Amphibian recovery after a decrease in acidic precipitation

Dag Dolmen, Anders Gravbrøt Finstad, Jon Kristian Skei

Dag Dolmen, Associate Professor, Norwegian University of Science and Technology, NTNU University Museum; NO-7491 Trondheim, Norway

Anders Gravbrøt Finstad, Associate Professor, Norwegian University of Science and Technology, NTNU University Museum; NO-7491 Trondheim, Norway

Jon Kristian Skei, Free-lance Researcher, Skei Biomangfold Konsult, Skeisvegen 16; NO-7510 Skatval, Norway

This is a preprint. Please cite the original article: Dolmen, D., Finstad, A.G. & Skei, J.K. Ambio (2017). https://doi.org/10.1007/s13280-017-0988-5

The printed version can be found on http://rdcu.be/y6BG (view only without subscription).

Abstract

We here report the first sign of amphibian recovery after a strong decline due to acidic precipitation over many decades and peaking around 1980–90. In 2010, the pH level of ponds and small lakes in two heavily acidified areas in southwestern Scandinavia (Aust-Agder and Østfold in Norway) had risen significantly at an (arithmetic) average of 0.14 since 1988–89. Parallel with the general rise in pH, amphibians (*Rana temporaria*, *R. arvalis*, *Bufo bufo*, *Lissotriton vulgaris* and *Triturus cristatus*) had become significantly more common: the frequency of amphibian localities rose from 33% to 49% (n = 115), and the average number of amphibian species per locality had risen from 0.51 to 0.88. In two other (reference) areas, one with better buffering capacity (Telemark, n = 21) and the other with much less input of acidic precipitation (Nord-Trøndelag, n = 106), there were no significant changes in pH or amphibians.

Corresponding author: Dag Dolmen, NTNU University Museum, NO-7491 Trondheim, Norway.
Phone: (+47) 73592108, 41686501
E-mail: dag.dolmen@ntnu.no

Keywords: Acidity Amphibian decline Habitat recovery Scandinavia Water chemistry

INTRODUCTION

During the 20th century, anthropogenic acidification became a serious environmental problem in limnic ecosystems in large parts of Europe and North America (e.g. Haines1981; Elvingson and Ågren 2004; AirClim 2011). On the Scandinavian Peninsula, the southwestern regions probably experienced the greatest damage, due to the large amounts of acidic precipitation and the poor buffering capacity of the bedrock (Henriksen et al. 1988; Mason 1989). Inland fish, primarily brown trout (*Salmo trutta*), were seriously affected in an area of approximately 52 000 km^2 (Hesthagen et al. 1999; see also Garmo et al. 2014). That amphibians in Scandinavia also suffered from acidic precipitation was reported by Hagström (1981) and Dolmen (1987), for example.

Amphibian declines have also been identified as a worldwide problem during the latest decades (e.g. Gardner 2001; Blaustein and Bancroft 2007; ICE 2014). It is assumed that nearly 170 amphibian species have become extinct and around 2500 additional species have declining populations. This amounts to about one-third of the world's amphibian species (Stuart et al. 2004; IUCN 2013). Declines and extinctions have been caused by a series of factors, the most significant being habitat alteration, such as fragmentation or direct destruction (Vos and Chardon 1998; Lehtinen et al. 1999; Cushman 2006). Other factors are predation by introduced species, chemical pollution, disease, parasites, climate change, increased UV-B radiation and over-exploitation (e.g. Beebee and Griffiths 2005; Hopkins 2007). In this article, we shall deal with anthropogenic acidification.

In many parts of the world, damage to amphibian habitats caused by acidic precipitation is probably one of the most important reasons for the amphibian decline (cf. Barr 2011). Low pH may harm amphibians (larvae) in several ways, including the direct toxicity of low pH, induced osmoregulation problems (Na^+ loss), gill-clogging by mucous, and the toxicity of dissolved metals like Al. The damage to amphibians by low pH has been described by numerous authors (e.g. Clark and Hall 1985; Leuven et al. 1986; Böhmer and Rahmann 1990; Pierce 1993; Blaustein et al 2003). The seriousness of acidic precipitation for amphibians in the wild has not been unchallenged, however. A symposium on "Amphibian declines and habitat acidification" held in Pennsylvania, USA, concluded that "No data are at hand which show directly that anthropogenic acidification causes amphibian populations to decline" (Dunson et al. 1992) (see also Pierce 1993 and references and summary by Wells 2007).

In order to acquire more knowledge of the status and ecology of the amphibians during the acidification and to better understand the extent of the problem, studies were performed in 1988–89 on the habitat of amphibians in the heavily acidified areas of southernmost and southeastern Norway (Dolmen et al. 2008). The data strongly suggested that acidification explained the absence of amphibians locally in the lowlands and had caused their extinction in inland and upland districts.

Today, more than 20 years after the first investigations, which took place when the impact of the acidification was most severe in southwestern Scandinavia, the situation is different as a result of the implementation of international agreements to reduce the industrial discharge of sulphur and nitrogen oxides to the atmosphere (Levy 1995; Elvingson and Ågren 2004; Air Pollution 2014). By 2010, there had been significant reductions in SO_4^{2-} in the precipitation (67–79 %) and in NO_3^- (29–41%) in southernmost and southeastern Norway. Also the negligibly acid-polluted central Norway had a significant (63%) reduction of SO_4^{2-} in the precipitation in 1990–2010, while the very low NO_3^- concentration was unchanged (Aas et al. 2010).

This new study therefore sought to learn whether the above-mentioned positive changes in acidic precipitation over southwestern Scandinavia have also brought positive changes to the amphibian fauna. Our hypotheses were:

1) Because of the decrease of SO_4^{2-} and increase of pH in the precipitation, it is also possible, more than two decades after the positive trend started, to see an increase of pH in the ponds and lakes, and

2) If there is an increase of pH in the ponds and lakes, it is also possible to see an improvement or recovery of amphibian populations following the improvement in the chemistry.

MATERIALS AND METHODS

The study areas and species

The study areas and localities (ponds and lakelets, i.e. small lakes) were the same as were investigated in 1988–89, except that 15 localities were excluded because they had been polluted or limed, or were too small and in danger of drying-up. The study was conducted in four areas, which are presented in Fig. 1: Aust-Agder (AA) in southernmost Norway, a heavily acidified area; Telemark (TE), a northerly and inland extension of AA but with higher buffering capacity and used as a less acidic "comparison area", Østfold (ØF) in southeastern Norway, another heavily acidified area; and an almost non-acidified reference area, Nord-Trøndelag (NT) in central Norway. The Solhomfjell sub-area (see below) in AA, had previously been selected as an international research area for acidic precipitation studies, and NT, having similar bedrock and altitude to Solhomfjell, as a reference area for these studies (Blakar and Hongve 1997; Dolmen et al. 2008). The selection of the individual localities was based on their (under normal conditions) potential as amphibian habitats.

A total of 242 permanent water bodies were investigated: ponds, pools and lakelets in *Sphagnum* bog or bog with scattered rock outcrops. The areas (with sub-areas) and the number of localities were: in AA (Moland/Froland, Vegårshei, Heiland/Felle and Solhomfjell) 88 localities, in TE (Huvestad/Tveitgrendi) 21 localities, in ØF (Kilsjø, Krokvatn and Hivatna) 27 localities, and in NT (Røyrtjørna, Vikafjellet/Flasnesfjellet and Kovaholet) 106 localities.

