The distribution of *Rana temporaria* L. (Amphibia) in an acidified and a non-acidified region of Norway

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The common frog *Rana temporaria* L. was absent from a poorly buffered, anthropogenically acidified, inland area (Solhomfjell) of Southern Norway where the pH values of ponds and lakes ranged from 4.3 to 4.8 and the Al_a concentration was usually below 150 µg/L. Closer to the coast, the calcium concentration was higher and *R. temporaria* was present, although not common. Successful spawning and larval development were recorded at pH values down to 4.6 and an Al_a concentration of 100-300 µg/L. In a reference area (Høylandet) in Central Norway with similar altitude, geology and flora, and a pH in the range of 4.6 to 6.8, *R. temporaria* was very common at all pH levels. The Al_a concentration was usually below 100 µg/L. The comparison between Solhomfjell and Høylandet strongly suggests that acidification explains the absence of *R. temporaria* at Solhomfjell. This was also demonstrated indirectly by the use of multivariate analyses, although chloride (NaCl) was shown to be the most important explanatory factor for the presence of *R. temporaria* in the acidified area. Also at Høylandet, where pH was not directly limiting, the lack of salts, and also increased humus content in the water, to some degree restricted the distribution of the frog. In both areas, a pH of approximately 4.6 may be critical for *R. temporaria* populations in small, poorly buffered, boggy, water bodies like those we have investigated.

Keywords: Rana temporaria, Amphibia, acidification, habitat, hydrography, bogs, Norway

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INTRODUCTION

European amphibians experience a general decrease in abundance due to factors like drainage and filling-in, fish release, pollution of breeding sites and possibly also more diffuse environmental variables (Honegger 1981, Corbett 1989, Wake 1991, Houlahan et al. 2000; cf. Dolmen 1986, 1987). One of the most important detrimental factors regionally is anthropogenic acidification.

During the 20th century, acidic precipitation became a serious environmental problem in Scandinavia (Rodhe et al. 1995). For instance, freshwater fish in Norway, primarily brown trout *Salmo trutta*, have been seriously affected in an area of approximately 52 000 km^2 (Hesthagen et al. 1999). Southern Norway is most damaged, due to the large amounts of acidic precipitation and the low buffering capacity of the bedrock (Overrein et al. 1980, Henriksen et al. 1988, Hesthagen et al. 1999).

With regard to amphibians, Hagström (1981) reported a gradual decrease and eventually disappearance of the common frog *Rana temporaria* L. 1758 over a period of six years in a small, acidified lake in southwestern Sweden. Likewise, Hagström (1981), Fjellheim & Raddum (1985) and Andrén et al. (1988) described experiments which demonstrated decreasing hatching success, survival and growth rates of various Scandinavian anurans with decreasing pH and/or increasing aluminium levels.

In the present study, two areas were chosen to investigate the effect of long-term acidification on natural *R. temporaria* populations; one area is undergoing anthropogenic acidification while the other is not (Figure 1). The first is in Aust-Agder, Southern Norway, where monitoring and research on the effects of acidic precipitation are being performed (Økland & Eilertsen 1996, Nilssen & Wærvågen 2002). The other area, further north, in Høylandet, Nord-Trøndelag, Central Norway, is an international reference area for non-acidified conditions (cf. Dahl 1997, Blakar & Hongve 1997).

R. temporaria seems to be distributed practically all over Scandinavia, and is definitely the most common amphibian and also the most eurytopic (Gislén & Kauri 1959, Ahlén et al. 1992, Dolmen & Strand 1997, Fog et al. 1997). By using multiple regression methods, we wanted to examine whether the presence or absence of the species can be explained by various environmental factors. Of special importance was the effect of acidic precipitation. We also tried to find the natural pH limit for successful reproduction of *R. temporaria* in the two areas; this might differ from the (physiological) tolerance limit found experimentally in the laboratory. Such a "limit" would be of great importance for evaluating any detrimental effects of anthropogenic acidification on *R. temporaria*. Some of the results were briefly reported by Dolmen & Blakar (1989).

