Scandinavian amphibians: their aquatic habitat and tolerance to acidic water – a field study

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To determine the impact that anthropogenic acidification has had on natural amphibian populations in Scandinavia and to trace the species' tolerance limits, in 1988-89 four poorly buffered areas in Norway were investigated; three were anthropogenically acidified and the fourth was not. The increasing acidification from the coastal to inland/highland region of Southern and Southeastern Norway (pH 7.2-4.1) was accompanied by a decreasing frequency of amphibian (*Rana temporaria*, *Bufo bufo* and *Triturus vulgaris*) localities. In the (anthropogenically) non-acidified Central Norway region (pH 6.8-4.6), *R. temporaria* was very common at all pH levels. The data strongly suggest that acidification explains the absence of amphibians locally in the acidified areas, and has caused their extinction in the inland/highland region of Southern Norway. A pH of 4.5-4.6 is the critical minimum for *R. temporaria* populations in small, poorly buffered, boggy, water-bodies as were investigated here. For *B. bufo*, the lowest pH recorded was 4.7, and for *T. vulgaris* 4.8. We did not find any signs of successful reproduction in *Rana arvalis* and *Triturus cristatus* below a pH of 5.2 and 5.3, respectively. Increased contents of Ca^{2+}, Na^+ (NaCl) or humus (NOM) had an ameliorating effect on the amphibians in acidic water. The presence of Al was of only minor importance for the amphibians in humic waters. In a strategy for the conservation of amphibians in acidified or acidifying areas, liming ($CaCO_3$) and/or NaCl treatment of the localities may work well in order to establish a satisfying aquatic environment for the species. The study may act as a baseline for further surveys in the future when acidic precipitation hopefully has decreased, looking for improvements of the habitats and possible recoveries of amphibian sites.

Keywords: amphibians, habitat, pH tolerance, Scandinavia

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INTRODUCTION

During the 20th century, anthropogenic acidification became a serious environmental problem in limnic ecosystems in Scandinavia (Rodhe et al. 1995). Inland fish in Norway, primarily brown trout *Salmo trutta* Linnaeus, 1758, have been seriously affected in an area of approximately 52 000 km^2 (Hesthagen et al. 1999). Southern and Southeastern Norway are the most damaged, due to the large amounts of acidic precipitation and the poor buffering capacity of the bedrock (Overrein et al. 1980, Henriksen et al. 1988, Hesthagen et al. 1999). To neutralise the detrimental effects of the acidification on fish, numerous small and large-scale liming projects have been accomplished quite successfully in southern Scandinavia (e.g.
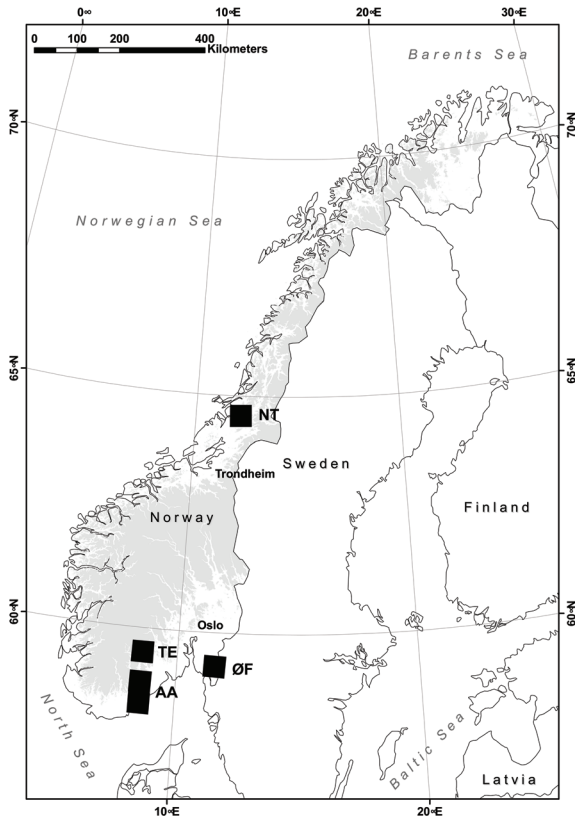
Figure 1. The locations of the areas investigated in Southern Norway (AA – Aust Agder, TE – Telemark), Southeastern Norway (ØF – Østfold) and Central Norway (NT – Nord-Trøndelag). Areas in Norway above 500 m a.s.l. are hatched.

Sandøy & Romundstad 1995).

Amphibians in Scandinavia also suffer from the acidification (e.g. Hagström 1981, Dolmen 1987, Dolmen et al. 2004) and have therefore become rare over large parts of for example Southern Norway (cf. Beebee & Griffiths 2005). Although acidic precipitation has been decreasing recently (Alewell et al. 2000; Direktoratet for Naturforvaltning 2005), it is still a threat to aquatic life in large parts of Scandinavia and will remain so for a long time (Stoddard et al. 1999, Driscoll et al., 2001). In order to understand the possible recovery of amphibian populations following chemical recovery in the future, as a result of liming or of natural causes, it is important first to know their status and ecology during the most severe impact of the acidification.

The occurrence of the common frog *Rana temporaria* Linnaeus, 1758 in an acidified area was studied by Dolmen et al. (2004). A pH value of approximately 4.6 was the lowest found for the successful reproduction of the species in natural bog ponds and pools with low calcium and aluminium concentrations. Laboratory experiments by Andrén et al. (1988) and Skei & Dolmen (2006 and unpublished) show that all the six amphib-

ian species tested had lethal or critical pH limits between pH 4 and 5. To obtain a more detailed picture of the impact of anthropogenic acidification on natural amphibian populations in northern latitudes, four areas (in three regions, Figure 1) were investigated in 1988 and 1989. One was strongly acidified (Aust-Agder), two somewhat less acidified (Telemark and Østfold) and the fourth (Nord-Trøndelag) was negligibly acidified. In addition to the common frog *R. temporaria*, the moor frog *R. arvalis* Nilsson, 1842, the common toad *Bufo bufo* (Linnaeus, 1758), the smooth newt *Triturus vulgaris* (Linnaeus, 1758) and the great crested (warty) newt *T. cristatus* (Laurenti, 1768) were also present.

The questions we addressed in this study were: 1) Which hydrochemical factors and other habitat characteristics (altitude and area of localities) are most important for the distribution of these amphibians in northern latitudes? We predicted that low pH might restrict the distribution of amphibians and possibly lead to their local extinction. 2) Which critical pH limits can be traced in nature for their successful reproduction? The various species were expected to differ in their tolerance or preference with respect to pH. 3) Is it possible to observe any ameliorating influence from other elements or chemical compounds on their pH tolerance? Physiologically important elements like Ca, Na and Al, and perhaps humus, should be expected to have an effect on the amphibians' pH tolerance. Some of them could also therefore be of interest for conservation work on amphibians in acidified areas.

METHODS

The study area

In 1988 and 1989, we conducted this study in four areas presented in Figure 1: Aust-Agder (AA; Southern Norway), Telemark (TE; north of Southern Norway), Østfold (ØF; Southeastern Norway) and Nord-Trøndelag (NT; Central Norway). In the paragraphs below, the source of information on the bedrock geology is Sigmond et al. (1984), the postglacial marine limit (ML) Holtedahl & Andersen (1960), the vegetation zones and timber lines (woodland limits) Moen (1999), the yearly runoff NVE (1987) and the pH in the precipitation Henriksen et al. (1988).

The first area (AA), was defined along a south-north transect from the coast at Arendal to Solhomfjell, a mountain 50-60 km inland. Monitoring of and research on the effects of acidic precipitation have been performed here previously (Økland & Eilertsen 1996, Nilssen & Wærvågen 2002, Dolmen et al. 2004). Its location is 58°32-58'N, 8°45'E. The bedrock consists mainly of intermediate to acidic gneisses, and the ML is around 100 m a.s.l. The area belongs in the boreonemoral and (Solhomfjell) southern boreal vegetation zones. It is entirely below the cli-

matic timber line, but the ecological timber line at Solhomfjell (coniferous forest) is about 500-600 m a.s.l. The yearly runoff is 500-1500 mm, and the average acidity in the precipitation (1988) is pH 4.2-4.3.