The altitude of the investigated ponds and lakes varied in the range 70–580 m a.s.l. in AA, 330–800 m in TE, 110–250 m in ØF and 160–650 m in NT. The bedrock of the four areas consists mainly of intermediate to acidic gneisses, except in TE which also has large occurrences of metamorphosed volcanic rocks of Proterozoic age. The three southern areas (AA, TE, ØF) belong to the boreonemoral and southern boreal vegetation zones, while the northern area (NT) belongs to the middle boreal and northern boreal zones. The great majority of localities were situated on small or large bogs, and the water was quite humic: yellowish or brownish. In 2010, the water colour in AA averaged 70 mg Pt L^{-1} (range: 35–130), while ØF had 157 (40–400), TE 71 (35–220) and NT 94 (5–200). The few clear-water lakelets in NT were situated on bare rock, usually in the mountains. In 1988, the average pH in the precipitation was 4.2–4.3 in AA, 4.3–4.4 in TE and ØF, and 4.9–5.0 in NT (Henriksen et al. 1988). In 2010, the pH in the precipitation had risen to an average of 4.7–4.8 in AA, 4.8–5.0 in TE and ØF, and 5.7 in NT (Aas et al. 2011). The postglacial marine limit (ML) in AA is about 100 m a.s.l. and in ØF about 200 m a.s.l. The position of the areas, their bedrock geology, quaternary deposits, vegetation zones and yearly runoff are described in more detail by Dolmen et al. (2008).

Species involved were the common frog (*Rana temporaria* Linnaeus, 1758), the moor frog (*R. arvalis* Nilsson, 1842), the common toad (*Bufo bufo* (Linnaeus, 1758)), the smooth newt (*Lissotriton vulgaris* (Linnaeus, 1758)) and the great crested (warty) newt (*Triturus cristatus* (Laurenti, 1768)).

Data and water sampling, and water analyses

The field investigations were comparable to those in 1988–89 with respect to method and timing; in the AA area they were carried out on 17–23 May 2010, with an additional check for amphibians on 16–19 June. They took place in TE on 23–24 May and 20–21 June, and in ØF on 11–13 May and 22–24 June. The localities in the (northern) NT area were studied later, on 7–11 July. The first round of investigations in each area, when also water samples were collected, was at the time when the pH of the water is usually at its lowest, when frogs' egg clusters or (in the lowlands) small tadpoles would be found and newts were breeding. The second round took place when larvae were big, but well before metamorphosis. Only one round was carried out in NT, where *R. temporaria* is the only amphibian species present. Amphibians were recorded by netting and by sight in accordance with the earlier investigations (Dolmen et al. 2008). Clusters of frogs' eggs are so easy to see in spring in the current biotopes that their chance of detection was regarded as 100 %; other life stages or species somewhat less. Since our study was concerned with the tolerance of amphibians, only their presence or absence was considered, not their numbers or densities. For the highly vagile anurans, the term "presence" denotes the successful breeding of a species, i.e. it includes healthy tadpoles. For the less mobile urodeles, the mere occurrence of adults was usually decisive, since it indicates a viable population there.

One water sample per locality was collected in 0.5 L polyethylene bottles at a depth of 10–15 cm approximately 1 m from the shore. pH, conductivity (K_{25}) and water colour were measured in the field the same day or the next. pH was measured with a WTW pH 3110 pH meter with a Hamilton electrode, conductivity with a WTW Cond 330i meter and water colour with a Hellige colour comparator and Nessler tubes. (Since the water colour had been analysed differently in 1988–89 and not on the spot, they were omitted in the comparisons.) The water samples were kept dark and cool until they were further analysed in the laboratory, after a couple of months. The following parameters were analysed using the methods employed by Blakar and Hongve (1997): SO_4^{2-}, Ca^{2+}, Mg^{2+} and Na^+. (Other parameters used by Dolmen et al. (2008) had been shown to be of no or only minor importance for the presence of amphibians.) Altitudes were found on topographical maps in the M-711 series (1:50 000).

Data analyses and statistics

We tested for different temporal development between acidified and non-acidified areas on hydrographic variables, the occurrence and the number of amphibian species at each location using linear and generalized linear mixed effect models with individual wetland as a random effect, and area (acidified, non-acidified) and measurement year, as well as the area x year interaction as fixed factors using the lme4 (Bates et al. 2015) package in R version 3.4.0 (R Core Team 2017). Altitude was included in the full model as a controlling variable (sensu Freckleton 2002). Model selection and model averaging on fixed effect structure were done by model comparison using the MuMIn library (Grueber et al. 2011) according to the Akaike information criterion (AICc). Models with hydrographical variables, occurrence of amphibians and the number of species were fitted using gausian, poisson or binominal link functions, respectively (lmer and glmer functions). Prior to the analyses, all variables were standardized by subtracting mean and dividing on standard deviation. Residuals of the final selected models were visually inspected for deviations from normality, heteroscedasticity, and spatial autocorrelation without finding evidence for violation of model assumptions. For all tested models, the interaction between area and year was the variable of interest, and in the following result section we report ΔAICc between models including this variable and models without.

Means for the hydrographical variables were compared for 2010 and 1988-89 using a paired t-test or a Mann Whitney U-test. We used the chi-square method to test the significance of an increased number of localities with certain hydrological or amphibian qualities compared to the number of localities with no change + a change in the opposite direction. A Spearman correlation test was used to explore the correlation between the change in pH and the change in the number of species at the various localities between 1988-89 and 2010. These analyses were performed in IBM SPSS Statistics for Windows Version 19.0 (IBM Corp., Armonk, NY, USA). The significance limit was set to $p < 0.05$.

Henriksen's acidification indicator

We have followed Henriksen's (1979) approach to acidification. 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. In a Ca^{2+} – pH diagram, acidified and non-acidified lakes can be distinguished by a line (Henriksen's empirical "acidification indicator" line) (see Fig. 4), with acidified lakes above (or to the right of) the line and non-acidified lakes below (or to the left).

RESULTS

Topography and hydrography

The altitudes and some of the most important hydrographical variables measured in the four areas are illustrated in Fig. 2, where the parameter values for each locality in 1988–89 and 2010 have been plotted against their successive locality number; within each area, the numbers are in keeping with increasing altitude.

The huge amount of industrial pollution, leading to acidification of southwestern Scandinavia in 1988–89, can best be illustrated by the different concentrations of the most typical anion component of acidic precipitation, SO_4^{2-}, in AA compared to NT (Fig. 2). The values are very high in coastal (lowland) AA, the area that receives most acidic precipitation, and decreases inland (decrease with altitude $r^2 = 0.77$). The SO_4^{2-} values are quite low for NT. An overview of all the hydrographic variables by area and year is shown in Table 1.

However, the SO_4^{2-} values in AA were significantly lower in 2010 than in 1988–89 (Table 1): average 1.4 vs. 3.0 mg L^{-1} (n = 88 and 77, respectively), only 46 % of the earlier values ($p <$ 0.001) (Mann Whitney U-test). Since SO_4^{2-} analyses for TE and ØF were not carried out in 1988–89, there is no basis for calculating any changes there, but in 2010 TE had quite low SO_4^{2-} values (1.0 mg L^{-1}), while ØF showed the same average (2.1 mg L^{-1}) as localities at similar altitudes (i.e. < 250 m a.s.l.) in AA (2.1 mg L^{-1}). NT also had significantly lower SO_4^{2-} values in 2010 compared to 1988–89: 1.0 mg L^{-1} reduced to 0.8 mg L^{-1} (n = 106 and 97, respectively) ($p < 0.001$).

