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pH Tolerance in Freshwater Bacterioplankton: Trait Variation of the Community as Measured by Leucine Incorporation

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pH is an important factor determining bacterial community composition in soil and water. We have directly determined the community tolerance (trait variation) to pH in communities from 22 lakes and streams ranging in pH from 4 to 9 using a growth-based method not relying on distinguishing between individual populations. The pH in the water samples was altered to up to 16 pH values, covering *in situ* pH ± 2.5 U, and the tolerance was assessed by measuring bacterial growth (Leu incorporation) instantaneously after pH adjustment. The resulting unimodal response curves, reflecting community tolerance to pH, were well modeled with a double logistic equation (mean $R^2 = 0.97$). The optimal pH for growth (pH_{opt}) among the bacterial communities was closely correlated with *in situ* pH, with a slope (0.89 ± 0.099) close to unity. The pH interval, in which growth was ≥90% of that at pH_{opt}, was 1.1 to 3 pH units wide (mean 2.0 pH units). Tolerance response curves of communities originating from circum-neutral pH were symmetrical, whereas in high-pH (8.9) and especially in low-pH (<5.5) waters, asymmetric tolerance curves were found. In low-pH waters, decreasing pH was more detrimental for bacterial growth than increasing pH, with a tendency for the opposite for high-pH waters. A pH tolerance index, using the ratio of growth at only two pH values (pH 4 and 8), was closely related to pH_{opt} ($R^2 = 0.83$), allowing for easy determination of pH tolerance during rapid changes in pH.

By measuring growth of a bacterium in a range of environmental conditions, such as temperature, pH, and salinity, the fundamental niche of the bacterium can be determined. The response to these varied conditions can also be seen as a descriptor of the tolerance of that strain to different environmental factors. In a similar way, the tolerance of a bacterial community in a habitat can be estimated by measuring the intrinsic growth of the community at a range of different environmental conditions. The response curves of a community can then be regarded as the trait distribution (community tolerance) of that community. In soil this approach has been commonly applied to study community tolerance to heavy metals (1–3), other toxicants (4, 5), temperature (6–8), and salinity (9, 10), but this method has been applied less often in aquatic habitats (11–13).

pH has been shown to be a decisive environmental factor determining the bacterial community composition in both soil (14–16) and water (17–22), often being the most important one compared to factors such as temperature and moisture in soil (15) and temperature, water retention time, organic matter, and nutrient concentrations in freshwater systems (17, 19–22). pH is also an environmental factor that can vary greatly in aquatic systems. Lake and stream waters can have pH values below 4 and above 9 even within small geographical areas (such as the area in southern Sweden in the present study). In highly productive lakes, surface pH can be as much as 2 U higher than in bottom waters, a variation driven by vertical differences in photosynthesis, respiration, and redox conditions (23). pH can also fluctuate rapidly. For example, during snow melt and rain storms, stream pH can decrease several units (24, 25), sometimes within a few hours (26). On the other hand, warm and sunny days can result in high photosynthetic activity with increasing pH. Accordingly, diel changes of 2 pH units and seasonal changes of 3 pH units may be found in highly productive aquatic environments (27–29). During episodes of rapid pH changes, the bacterial community may not be optimally adapted to the new pH condition, resulting in impaired functions. An indication of this was found in an earlier study in which bacterial communities from different environments were grown in batch cultures at neutral pH (30). Longer lag periods before onset of growth and lower bacterial abundances in the stationary phase were found with starting cultures emanating from low-pH lakes compared to lakes with a neutral pH, where in the latter case a better pH-adapted community was assumed to be present. However, the community adaptation to pH was not explicitly assessed.

In soil, the pH tolerance of the bacterial community has frequently been measured using Leu incorporation as a proxy of growth after adding buffers to alter pH. Usually, a correlation between optimum pH for growth and *in situ* pH has been found (31–33). The same methodology has also been used to monitor changes over time in community pH adaptation, when soil pH has been altered by liming (34). When there was not a perfect match of pH tolerance of the community and soil pH after liming, functions such as bacterial growth was impaired (35). Thus, there was a correlation between the function and the degree of maladaptation of the community to pH. This also means that the community tolerance to pH could be used to directly assess trait variation, without having to explicitly determine community composition.