The second area (TE), is in extension of the above-mentioned gradient, 50-60 km further inland, at Kviteseid. Its location is 59°25'N, 8°15'E. The bedrock resembles that of the foregoing area, but with large occurrences of metamorphosed volcanic rocks of Proterozoic age. The whole area, which is situated (far) above the ML, belongs to the southern boreal zone, and the climatic timber line (coniferous forest) is about 1000 m a.s.l. The yearly runoff is 500-1000 mm, and the average acidity in the precipitation (1988) is pH 4.3-4.4.

The third area (ØF), is situated in western Aremark. Its location is 59°15'N, 11°35'E. The bedrock resembles that of the AA area. The ML is about 200 m a.s.l. The area belongs to the boreonemoral zone, and is below the timber line. The yearly runoff is 250-500 mm, and the average acidity in the precipitation (1988) is pH 4.3-4.4.

The fourth area (NT), is at Høylandet, which is an international reference area for non-acidified conditions (Dahl 1997, Blakar & Hongve 1997, Dolmen et al. 2004). The area is located at 64°35'N, 12°05'E. The bedrock is mainly gneiss, resembling the other areas, and the ML is approximately 150-160 m a.s.l. The area belongs to the middle boreal and northern boreal zones, and the climatic timber line (coniferous forest) is about 400 m a.s.l. The yearly runoff is 1000-2000 mm, and the average acidity in the precipitation (1988) is pH 4.9-5.0.

A total of 261 permanent water-bodies (localities) were investigated: ponds, pools and lakelets in bog or bog with scattered rock outcrops. The number of localities in the AA area was 98 (in subareas Moland – Froland 28, Vegårshei 17, Heiland – Felle 12 and Solhomfjell 41), in TE (Huvestad – Tveitgrendi) 22, in ØF 34 (Krokvatn 8, Hivatna 11 and Kilsjø 15) and in NT 107 (Vikafjellet – Flasnesfjellet 19, Kovaholet 54 and Røyrtjørna 34). Only a few (two or three) of the highest localities (about 600 m a.s.l. and higher) in the first and last-mentioned areas, were under the influence of meltwater from ice and snow when they were investigated.

Data and water sampling, and water analyses

The field investigations were carried out in the AA area on 2-7 June 1988, and an additional check for amphibians on 18-22 July; the same localities and some additional ones were again investigated on 25-28 May 1989. In TE the investigations were carried out on 29-30 May 1989; in ØF they took place on 20-23 May 1989, and an additional check for amphibians on 6-7 July. In the NT area the localities were studied on 16-19 June

1988, and some additional localities on 14 June 1989. The first rounds of investigations in each area, when also water sampling was accomplished, coincided with the time when frog spawn or (in the lowland) small tadpoles would be found and newts were breeding. We also checked for possible dead/dried-in egg clumps on land. The water quality of ponds and lakes changes over the season; our water samples were taken in spring/early summer, when the pH is at its lowest. The second rounds took place when larvae were big, but in good time before metamorphosis. Amphibians were recorded by sight and by netting (Dolmen 1991). Each time, one person moved at a speed of 7-8 m/min with observations along the edge, and in addition carried out effective netting (10 samples) for 15 min. Thus, about 30 minutes were spent at localities of 1000 m², but more for larger localities. Only tadpoles or larvae on healthy spawn are considered, since metamorphosed amphibians (1[+] yrs and older) do not necessarily indicate successful spawning conditions at the site. Since our aim was to get a picture of the tolerance of the amphibians, only the occurrence or not of the species is considered, not their numbers or densities.

Water samples, one in each locality, were collected in polyethylene bottles at a depth of 10-20 cm approximately 1 m from the shore. They were kept in the dark at approximately 4°C until they were analysed in the laboratory, after a couple of months. In addition to altitude and area, the following parameters were analysed using the methods employed by Blakar & Hongve (1997): pH, alkalinity, Ca, Mg, Na, K, conductivity, turbidity, NOM (natural organic matter, measured as mg Pt l^{-1} or TCU), SO_4, Cl, NO_3-N and Al_a (acid-reactive aluminium concentration; the speciation of Al was not investigated). For use in the multiple regression analyses, we have calculated the concentrations of H$^+$, SSA (the salts of strong acids), Alk-H$^+$ (alkalinity minus H$^+$ concentration), the Σ cations (sum of cations), Σ base cations (Ca^{2+}, Mg^{2+}, Na$^+$ and K$^+$) and ANC (acid-neutralising capacity).

Data analyses

The presence or absence of each amphibian species was analysed by multiple logistic regression analyses. Of variables that were strongly correlated, all but one was excluded prior to the logistic regressions.

We first composed initial models including ten variables that were assumed to influence the probability of finding each species present. The final models were determined after a stepwise, backwards exclusion of the least significant covariates. When further exclusion of covariates did not improve the model, the selection stopped. The remaining covariates, representing the highest overall probability of finding the species, were then included in the final model. For the initial models, we selected Al_a, altitude, area, Ca^{2+}, NOM, H$^+$, Mg^{2+}, Na$^+$, NO_3-N and
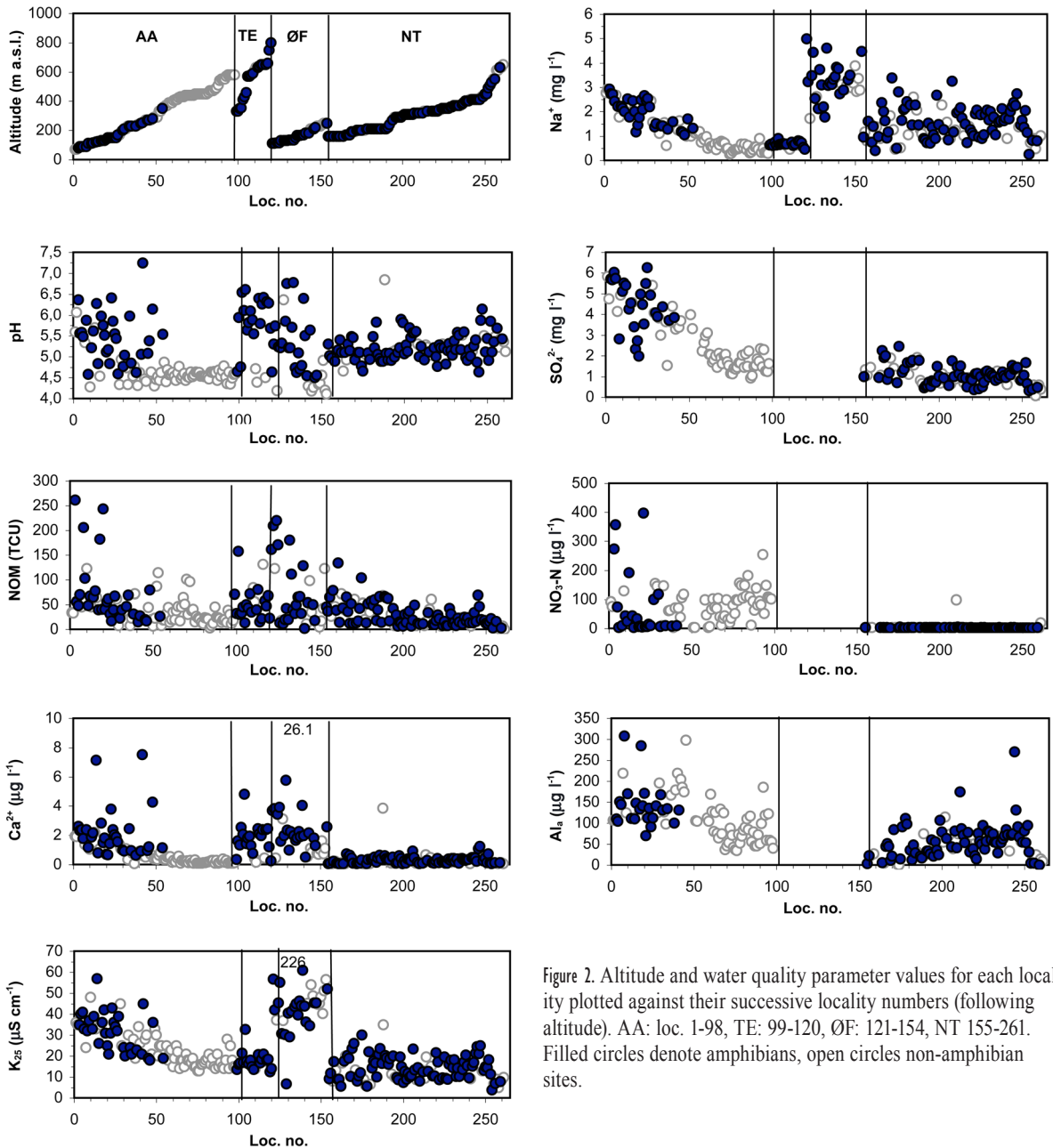
Figure 2. Altitude and water quality parameter values for each locality plotted against their successive locality numbers (following altitude). AA: loc. 1-98, TE: 99-120, ØF: 121-154, NT 155-261. Filled circles denote amphibians, open circles non-amphibian sites.