The reduction in SO_4^{2-} from 1988–89 to 2010 (seen in AA, Fig. 2) is accompanied by a significant increase in pH in the acidified regions (Table 2**).** This was also evident by the inclusion of an area x year interaction in the final selected mixed effect model with pH as depended variable, and year and area as fixed effects nested within sub-area (ΔAICc between best model including interaction and next best model = 2.10). The (arithmetic) average increases in pH for AA and ØF were 0.11 and 0.26, respectively. The numbers of localities with elevated pH values in AA and ØF were also significant (Table 3). In the reference areas (TE and NT), there was no detectable change in pH (Table 2). The (arithmetic) average (non-significant) decrease in pH was 0.06 and 0.01 for TE and NT, respectively. In TE and NT there were no significant numbers of localities with elevated pH (Table 3). On the contrary, there were a few more localities with lower pH, although not significant. Like pH, there was support for similar effects of H^+ (ΔAICc between best model including interaction and next best model = 6.66).

There was also strong evidence for different changes in the other related hydrographic variables between acidified and non-acidified regions (ΔAICc between best model including interaction and next best model > 10.44), with the exception of Na. Both AA and ØF showed decreases in conductivity, Ca^{2+} and Mg^{2+} (Table 1). Conductivity also decreased significantly in TE. In NT, on the other hand, conductivity increased, as did Ca^{2+}, Mg^{2+} and Na^+. For water colour, we have good data only for 2010.

The relationship between Ca^{2+} ions and pH for all localities in the four areas is shown in Fig. 3, where Henriksen's empirical "acidification indicator" line distinguishes acidified (above or to the right of the line) from non-acidified ponds and lakelets. As seen from the figure, all except one AA locality can be considered acidified in 1988–89, and most were strongly acidified, i.e. they are situated far above or to the right of the line. However, in 2010, the localities are clearly less acidified. In ØF, we see the same positive trend as in AA with respect to acidification. In TE, and also in the reference area (NT), the situation in 2010 was still about the same as in 1988–89.

The amphibians

Amphibians existed in all four areas in a number of localities above pH 4.5–4.7, especially at the relatively higher pH values (Fig. 4). Above pH 5.0, in AA, TE and ØF, amphibians occurred in 80–100 % of the localities in both investigation periods (1988–89 and 2010). In NT (above pH 5.0), the frequency of amphibian sites (*R. temporaria*) was about 90 % and 70 %, respectively, for 1988–89 and 2010.

There was an increase in the number of localities where amphibians were recorded in the acidified areas (AA and ØF), but not in the non-acidified areas (ΔAICc between best model with amphibian presence or absence as dependent variable, including interaction, and next best model, not including interaction > 9.29). In AA and ØF together (n = 115), the frequency of amphibian localities rose from 33 % in 1988–89 to 49 % in 2010. The increases in the number of localities for both *R. temporaria* and *L. vulgaris* separately were significant (Chi-square test: $X^2 = 4.90$, $df = 1$, $p < 0.05$ and $X^2 = 5.44$, $df = 1$, $p < 0.02$, respectively).

There was also an increase in the number of amphibian species recorded at each locality in the acidified areas during the study period; the average number of amphibian species per locality rose from 0.51 to 0.88, whereas no such difference was recorded in the non-acidified areas (ΔAICc between best model with number of species as dependent variable, including interaction between area and year, and next best model not including interaction > 7.53). For AA and ØF, there was a good correlation between the rise in pH and the increase of amphibian species (Spearman correlation test: $p = 0.01$). (For the two areas separately the Spearman correlation test showed $p = 0.023$ and $p = 0.230$ (Table 4).) In the TE area, there was an insignificant increase from 1.14 to 1.18 amphibian species per locality, while in the NT area, the frequency went down from 0.73 to 0.59 amphibian species (*R. temporaria*) per locality, but the decrease was not significant (Chi-square test: $X^2 = 1.4$, $df = 1$, $p < 0.3$). Correspondingly, in both these areas, there were no obvious correlations between changes in the pH and changes in the number of amphibian species (Table 4).

The lowest pH where *R. temporaria* was found to successfully reproduce was 4.5; *L. vulgaris* was also recorded at this pH. For *B. bufo*, it was 4.6. We found no signs of reproduction in

Rana arvalis and *T. cristatus* below a pH of 4.8 and 5.2, respectively, but the number of localities is low.

The Ca–pH relationship for ponds and lakelets in one or more areas seen together, and also the occurrence of amphibians can be seen in Fig. 5.

DISCUSSION

The regional decline and local survival of amphibians

In the past, the acidification of southwestern Scandinavia increased over a period of more than 100 years. In 1988–89, it was obvious that not only were amphibians rare in inland districts, they were also absent from large areas, like Solhomfjell in AA (Dolmen et al. 2008). However, many amphibians, like *Rana temporaria*, *Bufo bufo* and *Lissotriton vulgaris* had survived the period of acidification in the most coastal and lowland areas (below the ML) where buffering was better, and also in several refugia in inland districts where the bedrock was less acidic. Very small populations of the pool frog (*Pelophylax lessonae* Camerano, 1882), *Rana arvalis* and (possibly until recently) *Triturus cristatus* also survived in southernmost coastal Norway, which, for the sake of dispersal there, must have had been much larger earlier. This decline, too, may have been connected to and indicate the heavy impact of the acidification of the area (Dolmen 1996; Dolmen et al. 2008; D. Dolmen, unpubl.). In 2010, however, the pH of the precipitation was already markedly higher.

Acidification and the hydrochemical regime

The acidification of southwestern Scandinavia probably started before 1900 (Lippestad 2014), but accelerated after World War 2, also in other parts of Europe. In Scandinavia it peaked around 1980–90, and since then there has been a slow rise of the pH in the precipitation, also recorded in many lakes and rivers (Bouwman and Vuuren 1999; Aas et al. 2011; Moldan et al. 2013). Nevertheless, in some of our investigated localities the water had become even more acidic. The explanation is probably humic acids and natural acidification through cation uptake and ion exchange mechanisms by *Sphagnum* mosses, etc. (Dolmen et al. 2008).

The most acidified areas (AA and ØF) recorded significant decreases from 1988–89 to 2010 also in the water conductivity and the content of Ca^{2+} and Mg^{2+} ions in the water. Since H^+

ions make up a considerable part of the conductivity at pH < 5.0 (Busenberg and Plummer 1987; ASTM 2014), we ascribe much of the decrease in conductivity in this period to the increase of pH. Acidification usually also brings about strong dissolving processes and cation exchange relative to the bedrock. This leads to comparatively high concentrations of, for example, Ca^{2+} and Mg^{2+} in the water (Alewell et al. 2000; AirClim 2011). As the pH rises, this leaching from the bedrock slows down; hence the decrease in Ca^{2+} and Mg^{2+} from 1988–89 to 2010 (see also Garmo et al. 2014). The decrease of Ca^{2+} and Mg^{2+} ions brings about a further decrease in the conductivity. The two somewhat different hydrochemical regimes, in 1988–89 and 2010, can be seen from Fig. 2.

In NT, the opposite change is seen from 1988–89 to 2010: a small decrease in pH, although not significant, but the conductivity increased significantly, as did the concentrations of Ca^{2+} and Mg^{2+}. The increased acidity can probably be explained by natural acidification (see above). The small differences in Na^{+} seen in ØF and NT, although statistically significant, are thought to be merely the results of different predominant wind directions and rainfall.

Al may exist in various forms depending on water pH, temperature and chelating compounds, some of which are toxic to aquatic life, in particular the hydroxides $Al(OH)_2^{+}$ and $Al(OH)^{2+}$. These forms occur at their highest concentrations around pH 5.0–5.5 (Freda 1991; Gensemer and Playle 1999). However, since the great majority of ponds and lakes in the present study are on bogs with humic water (high water colour value, see Fig. 2), which binds and detoxifies the Al compounds, the presence of Al is regarded as being of only minor importance here (see Gensemer and Playle 1999; Dolmen et al. 2008).

