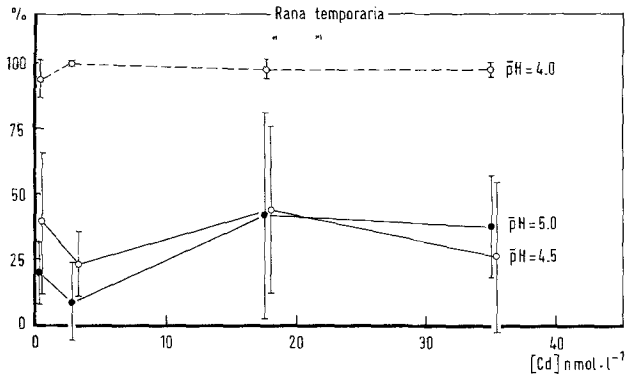

Figure 7. The mean mortality rate (±SD) of *Rana temporaria* eggs in relation to the cadmium content and pH of the culture medium (n = 4).

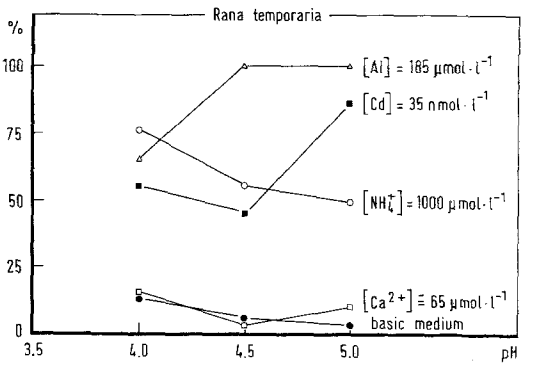

Figure 8. Mortality rates (%) of young larvae in relation to the pH of several culture media.

Table 9. Tolerance of amphibian embryos for acid media. Data from laboratory and/or field studies

Species	Lethal pH (Complete mortality)	Critical pH (50% mortality)	Source
Hylidae			
Acris gryllus	4.0–4.1	4.2–4.6	13
Hyla andersoni	3.4	3.6–3.8	13
Hyla crucifer	3.8	4.0–4.2	13
Hyla versicolor	3.8	3.9–4.3	13
Pseudacris nigrita	3.8	3.9–4.1	13
Pipidae			
Xenopus laevis	3.0–4.3	3.5–4.7	11,36,42
Ranidae			
Rana arvalis	3.5–4.3	4.0–4.5	*,41
Rana catesbeiana	3.9	4.1–4.3	13,36
Rana clamitans	3.7–3.8	3.8–4.3	13,36
Rana 'esculenta'	3.5–4.0	4.0–4.5	*,41
Rana palustris	3.9–4.0	4.2–4.3	13
Rana pipiens	5.5–5.8	–	39
Rana sylvatica	3.5	3.5–3.9	13,21,30,42
Rana temporaria	3.5–4.0	4.0–4.5	*,41
Rana utricularia	3.7	3.9–4.1	13
Rana virgatipes	3.4	3.6–3.8	13
Bufonidae			
Bufo bufo	3.5–4.0	4.0–4.5	*,41
Bufo calamita	< 3.8	–	41
Pelobatidae			
Pelobatus fuscus	4.5	–	41
Ambystomatidae			
Ambystoma jeffersonianum	4.0–5.0	4.0–4.6	5,31
Ambystoma maculatum	4.0–5.0	5.0–7.0	5,30,31

*This study.

significantly affected by the pH, the aluminium-, the cadmium- and also the ammonium content of the culture medium.

Only a few other studies elucidate the importance of pH-correlated factors. Assays using eggs of *Xenopus laevis* indicate that elevated aluminium concentrations result in higher mortality percentages at low pH[7,8]. Larvae of the salamander *Leurognathus marmoratus* were placed in containers in a creek above and below a pyritic road fill. Above the fill creek, pH was 6.9–7.2 and aluminium concentration less than 0.37 $\mu mol \cdot l^{-1}$. Below the fill the pH was 4.5–4.9 and the aluminium concentration 37 $\mu mol \cdot l^{-1}$. No larvae died above the fill. Although the pH below the fill was moderately acid most larvae died[19]. This field experiment also indicates that the increased aluminium concentration may contribute to the mortality rate.