SO_4^{2-}. [We chose H^+ instead of pH since the latter is measured on a logarithmical scale. Since Na^+ and Cl^- (NaCl from the sea) are strongly correlated ($r=0.94$, $n=183$, $P<0.01$), we also chose Na^+ before Cl^- because the number of Na^+ measurements was higher than that of Cl^-.] The other eleven variables (see above) were not included in the initial model, mainly due to strong, or fairly strong, intercorrelation among the different variables, to large numbers of missing values (turbidity), or to less biological importance for amphibians (K^+). Since Al_a, NO_3-N and SO_4^{2-} were not measured in TE and ØF, the initial model in these cases had seven variables.

Altitude was not included in the analyses when we combined areas; this was to avoid a possible source of error caused by the fact that the TE area had the highest altitude, but was less affected by anthropogenic acidification than the AA area at a lower altitude. However, we also ran separate analyses to see whether altitude made a difference to the results.

The multiple logistic regression analyses included only ponds and lakes that had complete information for all the explanatory variables (see above), and covered only regions where each species was observed in at least one locality. In the case of
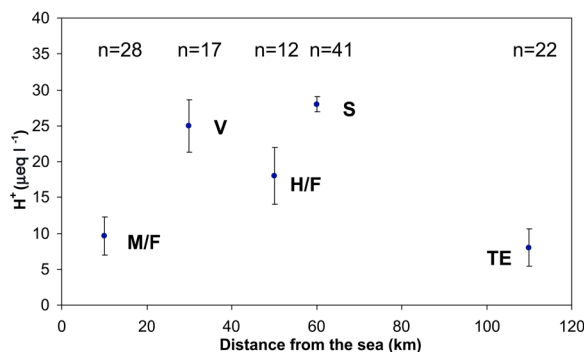
Figure 3. Mean [H$^+$] ± SE of ponds and lakes in the various sub-areas of AA (left to right: Moland/Froland, Vegårshei, Heiland/Felle, Solhomfjell) and TE.
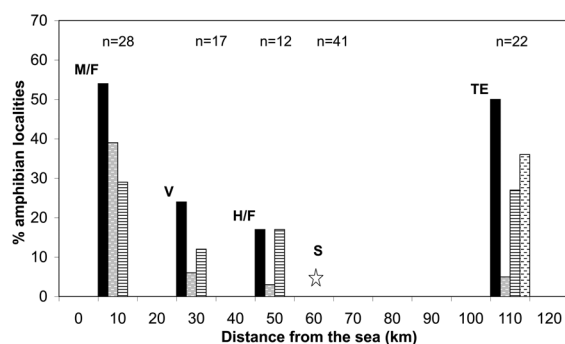

Figure 4. The percentage of investigated localities with amphibians in the various sub-areas of AA (left to right: Moland/Froland, Vegårshei, Heiland/Felle, Solhomfjell) and TE. *Rana temporaria* (black), *Bufo bufo* (grey), *Triturus vulgaris* (horizontally hatched), *T. cristatus* (horizontally lightly hatched); asterisk means no observations of amphibians.

Rana arvalis, only five localities were recorded, all in the ØF area (*n*=34), and this species was therefore not included in the analyses. The analyses of *B. bufo* and *T. vulgaris* included the AA, TE and ØF areas (*n*=153), whereas the analyses of *T. cristatus* included the TE and ØF areas (*n*=55). For *R. temporaria*, we made separate analyses for AA (*n*=84) and NT (*n*=98). In addition, we analysed all four areas together (*n*=260). SPSS-PC 10.00 was used for all the statistical analyses mentioned above. All tests of significance were two-tailed.

RESULTS

Biotopes and hydrographys

The altitude and some of the most important hydrographical variables in the four areas are illustrated in Figure 2, where the parameter values for each locality have been plotted against their successive locality number; within each area, the numbers are in keeping with increasing altitude. The most "coastal"

(lowland) parts of AA, in part also TE and lowland parts of ØF, stand out with relatively high values for pH, NOM and calcium. Sodium also has relatively high values in the lowlands. Inner (highland) parts of AA and the upland parts of ØF, on the other hand, show some extremely low pH values (down to 4.1 in ØF). The localities in NT usually have intermediate values of pH, intermediate to high for sodium, intermediate to low for NOM and low for calcium.

The huge amount of industrial pollution, leading to acidification of southernmost Norway, can best be illustrated by the different concentrations of the most typical anion components of acidic precipitation, SO_4^{2-} and NO_3^-, in AA compared to NT (Figure 2). The values are high in coastal AA, the area that receives most acidic precipitation, decreasing inland, and are low (SO_4^{2-}) or almost negligible (NO_3-N) for the non-acidified reference area in NT. [Since NO_3^- is an important nutrient for plant metabolism, the values observed show the excess of this ion which the plants have been unable to take up effectively.] Al_a (only measured in AA and NT) varies from <10 to 300 μg l^{-1}, but most localities in AA are well below 150 μg l^{-1} and in NT below 100 μg l^{-1} (Figure 2).

Within the acidified AA and ØF areas, there is a tendency to a decreasing gradient in the pH with increasing altitude (AA: *r*=-0.58, *n*=98, *P*=0.1; ØF: *r*=-0.55, *n*=38, *P*=0.05) and distance from the coast. A similar situation is found in ØF). Especially localities above the ML, i.e. approximately 100 m for AA and 200 m for ØF, are very acidic. Figure 3 shows the H$^+$ concentration for the AA sub-areas and TE. The increasing gradient in H$^+$ (decreasing gradient in pH) from the coastal lowland to Solhomfjell deviates at H/F because at least two localities there have been limed. Further inland, in TE, where the bedrock is more heterogenic, most pH values are again higher. The four most acidic localities were all recorded in the ØF area, with pH values of 4.1-4.2 and Ca^{2+} values of 0.6-1.1 mg l^{-1}. The most acidic locality in the AA area was 4.3 (Ca^{2+} 1.1 mg l^{-1}) and in TE 4.4 (Ca^{2+} 0.2 mg l^{-1}). For the northern, NT area, the most acidic localities were far less acidic. The four most acidic localities had a pH of 4.6-4.8 (Ca^{2+} 0.1-0.3 mg l^{-1}).