Acidification and the amphibians

The degree of acidification of the lakelets in the investigated areas in 1988–89 (at the maximum of the acidification), and also the much better situation in 2010, are depicted in Figs 3 and 5.

We also see that amphibians can often live without difficulty in quite heavily acidified biotopes; it is the acidity (pH) that sets the tolerance limit. In Fig. 5, the ameliorating effect of Ca^{2+} on the occurrence of *R. temporaria* and *B. bufo* in acidic water can likewise be discerned;

the higher the concentration of Ca^{2+} in the water, the lower pH can be tolerated. Also increased concentrations of Na^{+} (NaCl) and humus (high water colour value) has a similar effect (Dolmen et al. 2008).

Tolerance limits

Most of the current amphibian species, at least within the study areas, seem to have a tolerance limit for acidity around pH 4.5–4.8, depending on other environmental factors like Ca^{2+} and NaCl in the water. However, it seems to be higher for *Triturus cristatus* (although data are scarce); the lowest pH where it was found reproducing was 5.2 (Fig. 5). This is also the general impression of *T. cristatus*. It prefers ponds and lakelets with a higher pH and is also the most vulnerable of the species dealt with here (Strand 2002; Skei et al. 2006; Dolmen 2010). Most of the minimum pH values seen for species in the present study are supported by Strand's (2002) investigations of close to 1300 ponds and lakes in various parts of Norway. Only *R. arvalis* (n = 61) had a lower minimum (pH 4.4) in Strand's studies (cf. Andrén et al. 1988; Hangartner et al. 2011). The lower pH limit seen in our study coincides well with the overviews by Barr (2011) and EPA (2012) who mention that most amphibians require a pH higher than 4.5–5.0 for embryo survival and metamorphosis. Lower tolerance limits have been reported, but many of the studies have been achieved in the laboratory, not in the wild, and therefore should not be directly compared to natural conditions (Persson et al. 2007). However, populations from acidified areas tend to tolerate lower pH than populations from non-acidified areas (Glos et al. 2003; Hangartner et al. 2011).

Better survival and amphibian recovery

There is little doubt that acidic precipitation has been the one factor that has posed the greatest threat to amphibians in southwestern Scandinavia during the 20th century, at least outside urban areas. Reports also from other parts of the world include and emphasise the negative effect of acidification on amphibians there (see the Introduction), for example in the Netherlands, where acidic precipitation was considered by Fedorenkova et al. (2012) to be the most important pollutant responsible for amphibian decline.

However, in southwestern Scandinavia, we have shown that with less acidified rain since about 1980–90, the pH of ponds and lakelets in this area has again increased. Moreover, in the same period, at least up to the study in 2010, amphibians have (re-)expanded into localities and areas which have become suitable for them. Possibly, the opposite situation was seen in the northern reference area (NT), where acidity increased slightly and the number of amphibian localities decreased, although not significantly, between 1988-89 and 2010.

However, there is a considerable time lag in the healing process of the ecosystem because of the complexity of the soil and water chemistry. It may therefore take a long time for the fauna and flora of the acidified areas to recover completely; AirClim (2011) mentions one hundred years or more, depending on the depth and composition of the soil. Acidification must therefore still be considered a problem for aquatic life for a long time (Stoddard et al. 1999; Alewell et al. 2000; Garmo et al. 2014). Climate changes and forest practises may also contribute to acidification in the future (Moldan et al. 2013).

Perspectives

Since the pH of the precipitation in most parts of Europe and North America has now (2017) increased considerably compared with the 1980–90s (Alewell et al. 2000; Aas et al. 2011; Garmo et al. 2014), we will probably also see a positive trend in amphibian abundance and diversity in other acid-sensitive areas of the Western Hemisphere during the next few decades.

Nevertheless, the problems surrounding amphibians and acidification will continue to increase in the developing world, especially Asia. China became the world's largest emitter of SO_2 in 2005 (Su et al. 2011), but has reduced its emissions since about 2006. India is still increasing them (Klimont et al. 2013). Asian emissions of SO_2, NO_x and NH_3 are predicted to equal, or be greater than, the combined emissions from Europe and North America by 2020 (Galloway 1995).

Globally, there is a peculiar overlap between areas with high (or relative high) amphibian diversity and areas with (present and predicted) high annual deposition of sulphur and nitrogen (compare e.g. IUCN 2013 and GAA 2014 with Bouwman and Vuuren 1999). The bedrock in these areas is also very often vulnerable to anthropogenic acidification. In addition to eastern North America and Central Europe, we can mention Equatorial Africa, the Indian Peninsula and eastern China. This simply means that a great number of amphibians prefer the same land areas as humans, but it also means that these amphibians live under a serious threat from habitat destruction and pollution including acidic precipitation. Other parts of the world with high amphibian diversity, like Central and South America, southern Africa and the southeast Asian peninsula, including Malaysia and Indonesia, also have sensitive bedrock or soil, i.e. potential problems, but most of them so far suffer little anthropogenic acidification.

Conclusions

Regarding our hypotheses in the Introduction, after the decrease of SO_4^{2-} and increase of pH in the precipitation from 1988–89 to 2010 in southwestern Scandinavia, it was possible to see an increase of pH in the ponds and lakes two decades after the positive trend started. It was also possible to see an improvement in, or a recovery of, amphibian populations in the region following the improvement in the water chemistry.

Although the problems surrounding amphibians and anthropogenic acidification now may be less severe in parts of the "western" world compared to the situation a few decades ago, it is probably still increasing in the developing world, especially in Asia.

Acknowledgements

The hydrochemical analyses, except pH, water colour and conductivity, were carried out by Syverin Lierhagen at the NTNU Department of Chemistry. Ole Kristian Berg did the paired t-test and Lars Rønning the Spearman correlation test. The map was made by Marc Daverdin, and the English language consultant has been Richard Binns.

REFERENCES

Aas, W., S. Solberg, S. Manø, and K.E. Yttri. 2011. Overvåking av langtransportert forurenset luft og nedbør. Atmosfærisk tilførsel, 2010. (Monitoring of long-range transported air pollutants. Annual report for 2010.) Statlig Program for forurensningsovervåking rapport 1099, Klima- og Forurensningsdirektoratet, Oslo, Norway (in Norwegian, English summary.)

Air Pollution. 2014. 30 information sheets on Air Pollution. Retrieved 6 March, 2014, from http://www.air-quality.org.uk/index.php.

AirClim. 2011. Acidification. Air Pollution & Climate Secretariat. Retrieved 20 March, 2014, from http://www.airclim.org/publications/air-and-environment/acidification.

Alewell, C., B. Manderscheid, H. Meesenburg, and J. Bittersohl. 2000. Is acidification still an ecological threat? *Nature* 407: 856–857.

Andrén, C., L. Henrikson, M. Olsson, and G. Nilson. 1988. Effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina*. *Holarctic Ecology* 11: 127–135.

ASTM. 2014. Standard test method for on-line pH measurement of water of low conductivity. ASTM D5128–14. Retrieved 21 March, 2014, from http://enterprise.astm.org/filtrexx40.cgi?+REDLINE_PAGES/D5128.htm.

Barr, R. 2011. A look at the effects of acid rain on amphibians. Critters 360. Retrieved 15 March, 2014, from http://www.critters360.com/index.php/a-look-at-the-effects-of-acid-rain-on-amphibians-2773/.

Bates, D., M. Machler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48. doi: 10.18637/jss.v067.i01.