The pH tolerance (trait variation) of bacterial communities in aquatic environments has thus far not been systematically studied. We here determined the pH tolerance for communities from 22 lakes and streams encompassing a pH gradient between 4 and 9. We followed a similar procedure as that previously adopted in soil

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TABLE 1 Characteristics of 22 tested lake and stream waters[a]

Site	Lake or stream	Letter	*In situ* pH	Frozen or not frozen
Hjärtsjön	S	A	3.99	F
Fiolen	S	B	4.05	F
Älgarydssjön	S	C	4.26	F
Älgarydssjön	L	D	4.33	F
Skärlen	S	E	4.53	F
Hagesjön	S	F	4.53	F
Liasjön	L	G	5.11	F
Boasjön	S	H	5.21	F
Klintsjön	L	I	5.43	F
Tönnesjöfors	S	J	5.97	N
Hagesjön	L	K	6.41	F
Svanåssjön	L	L	6.51	F
Gyslättasjön	L	M	7.05	F
Helgeå	S	N	7.14	N
Hultasjön	L	O	7.41	F
Ecology stream	S	P	7.55	N
Källhultasjön	L	Q	7.60	F
Finjasjön	L	R	7.77	N
Håljasjön	L	S	7.89	N
Vombsjön	L	T	8.25	N
Oppmannasjön	L	U	8.43	N
Ivösjön	L	V	8.86	N

[a] Letters correspond to panel in Fig. 1. L, lake; S, stream; F, frozen; N, not frozen.

(31–33), where the growth rate, estimated using Leu incorporation, was measured instantaneously after altering pH. The resulting pH tolerance curves were fitted to different models, where a novel concept—treating extreme pH as a toxicant modeled by double logistic equations—turned out to provide the best fit. This model was then used to show the relation between optimum pH for growth (pH_{opt}) and *in situ* pH, as well as different effects of increasing and decreasing pH on bacterial growth in high- or low-pH waters. Finally, we compare estimation of pH_{opt} using the full data set with a simplified method using measurements at only two different pH values.

MATERIALS AND METHODS

Water samples. We studied 22 samples from lakes and streams in the south of Sweden (Scania and Småland) that varied in pH from 4.0 to 8.9 (Table 1). Waters varied broadly also in dissolved organic carbon concentration (3.7 to 27.9 mg liter^{-1}) and covered ultraoligotrophic to eutrophic waters (total phosphorus, 3.2 to 87 μg liter^{-1}). All samples were taken as grab samples from the surface with acid-washed polyethylene containers (1 to 2 liters). Some samples were taken fresh and used within 2 days after storage at 20°C. Some had been collected earlier and kept frozen (−18°C) until used. The latter were thawed, and Leu incorporation was measured daily to check that growth was high enough to be able to determine pH tolerance curves. This period never exceeded 4 days.

pH tolerance (trait distribution) of the bacterial community. The pH tolerance of the bacterial community was estimated by measuring Leu incorporation directly after altering pH of the waters either with buffers or by titration with KOH/H_2SO_4. The buffer method (31) is essentially the one described for measuring the pH tolerance of soil bacterial communities here, as modified by Fernández-Calviño and Bååth (32). We used two buffer types to cover as much of the pH gradient as possible (pH 2.5 to 8.5, differing by ~1 U): citrate-phosphate buffer (pH 2.5 to 5.5; citrate, 15.3 to 7.2 mM; phosphate, 2.8 to 18.9 mM) and phosphate buffer (pH 6.5 to 8.5; 66.6 mM), resulting in seven different buffers. A control with natural water was also included. Aliquots (1.35 ml) of sample water were added to 2-ml microcentrifugation tubes, together with 0.15-ml portions of the different buffers. The pH was measured in a buffer-water mixture with a glass electrode and subsequently used in the calculations instead of nominal buffer pH, since at low and high water pH levels the buffering capacity of the pH buffers was not enough to keep the sample at the nominal buffer pH. However, using higher concentrations to improve buffering capacity could lead to inhibition, especially for the citrate-phosphate buffer, and was therefore not an option. Leu incorporation was measured using one replicate per pH level directly after buffer addition.