The amphibians

The numbers of localities (ponds and lakelets) were 125 for *Rana temporaria*, 5 for *R. arvalis*, 25 for *Bufo bufo*, 23 for *Triturus vulgaris* and 11 for *T. cristatus*. In AA (total *n*=98), *R. temporaria* showed a gradual decrease in the relative number of localities from 54% in the coastal part of the transect to 0% in the mountainous subarea, Solhomfjell. The species thus becomes less common with increasing distance from the coast, or with altitude. In TE (total *n*=22), it occurred in 50% of the localities. A similar decrease in occurrence from coast to inland were seen for the rarer *B. bufo* and *T. vulgaris*, while *T.*

Table I. Multiple logistic regression analyses of the relative effects of each explanatory variable for the variation in amphibian occurrences. The initial model included the following variables: area, aluminium, calcium, NOM, magnesium, sodium, nitrate, sulphate and H^+. Thus, after successive exclusion of the least significant covariates, the final model is shown in the table. The inclusion of altitude in the initial model did not change the outcome of the analysis for *R. temporaria* or *T. cristatus*. For *B. bufo*, the inclusion of altitude in the initial model also included altitude ($P<0.05$, as a negative factor) in the outcome of the analysis. For *T. vulgaris*, the inclusion of altitude in the initial model also included Na^+ ($P=0.065$, as a positive factor) and altitude ($P=0.08$, as a negative factor) in the outcome of the analysis. (β is the slope of the best fit regression line, Wald is the Wald chi-square test statistics.)

Response variable and areas	Explanatory variable	β	SE	DF	n	Wald	P
R. temporaria	Intercept	1	0.362	1	260	7.652	<0.01
AA TE ØF NT	Area	-0.061	0.027	1		5.05	<0.05
	Ca^{2+}	-0.485	0.165	1		8.61	<0.01
	H^+	-0.122	0.019	1		41.298	<0.001
	Na^+	0.656	0.196	1		11.137	<0.001
R. temporaria	Intercept	-3.456	1.891	1	84	3.339	0.068
AA	Area	-0.331	0.258	1		1.645	0.2
	H^+	-0.08	0.036	1		4.78	<0.05
	NOM	0.021	0.012	1		3.034	0.082
	SO_4^{2-}	0.813	0.407	1		3.993	<0.05
R. temporaria	Intercept	-0.128	0.763	1	98	0.028	0.867
NT	Na^+	1.65	0.588	1		7.858	<0.01
	NOM	-0.029	0.016	1		3.281	0.07
	NO_3-N	-0.187	0.164	1		1.297	0.255
B. bufo	Intercept	-1.376	0.629	1	153	4.794	<0.05
AA TE ØF	H^+	-0.112	0.03	1		14.517	<0.001
	Na^+	1.675	0.494	1		11.503	<0.001
	Mg^{2+}	-4.141	1.772	1		5.458	<0.05
T. vulgaris	Intercept	0.365	0.388	1	153	0.885	0.347
AA TE ØF	Area	-0.122	0.057	1		4.624	<0.05
	H^+	-0.179	0.05	1		12.708	<0.001
T. cristatus	Intercept	1.875	1.029	1	55	3.317	0.069
TE ØF	Area	-0.236	0.14	1		2.847	0.092
	H^+	-0.207	0.117	1		3.126	0.077
	Mg^{2+}	-2.299	1.345	1		2.925	0.087

cristatus first appeared in TE, where it occurred in 32% of the localities (Figure 4). In ØF (total $n=34$), *R. temporaria* and *B. bufo* occurred at frequencies of 35% and 24%, respectively, of the investigated localities, while both *R. arvalis* and *T. vulgaris* occurred at 15%, and *T. cristatus* at 6%. In NT, *R. temporaria* was the only amphibian species present. The total count showed its occurrence in 76% of the investigated localities (total $n=107$). It was (1988) significantly more common in the hilly terrain (75% of the localities, $n=73$) than in the lowland peat bog localities (40%, $n=25$) (chi-square test for independence: $\chi^2=10.41$, df=1, $P<0.01$).

The environment connections

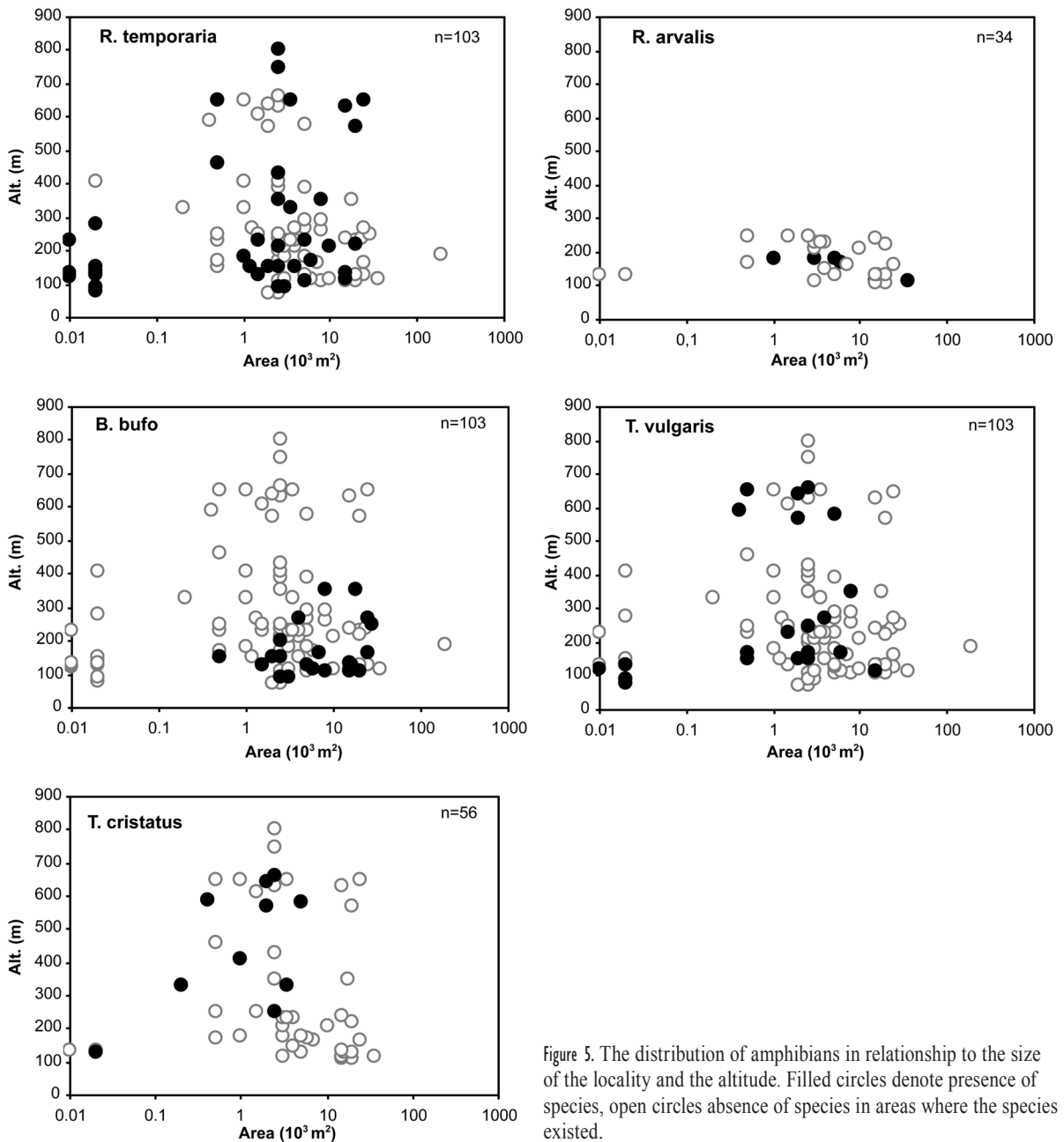*R. temporaria.* When all four areas (AA, TE, ØF, NT) were

Figure 5. The distribution of amphibians in relationship to the size of the locality and the altitude. Filled circles denote presence of species, open circles absence of species in areas where the species existed.

combined, the final multiple regression model for *R. temporaria* ended up with pond area, Ca^{2+}, H^+ and Na^+ as the most important variables explaining the occurrence of the species. The probability of finding *R. temporaria* increased with decreasing pond area, Ca^{2+} and H^+ and increasing Na^+ (Table 1).

For the most acidified region, AA, seen separately, the final multiple regression model included pond area, NOM, H^+ and SO_4^{2-} as the most important variables explaining the occurrence of *R. temporaria*, but only H^+ and SO_4^{2-} made a significant contribution. The probability of finding *R. temporaria* in AA increased with decreasing H^+ and increasing SO_4^{2-} (Table

1).