Beebee, T.J.C., and R.A. Griffiths. 2005. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* 125: 271–285.

Blakar, I.A., and D. Hongve. 1997. On the chemical water quality in Høylandet, a reference area for acidification research. *Hydrobiologia* 34: 37–47.

Blaustein, A.R., and B.A. Bancroft. 2007. Amphibian population declines: Evolutionary considerations. *BioScience* 57: 437–444.

Blaustein, A.R., J.M. Romansic., J.M. Kiesecker, and A.C. Hatch. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity & Distributions* 9: 123–140.

Böhmer, J., and H. Rahmann. 1990. Influence of surface water acidification on amphibians. In *Biology and physiology of amphibians*, ed. W. Hanke, 287– 309. Stuttgart: Gustav Fisher Verlag.

Bouwman, A.F., and D.P. van Vuuren. 1999. Global assessment of acidification and eutrophication of natural ecosystems. RIVM report 402001 012 (Research for man and environment; National Institute of Public Health and the Environment), Bilthoven, Netherlands.

Busenberg, E., and L.N. Plummer. 1987. pH measurement of low-conductivity waters. U.S. Geological Survey, Water-resources investigations Report 87–4060.

Clark, K.L., and R.J. Hall. 1985. Effects of elevated hydrogen ion and aluminium concentrations on the survival of amphibian embryos and larvae. *Canadian Journal of Zoology* 63: 116–123.

Cushman, S. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128: 231–240.

Dolmen, D. 1987. Hazards to Norwegian amphibians. In *Proceedings of the 4th Ordinary General Meeting of the Societas Europaea Herpetologica, Nijmegen 1987*, ed. J.J. van Gelder, H. Strijbosch, and P.J.M. Bergers, 119–122. Nijmegen: Catholic University (Faculty of Sciences).

Dolmen D. 1996. Damfrosk, *Rana lessonae* Camerano, oppdaget i Norge. *Fauna* (Oslo) 49: 178–180. (In Norwegian)

Dolmen, D. 2010. Amfibier og reptiler. Amphibia and Reptilia. In *Norsk rødliste for arter 2010. The 2010 Norwegian red* *list for species*, ed. J.A. Kålås, Å. Viken, S. Henriksen, and S. Skjelseth, 413–417. Trondheim (Norway): Artsdatabanken.

Dolmen, D., J.K. Skei, and I. Blakar I. 2008. Scandinavian amphibians: their aquatic habitat and tolerance to acidic water – a field study. *Fauna norvegica* 26/27: 15–29.

Dunson, W.A., R.L. Wyman, and E.S. Corbett. 1992. A symposium on amphibian declines and habitat acidification. *Journal of Herpetology* 26: 349–352.

Elvingson, P., and C. Ågren. 2004. Air and the environment. The Swedish NGO Secretariat on Acid Rain, Göteborg (Gothenborg), Sweden.

EPA. 2012. Amphibians and reptiles. Use as indicators. Water: Monitoring and assessments. United States Environmental Protection Agency. Retrieved 17 March, 2014, from http://water.epa.gov/type/wetlands/assessment/herps.cfm.

Fedorenkova, A., J.A. Vonk, H.J.R. Lenders, R.C.M. Cremers, A.M. Breure, and A.J. Hendriks. 2012. Ranking ecological risks of multiple chemical stressors on amphibians. – *Environmental Toxicology and Chemistry* 31: 1416–1421.

Freda, J. 1991. The effects of aluminium and other metals on amphibians. *Environmental Pollution* 71: 305–328.

GAA. 2014. Key findings on threat status and distribution of all amphibians known to science. Global amphibian assessment. Retrieved 12 March, 2014, from http://www.pacificbio.org/initiatives/ESIN/News/global_amphibian_assessment.htm.

Galloway, J.N. 1995. Acid deposition: Perspectives in time and space. *Water, Air, & Soil Pollution* 85: 15–24.

Gardner, T. 2001. Declining amphibian populations: a global phenomenon in conservation biology. *Animal Biodiversity and Conservation* 24 (2): 25–44.

Garmo, Ø.A., B.L. Skjelkvåle, H.A. de Wit, L. Colombo, C. Curtis, J. Fölster, A. Hoffmann, J. Hruška, et al. 2014. Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water, Air, & Soil Pollution* 03/2014; 225:1–14. doi: 10.1007/s11270-014-1880-6.

Gensemer, R.W., and R.C. Playle. 1999. The bioavailability and toxicity of aluminium in aquatic environments. *Critical Reviews in Environmental Science and Technology* 29: 315–450.

Glos, J., T.U. Grafe, M-O. Rödel and K.E. Linsenmair. 2003. Geographic variation in pH tolerance of two populations of the European common frog, *Rana temporaria*. *Copeia* 2003: 650–656.

Hagström, T. 1981. Reproductive strategy and success of amphibians in water acidified by atmospheric pollution. In *Proceedings of the European Herpetological Symposium at Cotswold Wild Life Park, Oxford 1980*, ed. J. Coborn, 55–57. Oxford: Cotswold Wild Life Park.

Freckleton, R.P. 2002. *On the misuse of residuals in ecology: regression of residuals vs. multiple regression. Journal of Animal Ecology* 71: 542–545.

Grueber, C.E., S. Nakagawa, R.J. Laws, and I.G. Jamieson. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *Journal of Evolutionary Biology* 24: 699–711.

Haines, T.A. 1981. Acid precipitation and its consequences for aquatic ecosystems: A review. *Transactions of the American Fisheries Society* 110: 669–707.

Hangartner, S., A. Laurila, and K. Räsänen. 2011. Adaptive divergence of the moor frog (*Rana arvalis*) along an acidification gradient. *BMC Evolutionary Biology* 11: 366. doi: 10.1186/1471-2148-11-366

Henriksen, A. 1979. A simple approach for identifying and measuring acidification of freshwater. *Nature* 278: 542–545.

Henriksen, A., L. Lien, T.S. Traaen, I.S. Sevaldrud, and D.F. Brekke. 1988. Lake acidification in Norway – present and predicted chemical status. *Ambio* 17: 259–266.

Hesthagen, T., I.H. Sevaldrud, and H.M. Berger. 1999. Assessment of damage to fish populations in Norwegian lakes due to acidification. *Ambio* 28: 112–117.

Hopkins, W.A. 2007. Amphibians as models for studying environmental change. *ILAR Journal* 48: 270–277.

ICE. 2014. Declining Amphibian Population Task Force (DAPTF). INFORMATION Center for the Environment (ICE), University of California. Retrieved 20 March, 2014, from http://ice.ucdavis.edu/project/daptf.

IUCN. 2013. Amphibians. The IUCN red list of threatened species 2013.2. Retrieved 18 March, 2014, from http://www.iucnredlist.org/initiatives/amphibians.

Klimont, Z., S.J. Smith, and J. Cofala. 2013. The last decade of global anthropogenic sulfur dioxide: 2000-2011 emissions. *Environmental Research Letters* 8 014003. doi: 10.1088/1748-9326/8/1/014003

Lehtinen, R.M., S.M. Galatowitsch, and J.R. Tester. 1999. Consequences of habitat loss and fragmentation for wetland amphibian assemblages. *Wetlands* 19 (1): 1–12.

Leuven, R.S.E.W., C. den Hartog, M.M.C. Christiaans, and W.H.C. Hijligers. 1986. Effects of water acidification on the distribution and the reproductive success of amphibians. *Experientia* 42: 495–503.

Levy, M.A. 1995. International co-operation to combat acid rain. In *Green globe yearbook of international co-operation on environment and development 1995*, ed. H.O. Bergesen, G. Parmann, and Ø.B. Thommessen, 59–68. Oxford: Oxford University Press.