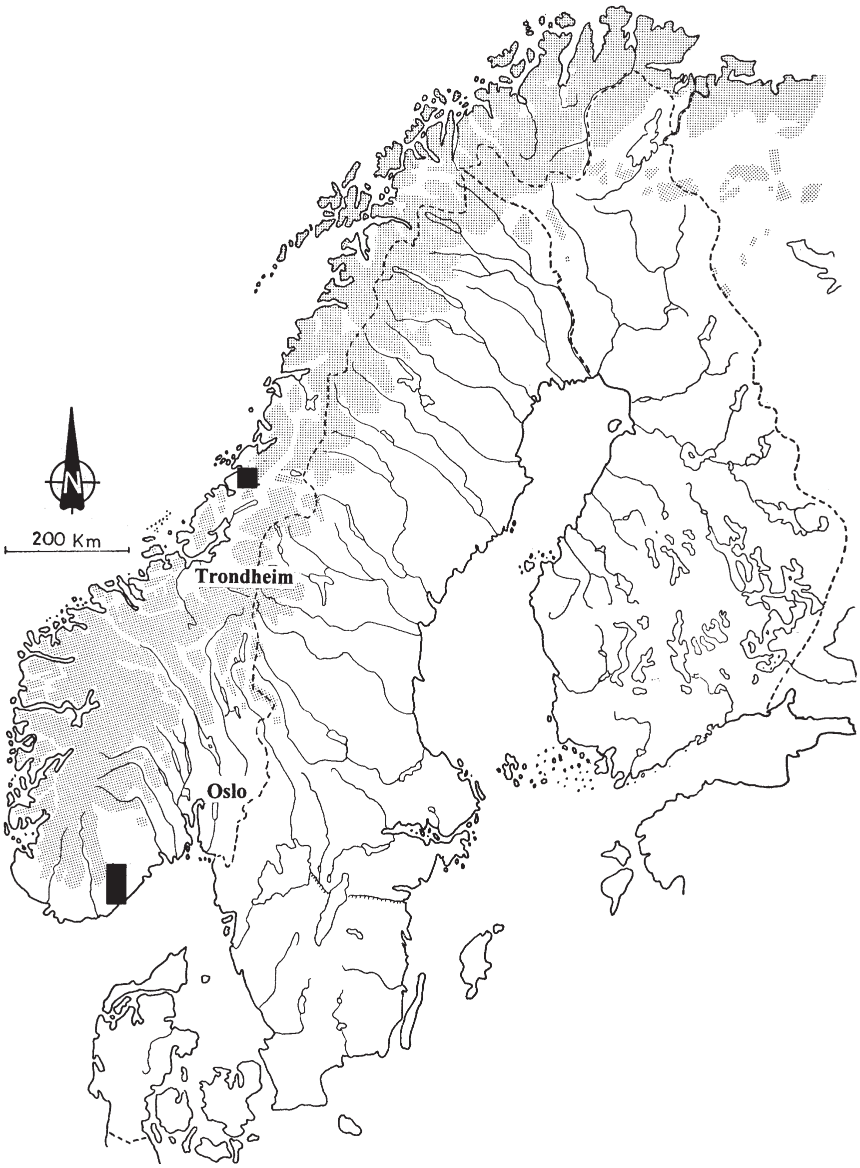

Figure 1
Map showing the locations of the areas investigated in Southern and Central Norway (black rectangles). (Arctic and alpine regions are hatched.)

STUDY AREAS

The southern area was defined along a south-north transect from the coast at Arendal to Solhomfjell, a mountain 50-60 km inland. It consists of three small sub-areas, from south to north, Moland – Froland, Vegårshei – Felle, and Solhomfjell, situated south and north of Vegår (58˚32-58′N, 8˚45′E) in Southern Norway. The bedrock consists mainly of intermediate to acidic gneisses (Sigmond et al. 1994), and the postglacial marine limit (ML) is around 100 m a.s.l. (Holtedahl & Andersen 1960). The timber line (conifer forest) is about 500-600 m a.s.l.

The northern area comprises three neighbouring sub-areas, from south to north, the Vikafjellet – Flasnesfjellet mountains, the mountains around Kovaholet, and the bogs surrounding a small lake, Røyrtjørna. The area is situated west of Høylandet (64˚35′N, 12˚05′E) in Central Norway. The bedrock consists mainly of intermediate to acidic gneisses (Sigmond et al. 1994), ML is about 150 m a.s.l., and the timber line (conifer forest) is about 300-400 m a.s.l.

Table 1 gives some details about the six sub-areas. Most of the localities were ponds and small pools and puddles in bog or bog with scattered rock outcrops, but a few tarns and small lakes were also included. Only a few (2-3) of the highest localities, about 600 m a.s.l. in both areas, were under the influence of meltwater from ice and snow when they were investigated.

The two areas are quite similar with respect to altitude, geology and flora, but the climate is more harsh in the northern area. The yearly runoffs are normally 500-1500 mm and 1000-2000 mm in the southern and northern areas, respectively (NVE 1987). The pH of the precipitation differs markedly, being below 4.3 in the southern area and above 4.9 in the northern area (Henriksen et al. 1988).

METHODS

Field investigations and water sampling were carried out on 2-7 June 1988 in the southern area, with an additional check for amphibians on 18-22 July. Sampling was carried out in the northern area on 16-19 June 1988.

Water samples were collected in clean polyethylene bottles at a depth of 10-20 cm approximately 1 m from the shore. They were kept in the dark at approximately 4^{o}C until they were analysed in the laboratory. The following parameters were analysed using the methods employed by Blakar & Hongve (1997): turbidity, natural organic matter (NOM) measured as colour (mg Pt/L or TCU), conductivity (K_{25}), pH, alkalinity, Ca, Mg, Na, K, SO_4, Cl, NO_3-N, acid-neutralising capacity (ANC) and reactive aluminium concentration (Al_a). (Some parameter data are missing for a few localities.) We also included altitude in the analyses.

Amphibians were registered by sight and by netting (cf. Dolmen 1991). About 15-30 minutes were spent at each locality. Only *R. temporaria* tadpoles, or larvae on healthy spawn, are considered, since metamorphosed frogs (1+ yrs and older) do not necessarily indicate successful spawning conditions. The number of individuals has not been dealt with here, only the occurrence or not of the frog.

The presence of *R. temporaria* in ponds and lakes can be considered to be a binomial process, in which the frog either is present with a probability p or is absent with a probability 1-p, where var(p)=p(1-p). Such data are therefore suitable for logistic regression analyses.