The result of the multiple logistic regression for NT showed that NOM, Na^+ and NO_3-N were the three variables that, combined, best explained the probability of finding *R. temporaria*, and only Na^+ made a significant contribution. The probability of finding the species increased with increasing Na^+ (Table 1).

Rana arvalis. Although *R. arvalis* was recorded in five localities, it was only proven to be breeding in four of them and in just one region, ØF.
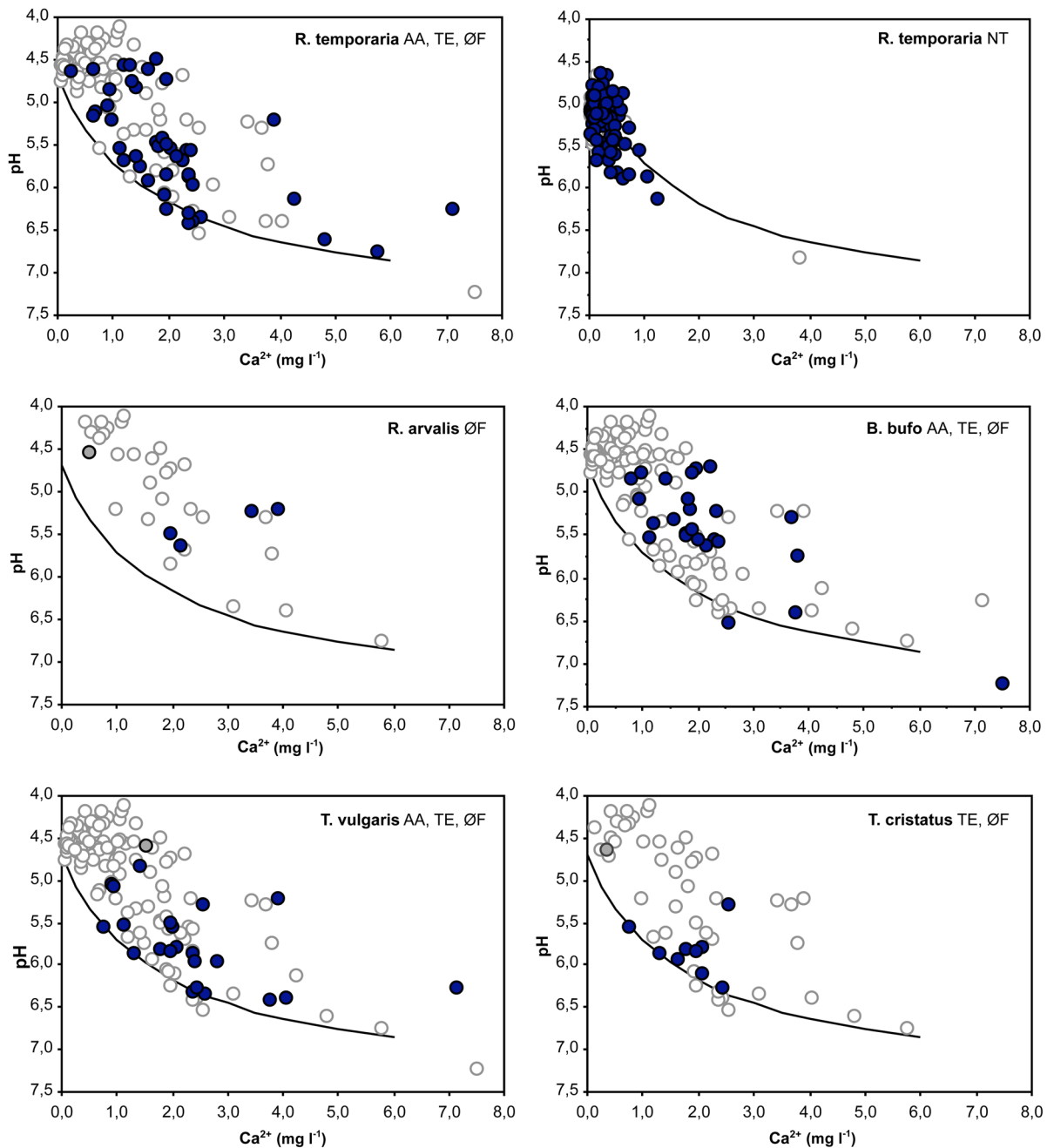
Figure 6. The relationship between Ca^{2+} and pH in the investigated ponds and lakes of the various areas. Henriksen's acidification line is inserted; localities above the line are considered to be acidified (Henriksen 1979). Black filled circles denote presence of species, grey filled circles that only adults were found, open circles absence of species.

Bufo bufo. The most important variables explaining the occurrence of *B. bufo* according to the final multiple regression model were H^+, Mg^{2+} and Na^+. The probability of finding *B. bufo* increased with decreasing H^+, Mg^{2+} and increasing Na^+. The same variables plus altitude gave the best explanation of the occurrence of *B. bufo*, when altitude was included in the initial model (Table 1).

Triturus vulgaris. Pond area and H^+ were the most important variables explaining the occurrence of *T. vulgaris* in the final multiple regression model. The possibility of finding *T. vulgaris* increased with decreasing pond area and H^+ (Table 1). When altitude was included in the initial model, Na^+ and altitude were also left in the table explaining the occurrence of the species. The possibility of finding *T. vulgaris* increased with decreasing pond area and H^+ and increasing Na^+ and altitude.
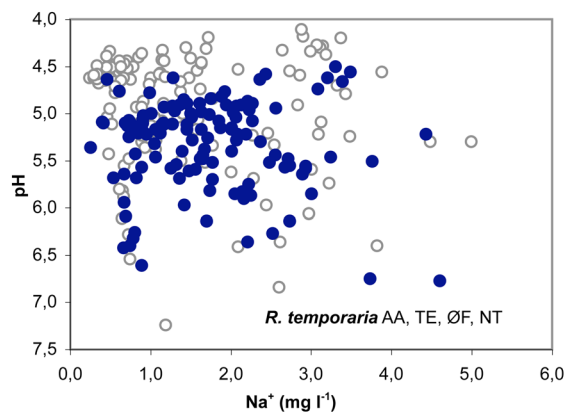
Figure 7. The relationship between Na$^+$ and pH in the investigated ponds and lakes. Black filled circles denote presence of *Rana temporaria*, open circles absence of the species.

Triturus cristatus. The final multiple regression model explaining the occurrence of *T. cristatus* included pond area, H$^+$ and Mg^{2+} as the most important variables. The possibility of finding *T. cristatus* increased with decreasing pond area, H$^+$ and Mg^{2+}; when all other covariates were held constant. The relationships were not significant, however (Table 1). (The number of *T. cristatus* localities was quite low.)

Altitude and size of locality

Some of the amphibians differed with respect to altitude and size of their water bodies (Figure 5). For example, while the highest locality for *R. temporaria* in these investigations was 800 m a.s.l., and both newts were found up to 660 m a.s.l., the highest *B. bufo* locality was only 352 m a.s.l. The difference in the distribution of *R. temporaria* and *B. bufo* above and below 400 m a.s.l. is significant (chi-square test for independence: χ^2=5.88, df=1, *P*<0.02). In addition, *B. bufo* is less often found in small water bodies like puddles and ponds, i.e. <2500 m^2 (χ^2=5.50, df=1, *P*<0.02). The multiple regressions found a negative effect of increasing pond area on *R. temporaria* occurrences. *T. vulgaris* is also more often found in ponds, and *T. cristatus* is almost exclusively found there, although not significantly different from *R. temporaria* (χ^2=1.57, df=1, *P*>0.20).