Lippestad, H. 2014. Meteorologisk instituttets historie. Norwegian Meteorological Institute. Retrieved 11March, 2014, from http://www.met.no/Om_oss/Instituttets_historie (In Norwegian).

Mason, J. 1989. Introduction. The causes and consequences of surface water acidification. In *Acid toxicity and aquatic animals* (Society for Experimental Biology Seminar Series 34), ed. R. Morris, E.W. Taylor, D.J.A. Brown, and J.A. Brown, 1-12. Cambridge: Cambridge University Press.

Moldan, F., B.J. Cosby, and R.F. Wright. 2013. Modeling past and future acidification of Swedish lakes. *Ambio* 42: 577–586. doi: 10.1007/s13280-012-0360-8.

Persson, M., K. Räsänen, A. Laurila, and J. Merilä. 2007. Maternally determined adaptation to acidity in *Rana arvalis*: Are laboratory and field estimates of embryonic stress tolerance congruent? *Canadian Journal of Zoology* 85: 832–838.

Pierce, B.A. 1993. The effects of acid precipitation on amphibians. *Ecotoxicology* 2: 65–77.

R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved 14 August, 2017, from https://www.R-project.org/.

Skei, J.K., D. Dolmen, L. Rønning, and T.H. Ringsby. 2006. Habitat use during the aquatic phase of the newts *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) in central Norway: proposition for a conservation and monitoring area. *Amphibia-Reptilia* 27: 309–324.

Stoddard, J.L., D.S. Jeffries, A. Lükeviler, T.A. Clair, P.J. Dillon, C.T. Driscoll, M. Forsius, M. Johannessen, et al. 1999. Regional trends in aquatic recovery from acidification in North America and Europe. *Nature* 401: 575–578.

Strand, L.Å. 2002. Reproduksjon hos amfibier i vann med ekstreme pH-verdier. (Breeding in amphibians in waters with extreme pH-values.) *Fauna* (Oslo) 55: 108–114 (in Norwegian, English summary).

Stuart, S., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fishman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1786.

Su, S., B. Li, S. Cui, and S. Tao. 2011. Sulfur dioxide emissions from combustion in China: from 1990 to 2007. *Environmental Science and Technology 45* (19): 8403–8410.

Vos C.C., and J.P. Chardon. 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *Journal of Applied Ecology* 35: 44–56.

Wells, K.D. 2007. *The ecology and behaviour of amphibians*. Chicago: The University of Chicago Press

Author Biographies

Dag Dolmen is an Associated Professor at The Norwegian University of Science and Technology, NTNU University Museum, in Trondheim, Norway. His main research interests are herpetology, biogeography and freshwater ecology, including acidification problems.
Address: NTNU University Museum; NO-7491 Trondheim, Norway.
e-mail: dag.dolmen@ntnu.no

Anders Gravbrøt Finstad is an Associated Professor at The Norwegian University of Science and Technology, NTNU University Museum, in Trondheim, Norway. His main research interests are community dynamics, biogeography and freshwater ecology.
Address: NTNU University Museum; NO-7491 Trondheim, Norway.
e-mail: anders.finstad@ntnu.no

Jon K. Skei is a Free-lance Researcher at Skei Biomangfold Konsult. His main research interests are herpetology, entomology, biodiversity and freshwater ecology.
Address: Skei Biomangfold Konsult, Skeisvegen 16; NO-7510 Skatval, Norway.
e-mail: Triturus@hotmail.no

Table 1 Mean values ± standard deviations and minimum and maximum values (in parenthesis) for hydrographic factors in the four areas of Norway in 1988–89 and 2010. Significant changes (Mann Whitney U-test) in bold. (For pH, the mean values are arithmetic means.)

Area	AA	TE	ØF	NT
H^+ (1988/89)	22.94±13.16	8.07±12.29	25.55±23.82	7.55±4.39
	(0.87-52.48)	(0.25-40.74)	(0.44-77.62)	(0.72-22.91)
(2010)	19.18±12.54	9.05±12.72	14.48±14.85	9.01±7.11
	(0.49-47.86)	(0.25-36.31)	(0.42-61.66)	(0.76-33.88)
pH (1988-89)	4.78 ±0.44	5.68 ±0.75	4.86 ±0.58	5.20 ±0.29
	(4.28-6.06)	(4.39-6.61)	(4.11-6.36)	(4.64-6.14)
(2010)	4.89 ±0.47	5.62 ±0.77	5.12 ±0.58	5.19 ±0.39
	(4.32-6.31)	(4.44-6.61)	(4.21-6.38)	(4.47-6.12)
SO_4^{2-} mg L^{-1}	3.03 ±1.56			1.03 ±0.45
(1988-89)	(0.92-6.26)			(0.06-2.46)
(2010)	**1.39 ±0.66**	0.95 ±0.28	2.09 ±0.67	**0.76 ±0.22**
	(0.61-2.92)	(0.50-1.35)	(0.60-3.28)	**(0.23-1.46)**
Conduct. µS cm^{-1}	23.7 ±8.27	17.7 ±4.25	44.8 ±6.62	13.7 ±5.33
(1988-89)	(13.0-48.0)	(13.0-33.0)	(31.0-57.0)	(4.0-30.0)
(2010)	**17.6 ±5.74**	**14.7 ±4.21**	**33.4 ±15.17**	**14.2 ±3.29**
	(9.0-33.7)	**(7.3-23.7)**	**(22.6-84.0)**	**(4.1-21.8)**
Ca^{2+} mg L^{-1}	0.70 ±0.66	1.66 ±1.09	1.83 ±1.05	0.27 ±0.23
(1988-89)	(0.07-2.44)	(0.16-4.81)	(0.45-3.92)	(0.03-1.24)
(2010)	**0.51 ±0.52**	1.42 ±0.99	**1.29 ±1.07**	**0.36 ±0.28**
	(0.05-2.45)	(0.07-3.17)	**(0.18-4.12)**	**(0.06-1.57)**
Mg^{2+} mg L^{-1}	0.23 ±0.20	0.28 ±0.10	0.73 ±0.24	0.19 ±0.12
(1988-89)	(0.04-0.70)	(0.06-0.50)	(0.32-1.35)	(0.02-0.50)
(2010)	0.18 ±0.15	0.22 ±0.10	**0.46 ±0.20**	**0.24 ±0.09**
	(0.04-0.63)	(0.04-0.40)	**(0.19-0.87)**	**(0.04-0.56)**
Na^+ mg L^{-1}	1.20 ±0.77	0.66 ±0.10	3.24 ±0.65	1.46 ±0.62
(1988-89)	(0.24-2.93)	(0.45-0.89)	(1.72-4.99)	(0.25-3.39)
(2010)	1.28 ±0.74	0.71 ±0.18	**3.28 ±2.20**	**1.72 ±0.43**
	(0.41-2.93)	(0.36-1.03)	**(1.80-10.73)**	**(0.38-2.79)**
Colour mg Pt L^{-1}	69.5 ±22.8	70.7 ±39.3	157.0 ±93.5	93.7 ±43.0
(2010)	(35.0-130.0)	(35.0-220.0)	(40.0-400.0)	(5.0-200.0)

Table 2. Paired t-test for means of the H^+ concentration and pH of the investigated localities in Aust-Agder (AA), Østfold (ØF), Telemark (TE) and Nord-Trøndelag (NT).