Using SPSS-PC 10.00, we first investigated each explanatory variable in separate tests on the probability of finding the frog present in a pond or lake. Then, to investigate the combined effect of multiple explanatory factors, we composed global models that accounted for the effects of several explanatory

Table 1. Characteristics of the sub-areas.

Area and sub-areas	Area (km^2)	Alt. (m a.s.l.)	No. of locs	pH range	pH m±SD
Southern, acidified area					
Moland – Froland	8	70-170	26	4.6-6.4	5.4±0.6
Vegårshei – Felle	5	189-330	18	4.6-6.0	4.9±0.4
Solhomfjell	10	291-580	41	4.3-4.8	4.6±0.1
Northern, non-acidified area					
Røyrtjørna surroundings	6	160-220	25	4.9-6.8	5.3±0.5
Vikafjellet – Flasnesfjellet	4	260-340	19	5.0-5.7	5.3±0.2
Kovaholet surroundings	10	190-650	54	4.6-6.1	5.3±0.3

variables on the probability of finding the frog present. We started with ten variables. After a stepwise exclusion of the least significant covariates, the final models were determined. For the analyses, we selected alkalinity, Al_a, altitude, ANC, Ca^{2+}, Cl^-, NO_3^-, H^+, NOM and SO_4^{2-}. [H^+ was chosen instead of pH since the latter is measured on a logarithmical scale.] We did not include the other five variables (see above), mainly due to strong, or fairly strong, intercorrelation among the different variables. [We excluded Mg^{2+} due to high correlation with Ca^{2+} (r^2=0.72) and Cl^- (r^2=0.59), K^+ due to high correlation with SO_4^{2-} (r^2=0.53) and Ca^{2+} (r^2=0.51), conductivity due to high correlation with Ca^{2+} (r^2=0.56), Na^+ due to high correlation with Cl^- (r^2=0.89), and turbidity due to high correlation with NOM (r^2=0.43).]

A "reduced global model" was also analysed containing only seven variables: alkalinity, Al_a, altitude, ANC, Cl^-, H^+ and NOM. Ca^{2+}, NO_3^- and SO_4^{2-} were omitted from this model because they are in the ANC calculation. Cl^-, however, was retained, since the result of the model with ten variables showed the importance of this variable.

Statistically, the two areas were analysed separately. For the multiple logistic regression analyses we only included ponds and lakes that had complete information for all the explanatory variables (see above). Hence, for these analyses, the sample size was reduced to 57 for Southern Norway and 80 for Central Norway. All tests of significance were two-tailed.

RESULTS

Water quality

The water quality regimes in both areas are illustrated in Figure 2, where some of the parameter values for each locality have been plotted against their successive locality number. In general, within each sub-area, the increasing numbers correspond with increasing altitude and (for the southern area) increasing distance from the sea (Figure 2). Except for a few relatively high values in the coastal parts of the southern area, there are relatively small differences in conductivity, NOM and turbidity (not in the figure) between the southern and the northern areas. However, more of the conductivity at Solhomfjell than at Høylandet is due to H^+ ions (cf. pH).

The decreasing gradient in conductivity from coast to inland in the southern area is mainly due to higher concentrations of Na^+ and Cl^- (NaCl from the sea) near the coast. Na^+ and Cl^- are highly correlated: r^2=0.90 and 0.92 in the southern and northern areas, respectively. In the southern area, Ca^{2+}, Mg^{2+} and K^+ (the last two are not in the figure), Al_a and SO_4^{2-} also occur in higher concentrations near the coast than inland. These ions are generally well correlated, for instance for calcium and magnesium r^2=0.86, and for calcium and potassium r^2=0.67.

The typical components of acidic precipitation, SO_4^{2-} and NO_3^-, have much higher levels in the southern area than in the northern area.

The Solhomfjell localities are clearly more acidic than the more coastal localities in the southern area, i.e. there is a decreasing pH gradient with increasing altitude or distance from the sea (r^2=0.48) (Table 1, cf. Figure 3a). The localities at Høylandet are notably less acidic, and there is no correlation between altitude and pH. The alkalinity is very low in both areas, usually below 50 µeq/L and often close to 0.

In the southern, acidified area, 80% of the localities had ANC <20 µeq/L and 72% had ANC <0 µeq/L. At Høylandet, 84% of the localities had ANC <20 µeq/L whereas only 21% had ANC <0 µeq/L.

NOM correlates poorly with the pH (r^2=0.29 and 0.01). The correlation is very good for pH and Ca^{2+} in the southern area (r^2=0.86), but poor in the northern area (r^2=0.26) (cf. Figure 3b). The southern area shows a generally higher level of reactive aluminium Al_a than the northern area. Values in both areas vary from about 0 to 300 µg/L, but most localities are well below 150 µg/L in the southern area and below 100 µg/L in the northern area (Figure 2).

Amphibians

R. temporaria was present in the two southernmost sub-areas, 54% (n=26) of the localities at Moland – Froland and 17% (n=18) at Vegårshei – Felle, but was totally absent from Solhomfjell (n=41) (Figure 2). It became gradually less frequent with increasing distance from the sea, or increasing altitude.

At Høylandet, *R. temporaria* occurred in 66% (n=98) of the localities investigated. It was especially common in the mountainous terrain, i.e. at Vikafjellet – Flasnesfjellet and Kovaholet (75%) (Figure 2), and significantly more frequent there than in the lowland peat bog localities at Røyrtjørna (40%) (P<0.01; chi-square test for two variables without expected values).