Since it was difficult to obtain pH levels in the high range with the chosen buffers, changing the pH by adding KOH/H_2SO_4 was compared to the buffer method for three different water samples. The former method was then used for all 22 water samples. This is basically a titration. The sampled water was divided into two subvolumes of 50 ml each and put on a magnetic stirrer. Before changing the pH, 1.5 ml of the natural water was transferred from each of the two subvolumes into a 2-ml microcentifugation vial. Acid (0.1 M) was then added dropwise to one of the subvolumes, and the pH was measured after 30 s to allow the pH to stabilize. Using seven increments, the pH was recorded, and 1.5 ml was transferred to microcentrifugation vials. This procedure was repeated with the second subvolume but with the addition of 0.1 M KOH and thus increasing the pH. In all, this resulted in 2 microcentrifugation vials with *in situ* pH (unmanipulated controls) and 14 vials with different pH levels. For each water sample, we aimed at a pH interval of ±2.5 U of the *in situ* pH. Usually, <0.5 ml of acid or base was required in 50 ml of water to achieve this. To test for any inhibiting concentration of the added KOH/H_2SO_4, K_2SO_4 was added to lake water at increasing concentrations, and the Leu incorporation was measured. No inhibition was found up to ~15 mM K_2SO_4, which is ~30 times more than the highest concentration in any of the pH tolerance measurements. The titration (at room temperature) took less than 30 min for each sample. Leu incorporation was then measured directly according to the method of Smith and Azam (36) at room temperature (22°C) over 1.5 h.

Calculations. Leu incorporation rates were normalized to that in the unmanipulated control with *in situ* pH. Initially, two equations previously applied to model pH tolerance of bacterial communities in soil were used (32, 33). First, we tested a second-degree equation, which can be used as a first approximation of a simple, unimodal model with three fitted parameters. However, as shown by Fernández-Calviño and Bååth (32), this model is not appropriate for nonsymmetrical pH tolerance curves, since it will bias the pH_{opt}. We then used a nonsymmetrical unimodal model, the cardinal pH model (CPM; with four fitted parameters) used for pure culture growth at different pH levels (37). Although this model could accommodate asymmetrical tolerance curves, it could not model "tailings" in bacterial growth found at very low or high pH.

We finally used a novel model consisting of a double logistic equation. The theoretical background is that the ecotoxicological response to increasing concentrations of a toxicant (on a log scale) is well modeled by logistic equations (38). This includes measuring toxicity as decreased Leu incorporation in aquatic conditions (13, 39). We could therefore treat increasing or decreasing pH as increasing concentrations of a toxicant inhibiting growth. To allow for a toxicity increase both for higher and lower pH, we used 2 logistic equations when regressing growth against pH, one for higher pH:

$$\text{Growth} = C_{hpH}/\{1 + \exp[B_{hpH}(pH - A_{hpH})]\} \quad (1)$$

and one for lower pH:

$$\text{Growth} = C_{lpH}/\{1 + \exp[B_{lpH}(pH - A_{lpH})]\} \quad (2)$$

"Growth" in these equations is the normalized Leu incorporation rate, while *pH* is the water pH during Leu incorporation. The parameter *C* is bacterial growth at pH_{opt}, i.e., the maximum growth rate, *B* is a slope parameter indicating the inhibition rate when pH is increasing or decreasing, and *A* is the pH resulting in 50% of growth at pH_{opt} (equivalent to the 50% inhibitory concentration [IC_{50}] in ecotoxicology). The index *hpH* indicates parameters that relate to a higher pH, and the index *lpH* indi-

cates parameters that relate to a lower pH compared to the *in situ* pH. Combining equations 1 and 2 and setting the growth rate at pH_{opt} (denoted C_{opt}) to be the same in the case of decreasing or increasing pH, that is, $C_{opt} = C_{hpH} = C_{lpH}$, resulted in the following double logistic equation covering the entire pH interval:

$$\text{Growth} = C_{opt}/\{1 + \exp(B_{lpH}[pH - A_{lpH})]\} + C_{opt}/\{1 + \exp[B_{hpH}(pH - A_{hpH})]\} - C_{opt} \quad (3)$$

The term C_{opt} at the end of the equation is introduced in order to achieve bacterial growth equal to C_{opt} at pH_{opt}. This equation has the largest number of parameters to be fitted (five parameters), but it has a theoretical base, allowing for both asymmetric response curves and "tailings" at high and low pH. Since each tolerance curve consisted of 16 data points, there was no problem in overfitting due to a low number of data points. Kaleidagraph was used to fit the models using nonlinear regression.