Area	parameter	paired differences	*n*	*t*	*df*	*p* <
AA	H+	3.75	88	4.42	87	0.001
	pH	-0.10	88	-4.35	87	0.001
ØF	H+	11.07	27	3.96	26	0.001
	pH	-0.26	27	-3.57	26	0.001
TE	H+	-0.98	21	-0.86	20	0.4
	pH	0.06	21	1.57	20	0.1
NT	H+	-1.46	106	-2.33	105	0.02
	pH	0.01	106	0.33	105	0.7

Table 3. Chi-square test. The number of localities with elevated pH values in Aust-Agder (AA), Østfold (ØF), Telemark (TE) and Nord-Trøndelag (NT).

Area	pH elevation	*n*	X^2	*df*	*p* <
AA	56	88	6.54	1	0.02
ØF	21	27	8.34	1	0.01
TE	8	21	1.2	1	0.3
NT	43	106	3.7	1	0.1

Table 4. Spearman correlation test (2-tailed) showing the relationship between changes in pH and the changing in the number of amphibian species.

Area	Spearman correlation	*n*	*p* =
AA	0.227	88	0.023
ØF	0.239	27	0.230
TE	0.191	21	0.408

NT	0.040	106	0.687

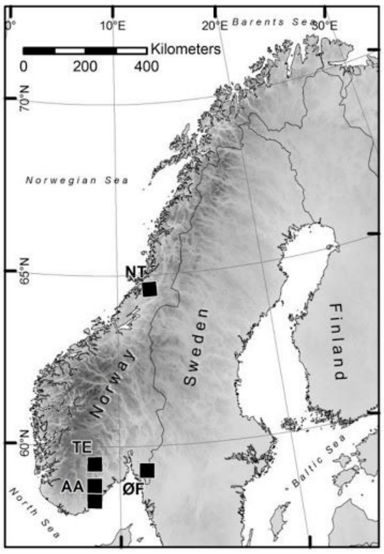

Fig. 1 The locations of the investigated areas: AA and ØF: heavily acidified areas, TE: better buffered, comparison area, NT: non-acidified reference area.

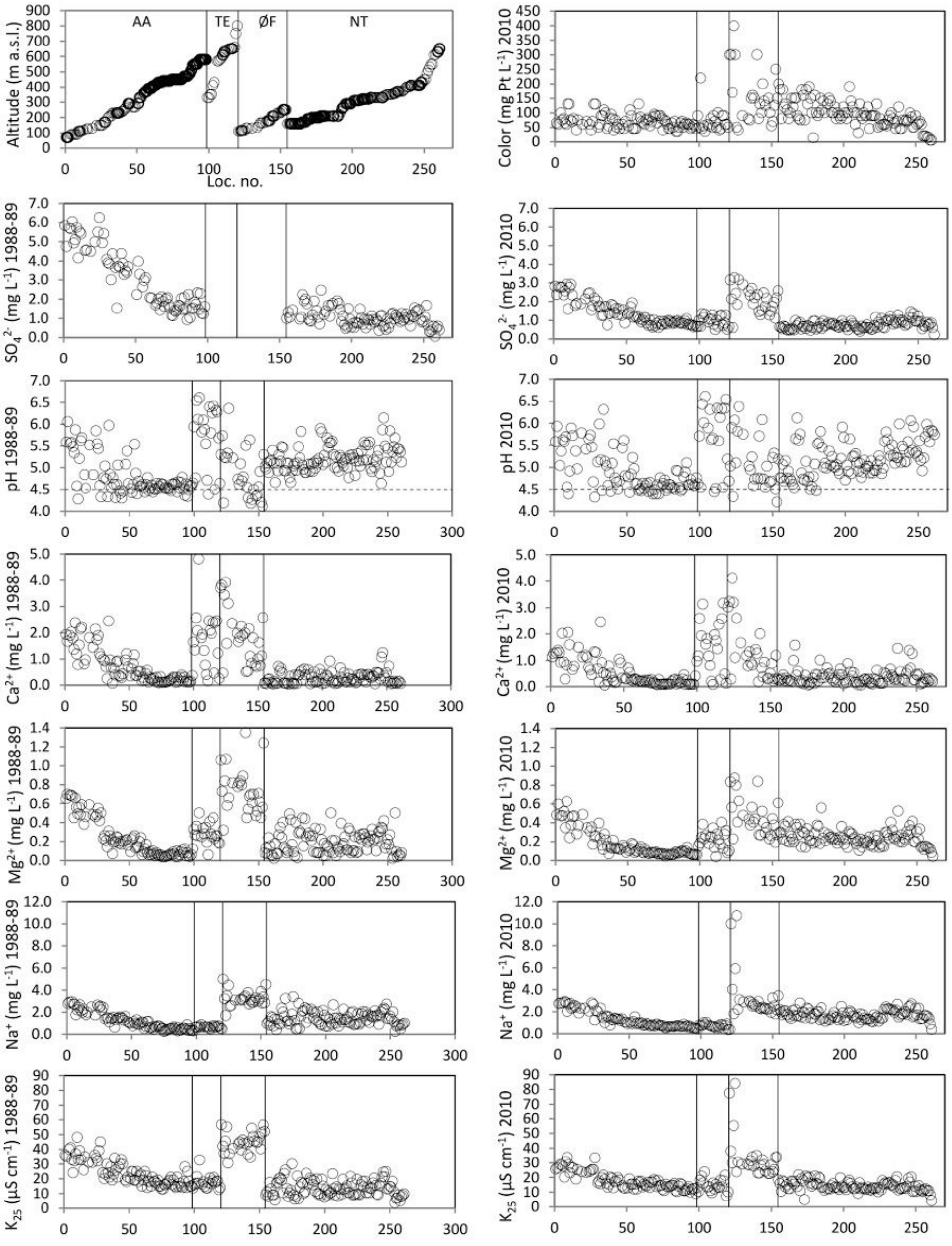

Fig. 2 Altitude and water quality variable values for each locality plotted against their successive locality numbers (following altitude). AA: loc. 1–98, TE: 99–120, ØF: 121–154, NT 155–261. (A few localities of the original number (261) from 1988–89 were excluded in 2010 (see the text), but the individual locality numbers are maintained.)