The three lowest pH values recorded at frog spawning sites in the southern, acidified area were in the range 4.6-4.8, with corresponding Ca values of 0.7-1.4 mg/L. The Al_a concentrations were in the range 70-150 µg/L and NOM was 30-100 TCU.

The three lowest pH values recorded at frog spawning sites in the northern, non-acidified area were also in the range 4.6-4.8. The corresponding Ca values were 0.2-0.3 mg/L, Al_a concentrations were 80-130 µg/L and NOM was 20-90 TCU.

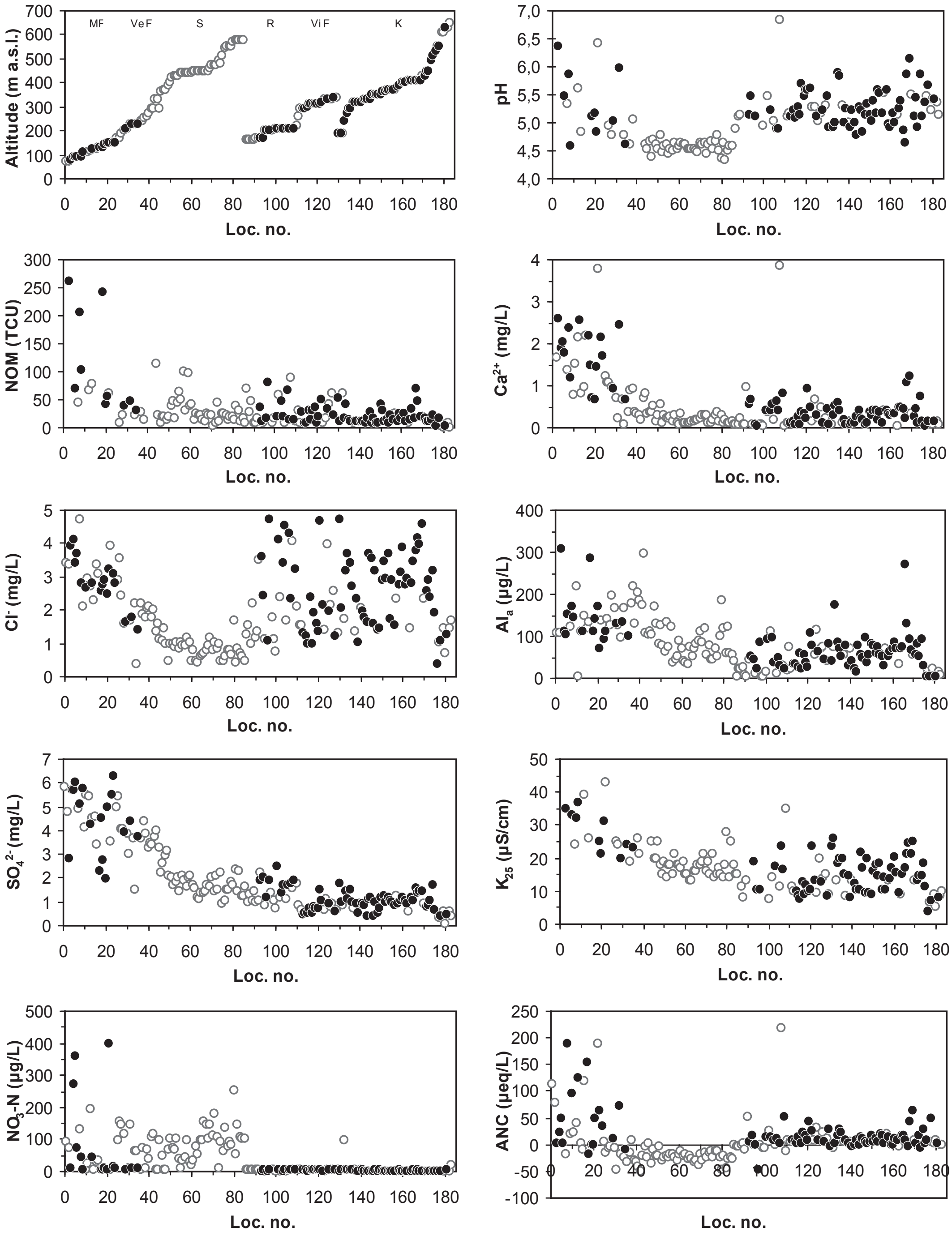

Figure 2
Water quality parameter values for each locality plotted against their successive locality numbers, which correspond to increasing altitudes and (for the southern area) distance to the sea within each sub-area. In Southern Norway: Moland – Froland 1-26, Vegårshei – Felle 27-44, Solhomfjell 45-85, and in Central Norway (Høylandet): Røyrtjørna 86-110, Vikafjellet – Flasnesfjellet 111-129, Kovaholet 130-183. Black dots denote localities with *Rana temporaria*, white dots denote localities without it.