Minimum pH values and modifying effects of Ca^{2+} and Na$^+$

A total of seven amphibian localities in all the areas had pH values below 4.7 (Figure 2). The most acidic amphibian localities (*R. temporaria*) recorded in ØF, AA and TE had pH/Ca^{2+} values of 4.5/1.8, 4.6/0.7 and 4.6/0.3, respectively. For the northern region, NT, where amphibians (*R. temporaria*) reproduced successfully even in the most acidic localities, the minimum
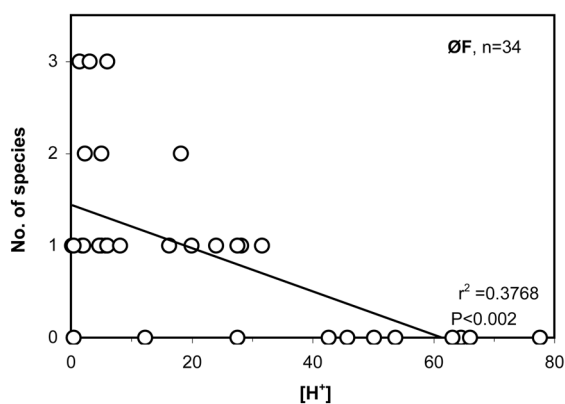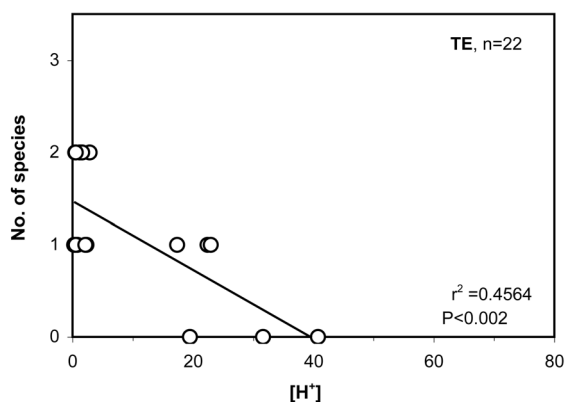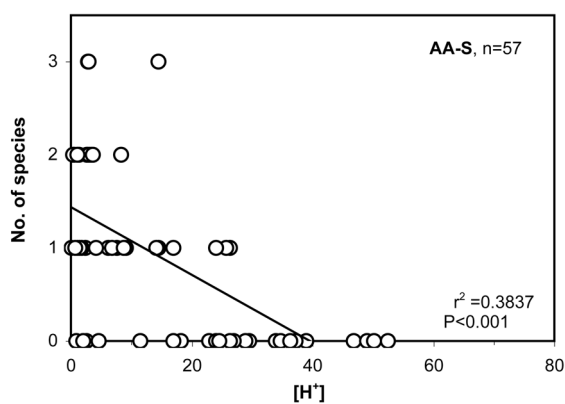

Figure 8. The relationship between [H$^+$] and the number of amphibian species in localities in AA (3 species exist) (except Solhomfjell), TE (4 species exist) and ØF (5 species exist).

value was 4.6/0.2. In two localities in AA, dead *R. temporaria* spawn (and no tadpoles) was found at pH 4.3 and 4.4 (Ca^{2+} values were 0.4 and 0.5 mg l^{-1}, respectively). A large proportion of the *R. temporaria* spawn was also dead in a locality in TE and only a few tadpoles had hatched, at a pH as high as 4.6 (Ca^{2+} 0.3 mg l^{-1}).

In the three acidified areas (AA, TE and ØF), *R. temporaria* tends to be absent from very acidic localities with a low concentration of Ca^{2+}. However, where the Ca^{2+} values are higher,

the species may be present at the same pH. This relationship becomes clearer when the three acidified areas are seen together (Figure 6). An imaginary line can actually be drawn between the most acidic localities in the upper part of the diagram from low Ca^{2+}/high pH to high Ca^{2+}/low pH, which separates sites with (below) and without (above) frogs. There is a strong correlation between Ca^{2+} and the lowest pH values within each 0.5 mg l^{-1} interval of Ca^{2+} (r=0.99, n=4, P<0.05).

The lowest pH/Ca^{2+} values at which *R. arvalis* tadpoles were recorded (ØF) were 5.2/3.4 and 5.2/3.9 (Figure 6). True enough, several juveniles (1^+ yrs old) and adults were found sitting in the water or on wet *Sphagnum* moss and rocks bordering a lakelet of clear water with a pH/Ca^{2+} of 4.6/0.5. (None of the neighbouring localities were found to keep a population of *R. arvalis*.) However, this does not prove reproduction, and the locality has therefore been given a grey (not black) symbol in Figure 6.

The lowest pH recorded for *B. bufo* tadpoles was 4.7 at 2.3 mg Ca^{2+} l^{-1} (ØF) (Figure 6). The correlation between Ca^{2+} and the lowest pH values within each 0.5 mg l^{-1} interval of Ca^{2+} is much lower than in *R. temporaria*, but still fairly good (r=0.66, n=4). The lowest pH recorded at a *T. vulgaris* locality where larvae were found had a pH/Ca^{2+} of 4.8/1.4 (Figure 6). However, adults were recorded at a locality with a pH/Ca^{2+} of 4.6/1.6 (grey symbol in the figure). Courting adults and healthy eggs of *T. cristatus* were seen at a pH/Ca^{2+} of 5.3/2.6 (ØF) (Figure 6). However, a few juveniles (2^+ or 3^+) were recorded at 4.7/0.4 in a pond in TE (grey symbol in the figure), close to a lakelet where the species was known to breed. A similar relationship as was found for Ca^{2+} was also found between Na^+ and pH: The higher values for Na^+, the lower the minimum values for pH which were associated with amphibians (Figure 7). For *R. temporaria* the correlation was strong (r=0.96, n=4, P<0.05). For *B. bufo* it was r=0.71 (n=4).

Since the various species seem to have different lower pH limits (but probably not upper limits within the pH range dealt with here), the average number of species at the localities also increases with the pH. However, of the six localities that had as many as three species (none had more), one had a [H^+] of 14, i.e. a pH as low as 4.8; the other five had a [H^+] of 1-6, i.e. pH 5.2-5.9 (Figure 8).

DISCUSSION

Acidification

The anthropogenic acidification process and its damage to aquatic life depend on a combination of strongly acidified precipitation and lack of adequate buffering capacity in the bedrock and/or soil (e.g. Mason 1989). This relationship is present over large parts of Aust-Agder (AA) and Østfold (ØF), especially above the ML. Telemark (TE), too, experiences acidic precipitation, but has better buffering capacity due to occurrences of metamorphosed volcanic rocks

According to Henriksen (1979), acidification can be defined as the difference between the 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. The presence of all five amphibian species in relation to pH and Ca^{2+} is shown in Figure 6, where Henriksen's (1979) empirical "acidification indicator" line distinguishes acidified from non-acidified lakes. Although it does not strictly apply to typical bog localities, which are often also influenced by (allochthonous) humic acids, it may offer a useful basis for discussion. We found that almost all the AA, TE and ØF localities (above the line) are more or less acidified (the few exceptions are in TE); some small ponds in ØF and the Solhomfjell localities in AA are highest in the figure, having very acidic water and a low calcium concentration (Figure 6).

The Nord-Trøndelag (NT) localities are mostly below Henriksen's line because, although they also lack good buffering capacity, the area receives only slightly acidic precipitation. However, humic acids, cation uptake and exchange from the thick mats of *Sphagnum* mosses bordering the pools, evaporation and possible ion exchange by Na^+ (from, for example, sea salt episodes) increase the acidity (pH 4.6-4.8) in some localities. Nevertheless, none of the Høylandet localities come close to the minimum pH values found at Solhomfjell, and *Rana temporaria* is present at all pH levels at Høylandet.

Four important biogeographical relationships were demonstrated: a) the increasing acidification from the coast to inland (highland) region of AA (pH 4.3-7.2), especially at Solhomfjell (pH 4.3-4.8), b) the decreasing frequency of amphibian (*R. temporaria*, *Bufo bufo* and *Triturus vulgaris*) localities along the same gradient and their absence from Solhomfjell. Further inland, in TE, the pH level was more favourable, and all three species, as well as *Triturus cristatus*, were present, c) similar clines to those in AA were seen in ØF (pH 4.1-6.8); the lowest pH level for *R. temporaria* was 4.5, d) in Central Norway, the pH levels were 4.6-6.8 and *R. temporaria* was very common at all pH levels. For *B. bufo*, the lowest pH recorded was 4.7, and for *T. vulgaris* 4.8. We did not find any signs of successful reproduction in *Rana arvalis* and *T. cristatus* below a pH of 5.2 and 5.3, respectively. Increased contents of Ca^{2+} or Na^+ (NaCl) lowered the minimum values for pH which were associated with amphibians.