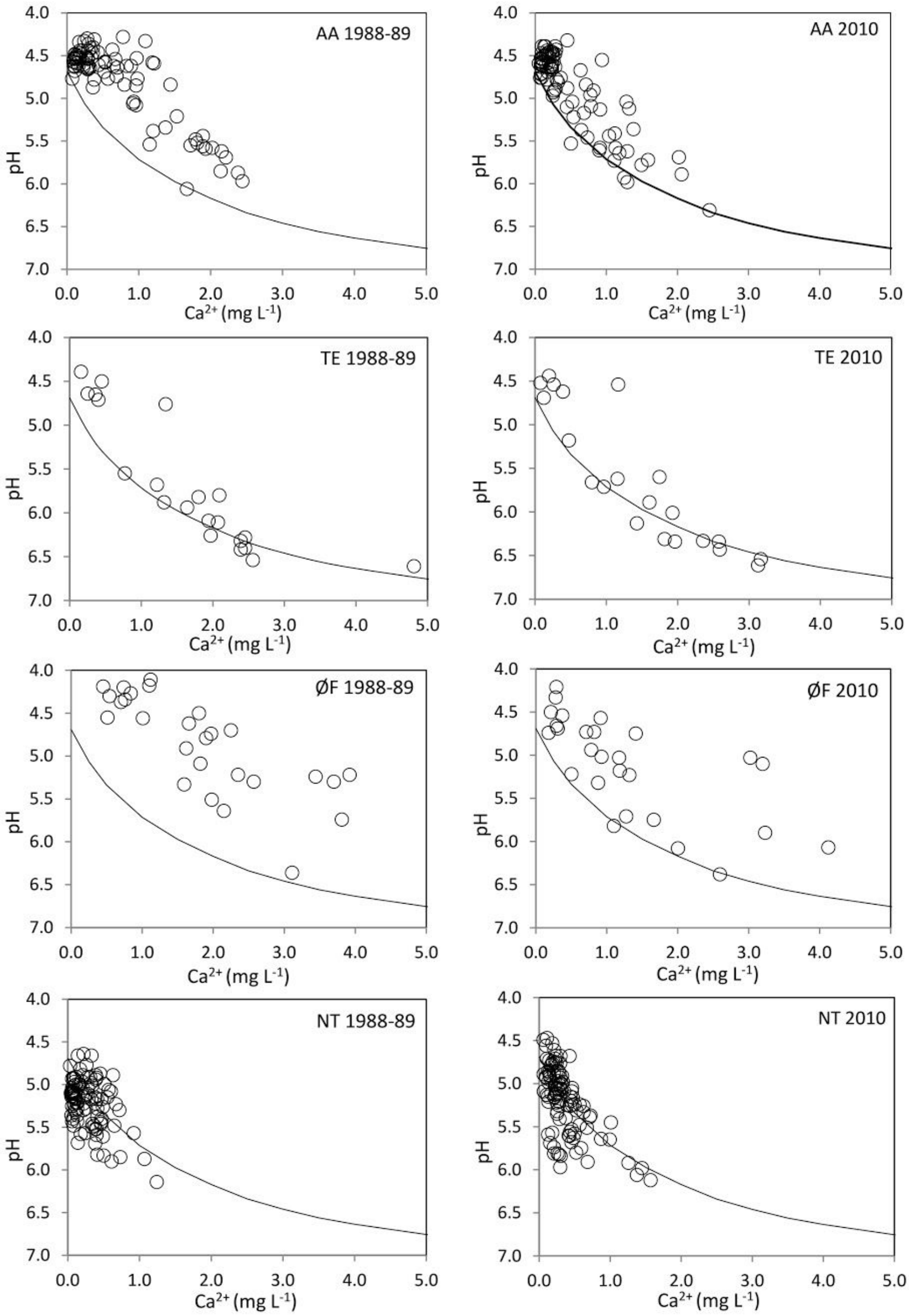

Fig. 3 The relationship between Ca^{2+} and pH in the investigated ponds and lakelets of the four areas investigated in 1988–89 and 2010. Henriksen's (1979) acidification indicator line is inserted; localities above and/or to the right of the line are considered to be acidified.

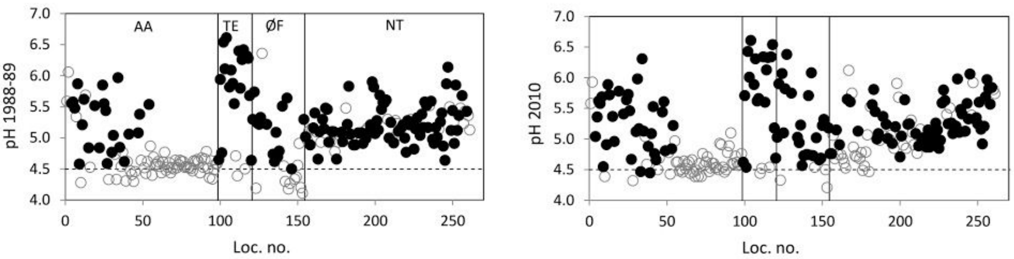

Fig. 4 The occurrence of amphibians in the investigated localities in relationship to pH. Amphibian localities are marked with dots, non-amphibian localities with open circles.

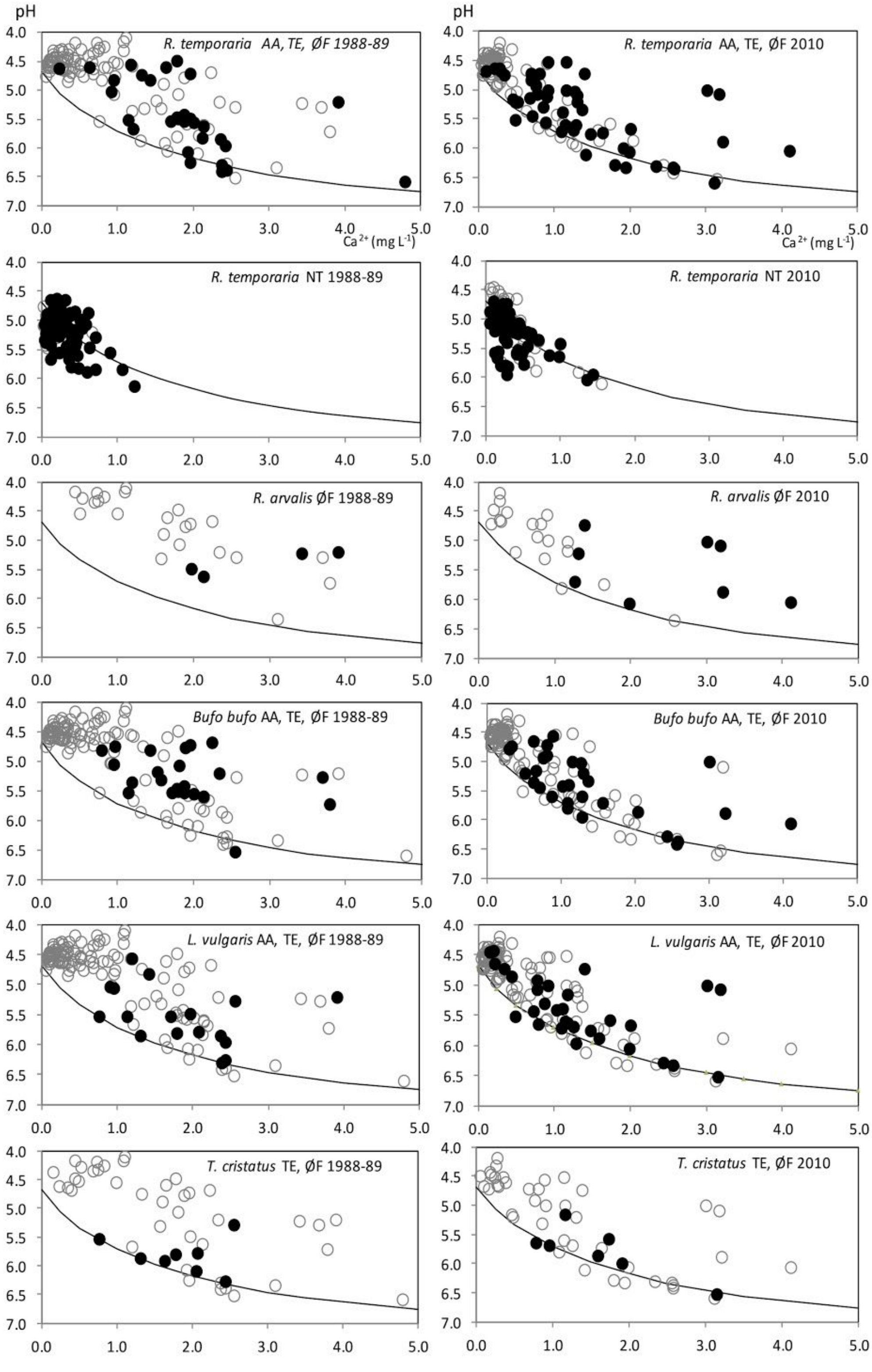

Fig. 5 The occurrence of amphibians in relationship to Ca^{2+}, pH and Henriksen's acidification indicator line. Black dots denote the presence of amphibians, open circles no amphibians.