The use of 16 measurements of Leu incorporation to achieve pH tolerance curves will result in numerous measurements when studying changes in pH tolerance over time at a high time resolution. Thus, a simplified protocol to estimate pH tolerance may be required. In soil, such a simplified protocol, using growth measurements at only two pH levels (with a wide span preferentially below and above pH_{opt}) and calculating the log ratio of growth at these pH values, has been shown to correlate well with pH_{opt} estimated from the entire pH tolerance curve (31, 33). We therefore calculated the logarithm of the ratio of growth at pH 8 divided by growth at pH 4 [denoted below as log pH(8/4)] and correlated that value to the pH_{opt}. We excluded the water from Lake Vombsjön (Fig. 1T), since extremely low values calculated for pH 4 made the determination very uncertain.

RESULTS

Good correspondence between methods were found for low- and high-pH waters (Fig. 1B, H, and P) when comparing the use of buffers to titration with dilute H_2SO_4/KOH to alter pH. Thus, both methods allowed for the determination of optimum pH for growth (pH_{opt}). However, for the high-pH stream (Fig. 1P), we could not find suitable buffers to obtain a pH of >8.5, making it impossible to determine full pH tolerance curves for high-pH samples. We therefore chose to use titration when comparing all 22 samples in the main study.

Except for 2 of the 22 samples (Fig. 1E and F), the bacterial community tolerance to pH was well modeled using the double logistic equation, accommodating relationships that were symmetrical (e.g., Fig. 1N, P, and R) or asymmetrical (e.g., Fig. 1A, C, H, and V) and that had narrow (e.g., Fig. 1G, T, and U) or broad (e.g., Fig. 1K and Q) optima. The model could also account for pronounced tailings at low and high pH (Fig. 1B and J). In two low-pH streams (Fig. 1E and F), we did not achieve a high enough pH to result in decreasing growth. These samples were therefore only modeled using equation 2 for decreasing pH, setting the pH_{opt} to the pH that yielded 99% of maximum growth. Overall, the fit to the logistic equation was good. In all cases except two, the R^2 was >0.90, and in 17 of the 22 samples the R^2 was >0.95. The mean R^2 was 0.97.

The use of the cardinal pH model (CPM) and second-degree equation resulted in less well modeled relationships. The mean R^2 for the second-degree equation was 0.85 (range, 0.37 to 0.98), and that for the CPM model was 0.90 (range, 0.71 to 0.99). The second-degree model was especially inadequate in low-pH waters with very asymmetrical pH tolerance relationships.