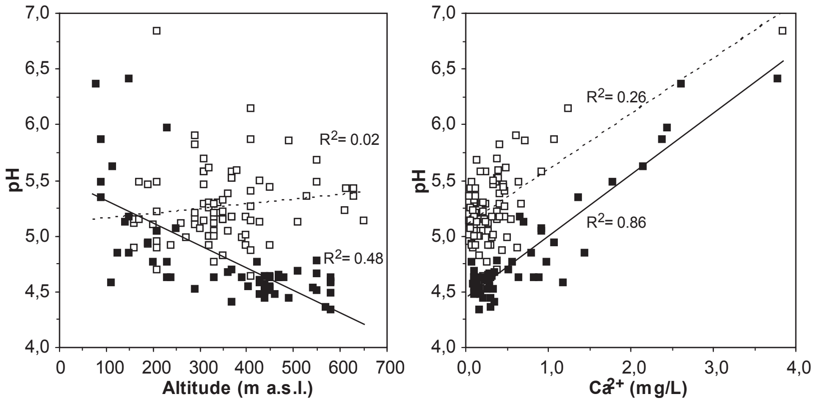

Figure 3
a) The relationship between altitude and pH, b) the relationship between Ca^{2+} and pH. Black dots and the solid line show the southern, acidified area; white dots and the stippled line show the northern, non-acidified area (the extreme pH/Ca value is not included in the r^2 value).

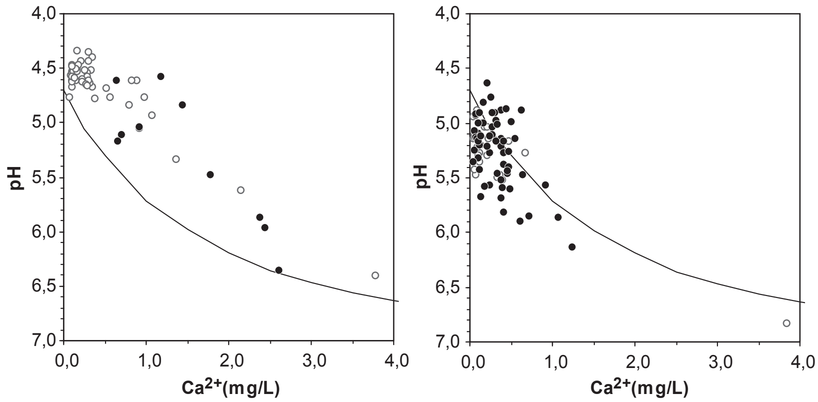

Figure 4
The relationship between Ca^{2+} and pH in a) the southern, acidified area, and b) the northern, non-acidified area. Henriksen's acidification line is inserted; localities above and to the right of the line are acidified. Black dots denote localities with frogs, white dots denote localities without frogs.

The presence of *R. temporaria* in relation to pH and Ca^{2+} is shown in Figure 4. In the southern area, apart from the most acidic localities, the calcium concentration is relatively high both for frog localities and non-frog localities. In the northern area, practically all localities, both with and without frogs, have very low calcium concentration.

In the southern, acidified area, 41% of the localities with *Rana temporaria* had ANC <20 µeq/L and 24% had ANC <0 µeq/L. The corresponding values for the northern, non-acidified area were 83% with ANC <20 µeq/L and 14% with ANC <0 µeq/L. The lowest ANC values for frog localities in the two areas were -17.4 and -48.3 µeq/L, respectively (Figure 2).

The single effects regression analyses (Table 2) showed that in both areas there was a significant, positive relationship between the presence of *R. temporaria* and conductivity, Mg^{2+}, Na^+, K^+, Al_a and Cl^-.

In the southern, acidified area, but not in the northern, non-acidified area, there was, in addition, a significant, positive relationship between the occurrence of *R. temporaria* and pH, Ca^{2+}, SO_4^{2-} and NOM, and a significant, negative relationship between the occurrence of the frog and altitude.

Multiple regression

The final multiple regression model developed by successive elimination of the least significant term concluded with only chloride as the most important variable explaining the occurrence of *R. temporaria* in the southern, acidified area (Table 3). As chloride increased, the possibility of finding *R. temporaria* present in localities also increased (when all other covariates were held constant). (The analyses of the "reduced global model " concluded with altitude as the only important variable explaining the probability of finding *R. temporaria*. This probability decreased with increasing altitude ($p<0.005$). In this model, chloride was removed on step number 3.)

The final model for the non-acidified area in Central Norway (Table 3) also concluded with chloride, and in addition NOM, as the most important variables. The possibility of finding *R. temporaria* present in localities increased as the chloride concentration increased (all other covariates held constant). Furthermore, there was a negative relationship between NOM and the probability of finding *R. temporaria*, although NOM had been non-significant in the single effect analysis (cf. Table 2). This was also the outcome of the analyses of the "reduced global model".