Additions of calcium to culture media at an otherwise lethal pH may facilitate hatching of *Xenopus laevis*. However, in experiment 5 calcium concentrations were varied within the normal range of poorly buffered systems (65–525 $\mu mol \cdot l^{-1}$) and no significant differences in the mortality of *Rana temporaria* eggs and larvae occurred.

Acidification of fresh waters causes deleterious changes in macrophyte communities[33,34]. The nature and structure of plant communities may play an important role in the habitat selection of amphibians[41]. Many amphibian species attach their spawn to the submerged vegetation. Acidifying systems often become dominated by mosses (*Sphagnum* spp.)[33,34]. Several authors have reported that *Sphagnum* negatively affected the reproductive success of amphibians[2,14,15,36]. Plant phenolics are known to be toxic to animals, and many bog plants (including *Sphagnum*) are known to be rich in these compounds[36].

In the acidifying lake Tranevatten (Sweden) with a pH between 4.0–4.5 *Triturus vulgaris* has increased remarkably[14,15]. In The Netherlands *Triturus helveticus, T. alpestris* and *Rana arvalis* occur relatively more in moderately acid waters than in less acid systems. Probably these amphibians are favoured in these lakes, due to the decreased or extinct fish fauna. Thus, the profound changes in the structure of the vegetation, but also in predator-prey relations, may also have a large influence on the presence and abundance of amphibians. However, continuing acidification, and the related changes in the physico-chemical environment, will finally lead to the extinction of all amphibians from the acidic systems.

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1 Beebee, T.J.C., and Griffin, J.R., A preliminary investigation into Natherjack toad *(Bufo calamita)* breeding site characteristics in Britain. J. Zool. Lond. *181* (1977) 341–350.
2 Brunström, B., Effects of egg hatching and early development of *Rana temporaria* and *R. arvalis* in waters of different pH values and ion contents. Snoken *8* (1978) 117–125 (in Swedish).
3 Carroll, E.J. Jr., and Hedrick, J.L., Hatching in the toad *Xenopus laevis:* morphological events and evidence for a hatching enzyme. Devl Biol. *38* (1974) 1–13.
4 Clausnitzer, H.J., Durch Umwelteinflüsse gestörte Entwicklung beim Laich des Moorfrosches (*Rana arvalis* L.). Beitr. Naturk. Niedersachs. *32* (1979) 68–78.
5 Cook, R.P., Effects of acid precipitation on embryonic mortality of spotted salamanders *(Ambystoma maculatum)* and Jefferson salamanders *(Ambystoma jeffersonianum)* in the Connecticut Valley of Massachusetts. Thesis, University of Massachusetts, Amherst 1978.
6 Cooke, A.S., and Frazer J.F.D., Characteristics of newt breeding sites. J. Zool. Lond. *178* (1976) 223–236.
7 Dale, J., and Freedman, B., Effects of acidity on Nova Scotia amphibians, in: Workshop Proceedings Kejimkujik Calibrated Catchments Program, p. 71. Ed. J. Kerekes. Life Science Centre, Dalhousie University, Halifax, Nova Scotia 1983.
8 Dale, J., and Freedman, B., Effects of acidity on Nova Scotia amphibians. First Annual Report. Prepared for the Canadian Wildlife Service 1983.
9 Dam, H. van, Suurmond, G., and Braak, C.J.F. ter, Impact of acidification on diatoms and chemistry of Dutch moorland pools. Hydrobiologia *83* (1981) 425–459.
10 Drabløs, D., and Tollan, A. (eds), Ecological Impact of Acid Precipitation. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project, Oslo-Aas 1980.
11 Dunson, W.A., and Connell, J., Specific inhibition of hatching in amphibian embryos by low pH. J. Herpetol. *16* (1982) 314–316.
12 Gellhorn, E., Studien zur Ionenphysiologie an den Spermatozoen und Eiern von *Strongylocentrotus lividus*. Pflügers Arch. ges. Physiol. *216* (1927) 198–219.
13 Gosner, K.L., and Black, I.H., The effects of acidity on the development and hatching of New Jersey frogs. Ecology *38* (1957) 256–262.
14 Hagström, T., The extinction of frogs in a lake acidified by atmospheric pollution. Sver. Nat. *11* (1977) 367–369 (in Swedish).
15 Hagström, T., Reproductive strategy and success of amphibians in waters acidified by atmospheric pollution. Proc. Eur. Herp. Symp. C.W.L.P. Oxford (1980) 55–57.
16 Haines, T.H., Acidic precipitation and its consequences for aquatic systems: a review. Trans. Amer. Fish. Soc. *110* (1981) 669–707.
17 Hall, R.J., and Likens, G.E., Ecological effects of experimental acidification on a stream ecosystem, in: Ecological Impact of Acid Precipitation, pp. 375–376. Eds D. Drabløs and A. Tollan. Proc. Int.

Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project, Oslo–Aas 1980.
18 Honegger, R. E., Threatened amphibians and reptiles in Europe. Akademische Verlags Gesellschaft, Wiesbaden 1981.
19 Huckabee, J. W., Goodyear, C. P., and Jones, R. D., Acid rock in the Great Smokies: Unanticipated impact on aquatic biota of road construction in regions of sulfide miniralization. Trans. Am. Fish. Soc. *104* (1975) 677–684.
20 Johnson, R. E., Ed., Acid rain/Fisheries. Proc. Int. Symp. Acid Precip. Fish. Imp., American Fisheries Society, Bethesda, Maryland 1982.
21 Karns, D. R., Toxic bog water in northern Minnesota peatlands: ecological and evolutionary consequences for breeding amphibians. Ph. D. Thesis, University of Minnesota 1983.
22 Lenders, A. J. W., Het voorkomen van de Knoflookpad (Pelobates fuscus [Laurenti]) in relatie met de zuurgraad van het voortplantingswater. Natuurh. Maandbl. *73* (1984) 30–35.
23 Leuven, R. S. E. W., and Schuurkes, J. A. A. R., Effecten van zure, stikstof- en zwavelhoudende neerslag op kalk en voedselarme wateren, vol. 47, pp. 1–131. Ministry of Housing, Physical Planning and Environment. Publikatiereeks Lucht 1985.
24 Leuven, R. S. E. W., Hartog, C. den, Christiaans, M. M. C., and Oyen, F. G. F., Effects of acid precipitation on amphibians and fish, in: Proc. Acid. Precip. Symp. 's-Hertogenbosch, pp. 184–186. Eds E. H. Adema and J. van Ham, Pudoc, Wageningen 1984.
25 Nielsen, C. L., Pough, F. H., and Gorn, J. A., The effect of acid precipitation on reproduction in salamanders: egg transplantation studies. Am. Zool. *17* (1977) 947.
26 Overrein, L. N., Seip, H. M., and Tollan A., Acid precipitation – effects on forest and fish. Final report of the SNSF project 1972–1980. Oslo–Aas 1981.
27 Petranka, J. W., Just, J. J., and Crawford, E. C., Hatching of amphibian embryos: the physiological trigger. Science *217* (1982) 257–259.
28 Peterson, R. H., Daye, P. G., and Metcalfe, J. L., The effects of low pH on hatching of Atlantic salmon eggs, in: Ecological Impact of Acid Precipitation, p. 328. Eds D. Drabløs and A. Tollan. Proc. Int. Conf. Ecol. Impact Acid Precip., Norway 1980, SNSF project, Oslo–Aas 1980.
29 Pierce, B. A., Hoskins, J. B., and Epstein, E., Acid tolerance in Connecticut wood frogs *(Rana sylvatica)*. J. Herpetol. *18* (1984) 159–167.
30 Pough, F. H., Acid precipitation and embryonic mortality of spotted salamanders, *Ambystoma maculatum*. Science *192* (1976) 68–72.
31 Pough, F. H., and Wilson, R. E., Acid precipitation and reproductive success of *Ambystoma* salamanders. Water, Air Soil Poliut. *7* (1977) 531–544.
32 Prescot, I., Cooke, A. S., and Corbett, K. F., in: The Changing Flora and Fauna of Britain, pp. 229–254. Ed. D. L. Hawksworth. Systematics Association special volume No. 6, Academic Press, London 1974.
33 Roelofs, J. G. M., Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. I. Field observations. Aquat. Bot. *17* (1983) 139–155.
34 Roelofs, J. G. M., Schuurkes, J. A. A. R., and Smits, A. J. M., Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. Aquat. Bot. *18* (1984) 389–411.