Unfortunately, there exist no old data on amphibian distribution in the investigated areas. However, Solhomfjell (in AA), where no amphibians were found, greatly resembles Høylandet (NT) with respect to altitude, topography, geology and flora (but the

climate is harsher in the latter area). The pH of the precipitation, however, was markedly different, being below 4.3 in the southern area and above 4.9 in the northern area (Henriksen et al. 1988). *R. temporaria* is very common at Høylandet, not least in biotopes in the mountainous parts that resemble those at Solhomfjell most. These relationships strongly suggest that acidification explains the absence of *R. temporaria* at Solhomfjell and has caused its local extinction there.

A similar situation as in AA was found in ØF. Even though the precipitation in ØF was less acidic (pH 4.3-4.4, similar to TE), the localities with the very lowest figures were found there, all without amphibians. However, one or more of the five amphibian species were found in localities with a higher pH, mainly below the ML.

Low pH and the modifying influence from other substances

Low pH was by means of logistic regression shown to significantly restrict the distribution of amphibians. Only in NT the pH of the ponds never reached below the tolerance limit for *R. temporaria*; hence, no restriction due to pH could be seen. At low pH, the control of the gill permeability is disturbed, mainly due to loss of Ca^{2+} from binding sites in the gill epithelium. This causes disturbances in ion regulation and extensive loss of body ions, that may be lethal (Freda & Dunson 1984, Rosseland & Staurnes 1994).

The logistic regressions showed no direct positive relationship between amphibians and Ca^{2+} in general. However, the positive influence of Ca^{2+} is important at extreme pH levels. It was seen that increased concentration of Ca^{2+} in a locality could compensate for higher pH, i.e. the amphibians tolerated low pH better. This is in agreement with Dale et al. (1985), Freda & Dunson (1985) and Cummins (1988), who found that the addition of Ca (and Mg) for instance prevented early mortality in anuran embryos in acidic water. Since it regulates the permeability of the gill membranes of aquatic animals, Ca^{2+} is also an important factor in the interplay between H^+ and Al in determining the toxicity of acidic water (Rosseland & Staurnes 1994, Gensemer & Playle 1999).

Our data on *R. temporaria*, *B. bufo* and *T. vulgaris* (from which we have the largest amount of data) also show that Na^+ (NaCl) has an ameliorating effect on amphibians in soft, acidic waters. A similar relationship for Cl^- (NaCl) has been demonstrated earlier (Dolmen et al. 2004) for *R. temporaria*. Moreover, Skei & Dolmen (2006 and unpublished) have shown experimentally that more Cl^- in the water results in more *R. temporaria* and *T. vulgaris* larvae surviving in acidified soft water. The reason why amphibian larvae were more rarely found where the NaCl concentration was low, may lie in the additional cost for tad-

poles 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 gills and skin) (Boutilier et al. 1992). 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 on the survival of fish living in acidic water (Overrein et al. 1980).

NOM may possibly also influence the presence or absence of the amphibians, as indicated for *R. temporaria* in AA. Humus binds (toxic) aluminium. The opposite trend is seen in the non-acidified NT, where *R. temporaria* is more common in relatively clear water. Clear water is probably normally preferred, but in the acidified areas, where the aluminium concentration is higher (Figure 2), amphibians may have problems unless the aluminium is bound and detoxified by humus.

Non-biological relationships

As seen from Table 1, when all four areas were pooled, for *R. temporaria,* a significant negative relationship was found between the occurrence of the species and Ca^{2+}. This surprising result is most likely explained by the high frequency of the species in the non-acidified NT, where low Ca^{2+} levels were common. Moreover, in AA, a positive connection was seen between *R. temporaria* and SO_4^{2-}, but this has no biological significance. It just means there is more SO_4^{2-} in the precipitation in the lowlands, where amphibians also occur most frequently provided there is sufficient buffering capacity against the acidification. The seemingly "negative influence" of Mg^{2+} on *B. bufo* can be explained by the fact that the highest Mg^{2+} concentrations were found in small ponds, while *B. bufo* prefers larger localities (see below).

A pH limit for successful reproduction in *R. temporaria*
We have demonstrated pH values, down to pH 4.5 at 1.8 mg Ca^{2+} l^{-1}, where populations of *R. temporaria* successfully have reproduced within the low-Ca and moderate-Al regimes in an anthropogenically acidified area (ØF). Similar pH results were also found by Strand (2002) in his survey of 1291 localities in various parts of Norway; he recorded *R. temporaria* two times at pH 4.5 in Western Norway, and at the lowest pH values measured, i.e. down to pH 4.6, in Central Norway.

However, *R. temporaria* does not seem particularly selective with respect to the acidity of its breeding localities. It may breed in acidic water, not only down to the limit for the survival of eggs and larvae, but even below, as in some small lakelets in AA, where the eggs failed to develop at pH/Ca^{2+} values of 4.3/0.4 and 4.4/0.5, respectively. Strand (2002), too, found hatching egg clutches of *R. temporaria* at a pH of 4.3 in two small woodland lakes in Western Norway. Embryos in the eggs

were alive, but (hatched) larvae on top of the eggs were motionless, and no tadpoles were found there three weeks later.

Freda (1986), based on information from Beebee & Griffin (1977) and Leuven et al. (1986), discriminated between lethal pH and critical pH for *R. temporaria*, these levels being 4.25 and 4.5, respectively. This critical pH value is exactly the same as the lower pH limit for *R. temporaria* sites with successful reproduction in Norway, as recorded in the present investigation. However, a tolerance limit is rarely exact and depends on life stage, and genetic and environmental factors. For example, the amphibians seem to tolerate a low pH better when the Ca^{2+} concentration is high (see above) (Figure 6). However, near optimum levels for reproduction are probably first reached at a pH of approximately 5.0, or even higher, depending on the aluminium concentration and speciation, ionic score etc. Older larvae are also somewhat more resistant to acidic water (Andrén et al. 1988, Skei & Dolmen unpublished).

pH minima for the other species
R. arvalis was not proved to be breeding below a pH of 5.2, but the number of records is small. There was no sign of successful breeding in a little lake with extremely clear water where the pH/Ca^{2+} was 4.6/0.5 and where many metamorphosed animals (1^+ and older) were seen along the water's edge. Very little humus was present to detoxify any aluminium. However, Dolmen (unpublished) found *R. arvalis* tadpoles in a bog pool in Arendal (AA) which, in June 2003, had a pH of 4.6 and high humus values (NOM 120 TCU). Likewise, Strand (2002) recorded *R. arvalis* at pH values as low as 4.4 in Akershus (Southeastern Norway), and thus in agreement with the experiments of Andrén et al. (1988), which showed that *R. arvalis* can tolerate a slightly lower pH than *R. temporaria*.

Although it is not included in the present study, it can be mentioned that the third frog species in Norway, the newly-discovered pool frog *Rana lessonae* Camerano, 1882, also seems to tolerate a fairly low pH. The pH level in May/June in its most acidic locality, a bog pool, varied between 5.1 and 5.4 in 1996-2004 (*n* = 9) (Dolmen, unpublished data).

For *B. bufo*, 4.7 was the lowest pH recorded, at 2.3 mg Ca^{2+} l^{-1}. Strand (2002) also recorded *B. bufo* close to this minimum value, at 4.7, and he found a dead *B. bufo* tadpole at a pH of 4.4. These two localities were the only ones in Strand's survey where *B. bufo* was recorded at a pH <5.0. In Norway, *B. bufo* is usually found in large localities, which usually have higher pH values than bog ponds (Rønning 1995, Strand 2002). And in experiments, Skei & Dolmen (2006 and unpublished) found a lower tolerance to acidic water in *Bufo bufo* tadpoles than in *R. temporaria* tadpoles.