Table 2. Logistic regression analyses of the separate effects of each explanatory variable for the variation in *Rana temporaria* occurrence in a) Southern Norway and b) Central Norway. (β is the slope of the best fit regression line, Wald is the test statistics.)

a)

Response variable	Explanatory variable	β	SE	df	n	Wald	p
R. temporaria	Alkalinity	0.076	0.052	1	82	2.105	n.s.
	Altitude	-0.016	0.005	1	85	12.162	<0.001
	Aluminium	0.010	0.005	1	84	4.792	<0.05
	ANC	0.022	0.006	1	85	12.247	<0.001
	Calcium	1.947	0.452	1	84	18.597	<0.001
	Chloride	1.256	0.320	1	85	15.429	<0.001
	Conductivity	0.175	0.057	1	61	9.568	<0.01
	H+	-0.155	0.044	1	61	12.286	<0.001
	Magnesium	5.825	1.394	1	85	17.462	<0.001
	Nitrate	-0.001	0.004	1	85	0.024	n.s.
	NOM	0.031	0.012	1	61	6.899	<0.01
	pH	2.570	0.833	1	61	9.517	<0.01
	Potassium	10.429	2.574	1	74	16.419	<0.001
	Sodium	1.872	0.472	1	82	15.757	<0.001
	Sulphate	0.871	0.230	1	85	14.272	<0.001
	Turbidity	1.335	0.760	1	61	3.084	n.s.

b)

Response variable	Explanatory variable	β	SE	df	n	Wald	p
R. temporaria	Alkalinity	-0.017	0.020	1	94	0.745	n.s.
	Altitude	0.002	0.002	1	98	1.498	n.s.
	Aluminium	0.025	0.008	1	98	9.041	<0.01
	ANC	-0.000	0.008	1	98	0.003	n.s.
	Calcium	0.258	0.577	1	98	0.200	n.s.
	Chloride	0.863	0.255	1	98	11.497	<0.001
	Conductivity	0.110	0.054	1	86	4.189	<0.05
	H+	0.030	0.061	1	86	0.238	n.s.
	Magnesium	5.992	2.128	1	98	7.925	<0.01
	Nitrate	-0.121	0.126	1	98	0.919	n.s.
	NOM	+0.000	0.012	1	97	0.001	n.s.
	pH	-0.178	0.712	1	86	0.063	n.s.
	Potassium	10.202	5.129	1	90	3.957	<0.05
	Sodium	1.207	0.439	1	97	7.565	<0.01
	Sulphate	0.693	0.510	1	97	1.844	n.s.
	Turbidity	1.396	1.055	1	47	1.752	n.s.

Table 3. Multiple logistic regression analyses of the relative effects of each explanatory variable for the variation in *Rana temporaria* occurrence. Initially, the global model included the following variables: alkalinity, aluminium, altitude, ANC, calcium, chloride, H^+, nitrate, NOM and sulphate. Thus, after successive exclusion of the least significant covariates, the final models for Southern Norway and Central Norway, respectively, are shown in the table.

Response variable	Explanatory variable	β	SE	df	n	Wald	p
R. temporaria	Intercept	-6.633	1.787	1	57	13.776	<0.001
(Southern Norway)	Chloride	2.751	0.818	1		11.316	<0.001
R. temporaria	Intercept	-0.711	0.659	1	80	1.164	n.s.
(Central Norway)	Chloride	1.179	0.371	1		10.093	<0.01
	Pt	-0.041	0.017	1		5.844	<0.05

DISCUSSION

One of the most important relationships recorded was the decreasing pH from the coast to inland in Southern Norway, and especially the low values at Solhomfjell – accompanied by a decrease in the abundance of *R. temporaria* along the same gradient and its absence from Solhomfjell. Moreover, at Høylandet, Central Norway, which is not anthropogenically acidified, *R. temporaria* was very common, especially in biotopes in the mountainous parts that closely resemble those at Solhomfjell. These relationships strongly suggest that acidification explains the absence of *R. temporaria* at Solhomfjell and has caused its extinction in this area. Being a very eurytopic and quite easily dispersed species, but sensitive to low pH and with a relatively short reproductive cycle and life span (cf. Elmberg 1990), *R. temporaria* is thus probably a useful indicator species for acidification, but has so far been much underrated.

We wanted to investigate in more detail the influence of the pH and also the possible effects of various other environmental factors. In the logistic regression analyses of the separate hydrographical and topographical variables, the presence of *R. temporaria* in a pond was shown to have a significant, positive relationship with each of several of them (Table 2). Each such variable may accordingly be part of the explanation of the frog's presence or not.

However, in the multiple logistic regression analyses for the southern, acidified area (Table 3), the presence of *R. temporaria* was significantly related only to chloride, i.e. only the chloride concentration could explain a significantly high proportion of the occurrences of the species, whereas the analyses of the "reduced global model " revealed that altitude was the only important variable. As the altitude increased, the probability of finding the frog decreased. In the northern, non-acidified area, chloride and NOM were both significantly related to the presence of *R. temporaria*. This was the outcome of both logistic regression models. However, this does not mean that the other environmental variables are without effect, separately or in combination (cf. Table 2).

The southern, acidified area

The reason why frog tadpoles were more rarely found where the Cl^- concentration was low, may lie in the additional cost for tadpoles of taking up enough salt in an extremely hypotonic environment where salt is scarce (limiting) and the acidity of the environment puts additional stress on the ion transport mechanism (through the skin) (cf. Boutilier et al. 1992). (As mentioned above, there is a high correlation between Cl^- and Na^+ (r^2=0.92), which in reality is NaCl from the sea.) Besides, NaCl facilitates compensation of pH during hypercapnia in amphibians (Boutilier et al. 1992). An increased salt content in the water has also been found to have a positive effect for the survival of fish living in acidic water (cf. Overrein et al. 1980).