35 Runn, P., Johansson N., and Milbrink, G., Some effects of low pH on the hatchability of eggs of perch, *Perca fluviatilis*. L. Zoon *5* (1977) 115–125.
36 Saber, P. A., and Dunson W. A., Toxicity of bog water to embryonic and larval anuran amphibians. J. expl Zool. *204* (1978) 33–42.
37 Salthe, S. N., Increase in volume of the perivitelline chamber during development of *Rana pipiens* Schreber. Physiol. Zool. *38* (1965) 80–98.
38 Salthe, S. N., and Mecham, J. S., Reproductive and courtship patterns, in: Physiology of the Amphibia, vol. II, pp. 310–521. Ed. B. Lofts. Academic Press, New York 1964.
39 Schlichter, L., Low pH affects the fertilization and develoment of *Rana pipiens* eggs. Can. J. Zool. *59* (1980) 1693–1699.
40 Schoots, A. F. M., and Denucé, J. M., Purification and characterization of hatching enzyme of the pike *(Esox lucius)*. Int. J. Bioch. *13* (1981) 591–602.
41 Strijbosch, H, Habitat selection of amphibians during their aquatic phase. Oikos *33* (1979) 363–372.
42 Tome, M. A., and Pough, F. H., Responces of amphibians to acid precipitation, in: Acid Rain/Fisheries, pp. 245–254. Ed. R. E. Johnson. Proc. Int. Symp. Acid Precip. Fish. Imp., Am. Fish. Soc., Bethesda, Maryland 1982.
43 Urch, U. A., and Hedrick, J. L., Isolation and characterization of the hatching enzyme from the amphibian, *Xenopus laevis*. Archs Biochem. Biophys. *206* (1981) 424–431.
44 Vangenechten J. H. D., Puymbroeck, S. van, Vanderborght, O. L. J., Bosmans, F., and Deckers, H., Physico-chemistry of surface waters in the Campine region of Belgium, with special reference to acid moorland pools. Archs Hydrobiol. *90* (1981) 369–396.
45 Witschi, E., Development of Vertebrates. Sounders Company London 1956.

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The biological indication of SO_2

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Key words. Air pollution; SO_2; biological indicator; environment monitoring; plant sensitivity .

Introduction

In the course of the so-called 'acid rain' debate the question of the direct effects of SO_2 and other gaseous pollutants has gained increasing attention. Obviously, the total reaction of ecosystems is a result of the combined impact of direct effects of air pollution and of indirect ones, like those due to changes in soil properties – overlaid by climate variation and cultivation practices. Measurements of air pollutants like SO_2, NO_2 and O_3 in rural areas have been scarce until now; this complicates the task of deducing the resulting impact on e.g. terrestrial vegetation from a dose-response kind of approach, as the immission values are normally too crude or are nonexistent. Consequently, an alternative means of assessment of direct effects separately was looked for, and the application of biological indicators of SO_2 and other pollutants came into the picture. It is well-known that some plant groups possess a high sensitivity to changes in air quality, and some species react quite spe-