Although *T. vulgaris* larvae were not found below a pH of 4.8 (Ca^{2+} 1.4), adults were recorded in early summer (possibly indi-

cating successful reproduction) at 4.6/1.6. Dolmen (1981, 1983) refers to records of *T. vulgaris* (larvae and adults) at pH 4.5 and 4.7. However, these measurements were made colorimetrically and are therefore more uncertain (Blakar & Digernes 1984). In Strand's (2002) survey, *T. vulgaris* larvae were found down to pH 4.6, although in general the species seems to be more "alkalic". This is much lower than the lowermost limit of occurrence in England proposed by Cooke & Frazer (1976), who rarely encountered *T. vulgaris* in water with a pH <6.0 (see also Beebee 1981, Denton 1991). Experimentally, Böhmer (1988; see also Haidacher & Fachbach 1991) found that pH 4.0-4.4 was lethal for *T. vulgaris* embryos, while pH 4.3-4.6 was critical, i.e. it resulted in 50% mortality.

The minimum pH value for successful reproduction in *T. cristatus* in our study was pH/Ca^{2+} 5.3/2.6, although metamorphosed specimens were recorded at 4.7/0.4. Dolmen (1981, 1983) recorded *T. cristatus* larvae at a pH of 4.9 and adults at a pH of 4.8-4.9 (colorimetric method, see above), but in general *T. cristatus* seems to prefer – or tolerate – less acidic water. At least, *T. cristatus* embryos are unable to hatch at a pH of 4.5 (Griffiths 1993). In Strand's (2002) survey, *T. cristatus* was recorded first at a pH of 5.2 and in Skei et al. (2006) not below pH 5.5, and it clearly preferred much higher values (>6.0). Denton (1991) also found *T. cristatus* much less than expected by chance at pH <6.0 in Cumbria, England; only three localities had a pH <6.0 (down to pH 4.8), but these ponds had only small populations, and the reproduction status was not mentioned.

Although there are certain pH limits for successful reproduction, it seems likely that juveniles and adult amphibians outside the breeding season sometimes may use localities of lower pH for feeding, as seen from two examples of *R. arvalis* and *T. cristatus* above.

Size of locality, altitude and seasonality
For *R. temporaria* and the two *Triturus* species, but not *B. bufo,* it was seen that the maximum size of the breeding locality is also important (Figure 5). This is first of all explained by the presence of predatory fish in the larger localities, and their predation on amphibian larvae, while small localities are usually devoid of fish (Økland 1990). Not least the newts, especially *T. cristatus*, are vulnerable to fish predation because of the exposed way of life of the larvae (Dolmen 1988), and they are therefore confined to small and medium-sized water bodies, i.e. without fish (Dolmen 1992, Skei et al. 2006). *B. bufo* tadpoles, on the other hand, tolerate the presence of fish, since they are not readily eaten (Fog et al. 1997). This ability to live in large localities (fishponds etc.) reduces its competition with other amphibians (which are more vulnerable to predation by fish) and also lowers the species' risk of death by desiccation.

But there are also connections to, for instance, acidity: the ability of *R. temporaria* to breed successfully early in spring

and to colonize all kinds of locality sizes, including those more acidic in high altitudes, also reflects its relatively high tolerance to acidic conditions. The pH in ponds and lakes is usually at its lowest during the spring thaw (in April to early May) and then rises throughout the summer (Hagen & Langeland 1973, Haapala et al. 1975).

In general, it seems that the newts' tolerance limit – or preference – is at a higher pH level. The newts usually also arrive at their breeding ponds later in spring. Skei et al. (2006) therefore suggest that there may be an adaptive connection between the relatively late arrival of newts at a locality and their higher sensitivity to a low pH. Their most pH-sensitive stages, the embryos and tiny larvae (see Pough & Wilson 1977, Pierce et al. 1984, Clark & LaZerte 1985, Böhmer & Rahmann 1990), are accordingly not found until later, from late May onwards, when the pH has risen considerably. Late reproduction (higher pH) is especially important for the newts, because their habitat (ponds and lakelets) usually have lower pH, and also are more unstable with respect to pH, than larger lakes. *B. bufo* avoids acidic stress by breeding in larger localities in the lowland, where the pH is higher.

The Al regime

In general, chronically sub-lethal effects of low pH on amphibians are seen in acidic water far above the critical or lethal pH level for the species, i.e. low food consumption, growth and mobility, and low population density (Clark 1986, Pahkala et al. 2002, Räsänen et al. 2002, Strand 2002). One reason may be toxic aluminium. The combination of low or fairly low pH and high aluminium concentration as a cause of fish death has been in focus for several years in Scandinavia (Rosseland & Staurnes 1994, Åtland 1998). The toxicity of aluminium in the water differs with the pH, and the hydroxides, Al $(OH)^{2+}$ and Al $(OH)_2^+$, are most toxic and have their maxima at pH 5.3 and 5.9, respectively (Lydersen 1991). The main mechanisms of Al toxicity appear to be osmoregulatory disturbances caused by increased loss and decreased uptake of ions, respiratory disturbances due to Al precipitation on gill surfaces, mucous clogging and reduced membrane fluidity, and circulatory disturbances with reduced blood plasma volume, increased blood protein concentration and erythrocyte swelling (Rosseland & Staurnes 1994).

In accordance with this, Skei & Dolmen (2006 and unpublished) also show that larvae of *R. temporaria* and *T. vulgaris*, but not *B. bufo*, displayed increased mortality in soft water in the pH interval of 5.1-5.9. Andrén et al. (1988) showed that high concentrations of aluminium in low-humic/high-calcium (12.6 mg Ca^{2+} l^{-1}) water did not affect the hatching of *R. temporaria* and *R. arvalis* eggs, but increased larval mortality and abnormal behaviour, especially at a pH of 4.0, but also at 5.0.

Aluminium values (reactive Al) for the present localities

investigated in AA and NT, which were mostly bog ponds and pools, were usually quite low. (We have no data for TE and ØF.) However, some localities (with or without frogs) in the AA area had Al_a values above 150 µg l^{-1}, and a few at Solhomfjell (AA) and in the NT area had values above 100 µg l^{-1}. There was a tendency for the most acidic ponds to have the highest aluminium concentration, and for the three most acidic frog localities it varied from low to moderately high (70-145 µg l^{-1}).

Since NOM (often 30-100 TCU or more) and/or turbidity (1-2 FTU) have quite high values, it is reasonable to believe that aluminium is largely bound to dissolved organic material and/or mineral and organic particles in the water and therefore has only a minor toxic effect on animal life at these sites, as described earlier.

Conclusions and perspectives

1) Low pH clearly restricted the distribution of amphibians, and acidic precipitation has probably, at least in the case of *Rana temporaria*, led to its local extinction, for example in parts of Aust-Agder (AA, at Solhomfjell). 2) A pH of 4.5-4.6 seems to be the lowest value for successful reproduction of *R. temporaria*. For *Bufo bufo*, *Triturus vulgaris*, *R. arvalis* and *T. cristatus*, the lowest pH values found were 4.7, 4.8, 5.2 and 5.3, respectively. 3) We saw a significant, positive influence of Ca^{2+} and Na^+ (NaCl) on the tolerance of amphibians to acidic conditions. It was not possible to see any clear influence of (reactive) Al and humus, but humus has the ability to bind and detoxify Al and may therefore be important for amphibians in small, humic localities as those studied here. In a strategy for the conservation of amphibians in acidified or acidifying areas, in addition to liming ($CaCO_3$) (Dolmen 2005), also NaCl treatment of the localities may possibly work well in order to establish a more satisfying aquatic environment for the species.

The field-work of the present study was carried out while the acidic precipitation was at its heaviest. As a result of international agreements, it has now, during the past few years, decreased somewhat (Alewell et al. 2000, Direktoratet for Naturforvaltning 2005). In 2008-2009, another investigation in the same areas will be carried out in order to look for improvements of the habitats and possible recoveries of amphibians at the sites presented here.

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