Since the concentration of NaCl in the precipitation decreases with distance from the sea, there is also a strong, negative relationship between Cl^- and altitude (r^2=0.76) in the southern area, – which again corresponds well with the decline and disappearance of *R. temporaria*. The Cl^- values of the ponds varied from 0.4 to 4.7 mg/L (m=1.7±1.1, n=85), i.e. 0.7-7.8 mg/L of NaCl. The values are for the most part low. For comparison, amphibian-bearing ponds in agricultural areas in the county of Østfold, Southeastern Norway, had Cl^- values from 4 to 193 mg/L (m=27±35, n=30), i.e. 7-319 mg/L of NaCl (Dolmen 1991).

In the southern, acidified area, chloride was thus the only factor of significance for frog distribution. In the "reduced global model", however, altitude came out as the only significant factor. To understand the cooperation of factors that may exclude *R. temporaria* from Solhomfjell, for example, we should therefore look at the hydrographical regime that characterises the high-altitude localities in this area. Briefly, these ponds are highly acidic (low pH due to acidic precipitation, as also indicated by relatively high values for SO_4^{2-} and NO_3^-) and they almost lack important cations like Ca^{2+} (cf. ANC, described later), and also NOM buffer systems (cf. Figure 2). The crucial factor for the presence or not of frogs is therefore not altitude in itself (cf. Høylandet), but rather the "total acidic condition" or the combined set of variables which results from the acidification of the high-altitude localities, of which the low NaCl concentration is also of great importance (cf. Figure 2). The relatively low levels of Al_a are probably not important in this connection (see below).

The northern, non-acidified area

In the northern, non-acidified area, no pH values were lower than 4.6, and frogs were present at all pH levels. Varying pH is therefore probably not a very important factor, at least not directly, for the distribution of *R. temporaria* in this area. However, here too there was a significant, positive relationship between frogs and Cl^-. The Cl^- values of the ponds in the northern area varied from 0.4 to 4.7 mg/L (m=2.4±1.1, n=98), i.e. 0.7-7.8 mg/L of NaCl. There is a slight tendency (r^2=0.39) for the high-altitude ponds in the northern area to have more Cl^- than the lowland ponds, which may also explain why the species was more common in the highland part. A possible alternative explanation is that the large bogs in the lowland around Røyrtjørna have few hibernation sites close by and are a less favourable habitat for terrestrial frogs than the more mountainous or hilly, and partly forested Vikafjellet – Flasnesfjellet and Kovaholet sub-areas, where frogs were abundant.

A significant, negative relationship was also seen between frogs and NOM. Although NOM may in part act as an organic buffer system in acidic water (see below), Skei (unpubl.) found experimentally that the mortality of *R. temporaria, Bufo bufo* and *Triturus vulgaris* larvae increased with increasing levels of NOM (0-150 TCU) at pH 4.3 (cf. Saber & Dunson 1978). There is also a slight tendency for the highland ponds to have less NOM (r^2=0.11) than the lowland ponds.

Acidification; pH, calcium, ANC, aluminium and NOM

From what has been said above, many cooperating environmental factors may be important for the presence of the frog in a pond. According to Henriksen (1979), acidification can be defined as the difference between pre-acidification alkalinity and present-day alkalinity, and the degree of acidification at a locality can be determined from the relationship between Ca^{2+} ions and pH.

In Figure 4, where the presence of *R. temporaria* in relation to pH and Ca^{2+} is shown, Henriksen's (1979) "acidification indicator" line has also been inserted. This empirical line distinguishes acidified from non-acidified lakes. Although it does not strictly apply to typical bog localities, which are often also influenced by natural organic acids, it may offer a useful basis for discussion. This figure (Figure 4a) shows that all the Southern Norwegian localities (above the line) are acidified to some extent, the Solhomfjell localities topping the figure, having very acidic water and a low calcium concentration.

The Central Norwegian localities (Figure 4b) are for the most part below Henriksen's line, because the area receives only slightly acidic precipitation, i.e. the yearly average pH is approximately 4.9 (Henriksen et al. 1988). However, humic acids, cation uptake by the thick mats of *Sphagnum* mosses embedding the pools, evaporation and possible ion exchange by Na^+ (from sea-salt deposition episodes, for example) increase the acidity (pH 4.6-4.8) in a few localities. Nevertheless, none of the Høylandet localities come close to the minimum pH values found at Solhomfjell, and *R. temporaria* is present at all pH levels at Høylandet.

It was seen that alkalinity was generally very low in both areas. The values for ANC were also low. A critical ANC limit for trout *Salmo trutta*, and in general for fish and invertebrates in Norway, is empirically defined at 20 µeq/L (Lien et al. 1992, 1996). However, in our study, as many as 41% and 83% of the frog localities in the southern and northern areas, respectively, had values below 20 µeq/L (cf. Figure 2). This means that the ponds are very unstable environments with respect to pH.

Only a few localities (with or without frogs) in the southern area had Al_a values above 150 µg/L (range approximately 100-300 µg/L). The aluminium concentration of the three most acidic frog localities varied from low to moderately high values (70-150 µg/L). Since NOM (30-100 TCU) has quite high values, it is reasonable to believe that aluminium is largely bound to natural organic material in the water and therefore has only a minor toxic effect on animal life at these sites. Høylandet localities have approximately the same ranges of aluminium and NOM as the Solhomfjell localities. In this non-acidified region, toxic aluminium has been demonstrated in only minor concentrations (Blakar & Hongve 1997; Frode Kroglund, pers. comm.) and is probably of even less importance here than in Southern Norway.

Boggy localities, characterised by high values of NOM, in general seem to be an advantageous biotope for amphibian reproduction for three reasons: 1) NOM takes part in a relatively important buffering system against acidification, 2) pools in the bog are often not in direct contact with mineralogical sources of Al, such as bedrock, and 3) a large proportion of the aluminium becomes bound and detoxified in humic water.

pH limit for successful reproduction

In the southern, anthropogenically acidified area, the very acidic localities with Ca <1 mg/L usually had no *R. temporaria* tadpoles, whereas some of the localities with Ca >1 mg/L had tadpoles (Figure 4a). A high calcium concentration to some degree seems to compensate for a low pH, as shown by, for example, Hesthagen et al. (1992) for fish fry. Dale et al. (1985) and Freda & Dunson (1985) also found that the addition of calcium (and magnesium) prevented early mortality in anuran embryos in acidic water.

The three lowest pH values recorded at frog spawning sites were in the range 4.6-4.8 in both areas. Corresponding calcium values were 0.7-1.4 and 0.2-0.3 mg/L for the southern and northern areas, respectively. When breeding takes place, just after the spring thaw, ponds and small pools are possibly even more acidic. Nevertheless, a pH value of about 4.6 is probably close to the "limit" of successful reproduction of *R. temporaria* within low-Ca and moderate-Al regimes.

Hagström (1981), supported by laboratory experiments, reported a gradual yearly decrease of *R. temporaria* between pH levels 5 and 4 in southwestern Sweden. Likewise, Fjellheim & Raddum (1985) found experimentally that when the pH was changed step by step from pH 6.4 to 4.5 (Al in the range of approximately 100-270 µg/L), the mortality of *R. temporaria* eggs increased significantly. In the thorough laboratory experiments reported by Andrén et al. (1988), 79% of the eggs of *R. temporaria* died at pH 4.0 compared with 4% at pH 5.0 and 100 µg Al/L. None of the surviving larvae at pH 4.0 were healthy, shown by deformities or abnormal behaviour. At pH 5.0, 86% of the surviving larvae were healthy. Increased Al concentration (100-800 µg/L) did not affect hatching, but increased larval mortality, etc., especially at pH 4.0, but also to a lesser degree at pH 5.0. Older tadpoles were somewhat more resistant to acidic water. (These experiments were, however, conducted with water of higher calcium concentration (12.6 mg/L) than in our study.)

Freda (1986), based on information from Beebee & Griffin (1977) and Leuven et al. (1986), discriminated between lethal pH and critical pH for *R. temporaria*, at levels of 4.25 and 4.5, respectively. This critical pH value is quite close to the lower pH limit for *R. temporaria* sites with successful spawning in Norway.

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SAMMENDRAG

Forekomsten av buttsnutefrosk *Rana temporaria* L. i et forsuret og et ikke-forsuret område av Norge

Den vanlige buttsnutefrosken *Rana temporaria* L. i Aust-Agder finnes i minskende forekomst langs en forsurningsgradient mot lavere pH innover i landet. Undersøkelsesområdet er forsuret gjennom forurenset nedbør. På Solhomfjell, i grensestrøkene til Telemark, mangler arten fullstendig. pH lå der i området fra 4.8 ned til 4.3 og med aluminiumsverdier (Al_a) vanligvis under 150 µg/L. Nærmere kysten forekom vellykket gyting og larveutvikling ned til pH 4.6 og aluminiumsverdier på 100-300 µg/L.

I et ikke-forurenset referanseområde, med liknende høyde over havet, topografi, geologi og flora i Midt-Norge (Høylandet i Nord-Trøndelag), varierte pH fra 6.8 ned til 4.6, og med aluminiumsverdier under 100 µg/L, i naturlig forsurete dammer. *R. temporaria* var der svært vanlig ved alle pH-nivåer, og aller hyppigst forekommende i fjellområdene (biotop svært lik Solhomfjell). Disse forholdene gir sterke indikasjoner på at fraværet av *R. temporaria* på Solhomfjell skyldes sur nedbør.

Multivariat analyse av ulike miljøparametere viste at det var en signifikant positiv sammenheng mellom froskens utbredelse i forsurningsområdet i Aust-Agder og saltinnholdet (NaCl) i dammene, noe som igjen avtok med høyde og avstand fra havet. Froskens fravær på Solhomfjell må sees i lys av det totale forsurningsmiljøet (lav pH, lite saltinnhold og mangel på buffersystemer m.m.), som er mest ugunstig i de høytliggende innlandsområdene. Også på Høylandet, der pH ikke setter direkte begrensninger, hadde lavt saltinnhold, men dessuten også økt innhold av organisk materiale i vannet, negativ innvirkning på tilstedeværelsen av frosk.

Myrvannslokalitetene er ellers ofte gode gytebiotoper for frosk fordi humus til dels bufrer sterke syrer (under forsurning); vannet har dessuten lite eller ingen direkte kontakt med berggrunnen og aluminiumsholdige mineraler; i tillegg binder og avgifter humusen aluminium.

En pH i nærheten av 4.6 og lavere synes kritisk eller letal for *R. temporaria* i små, lavbufrete myrvannslokaliteter som her undersøkt.

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