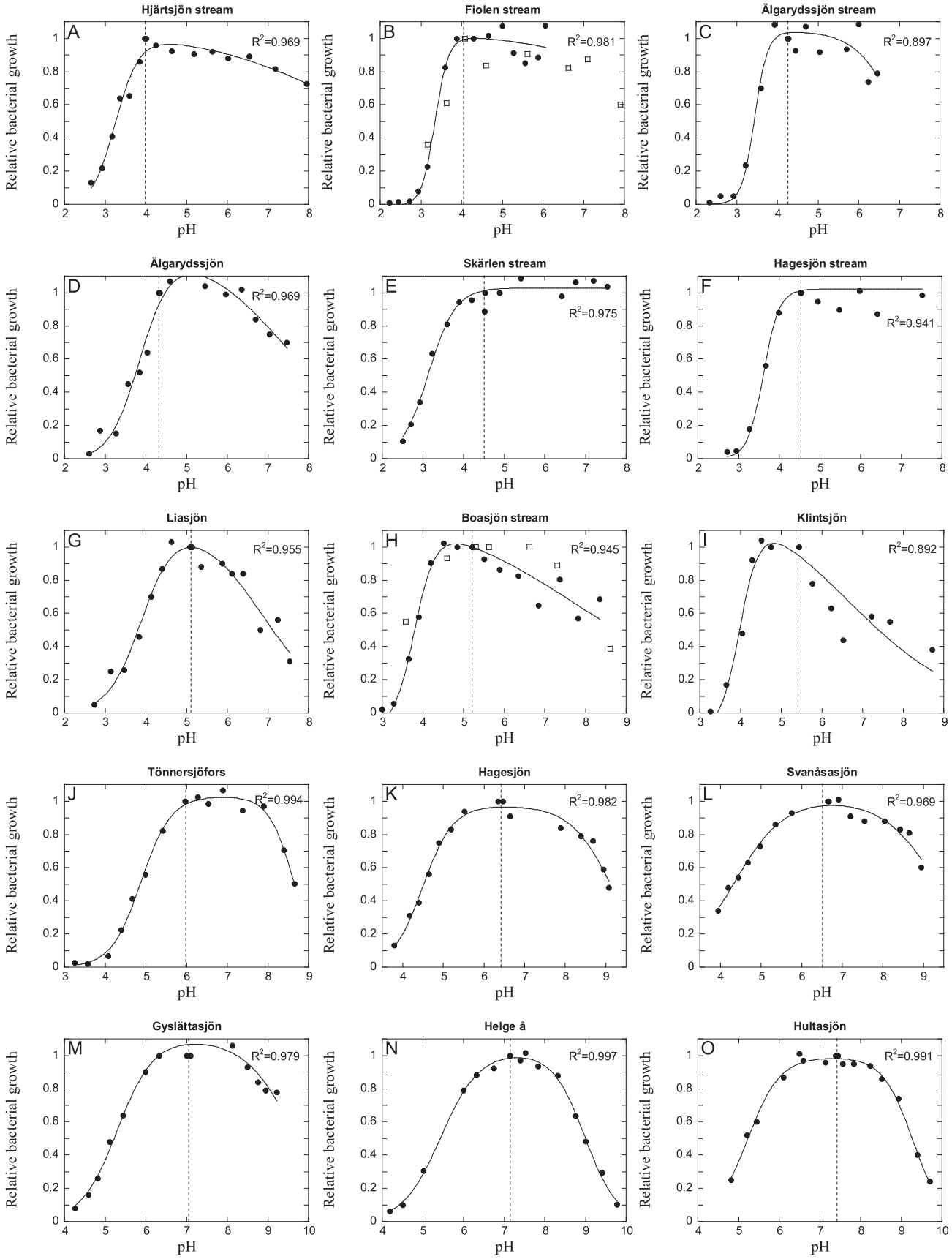
The pH_{opt} for growth differed dramatically between water samples (Fig. 1) and was low (pH ~4) in low-pH water, increasing to pH 9 in the water with the highest pH. The pH_{opt} calculated using the double logistic equation thus resulted in a strong correlation with *in situ* pH ($R^2 = 0.94$), with a slope close to unity (Fig. 2; slope, 0.89 ± 0.099 [95% confidence interval]). The reason for the slope being slightly less than 1 was the low-pH waters, where the asymmetric pH response curves resulted in difficulties in determine the exact pH_{opt}. However, the pH span in which ≥90% of growth at pH_{opt} was found always overlapped with *in situ* pH (Fig. 2), suggesting that the pH_{opt} of the bacterial community growth was similar to the *in situ* pH in all waters.

Although there was a good correlation between pH_{opt} determined with the three different models (double logistic versus CPM, $R^2 = 0.97$; double logistic versus second degree, $R^2 = 0.93$), the CPM and second-degree model deviated more from a 1:1 line when regressed against *in situ* pH. This was especially the case for the second-degree equation (slope of the regression = 0.64), but also for the CPM (slope 0.76). In neutral- to high-pH waters, the use of the three models resulted in very similar pH_{opt} levels. The less-steep slope of the regression, especially for the second degree, but also to some extent for the CPM, was thus due to overestimation of pH_{opt} in low-pH waters.

We calculated 90% of optimal growth as the range in which the community could be assumed to be competitive. This range varied between 1.1 (Fig. 1T) and 3 (Fig. 1B) pH units, with a mean of 2.0 pH units (excluding Fig. 1E and F, with no decrease at high pH). The 90% span using the CPM was similar, with a mean of 1.9 U. Thus, the mean 90% of growth was at pH_{opt} ± approximately 1 pH unit.

Although most pH tolerance curves were symmetrical (e.g., Fig. 1N, P, and R), some curves were distinctly asymmetrical (e.g., Fig. 1A and V). To elucidate whether the degree of symmetry was systematically related to pH_{opt}, we compared the parameters estimated in the double logistic equation over the entire pH range (Fig. 3). The parameters A_{hpH} and A_{lpH} indicate 50% inhibition of optimal growth at pHs higher and lower than pH_{opt}, respectively. This is similar to IC_{50}s estimating inhibition of toxic substances. A small divergence from pH_{opt} (shown by the thin line in Fig. 3A) indicates a higher toxicity than does a large difference. This 50% inhibition value varied systematically with pH_{opt}. At high pH_{opt}, the difference between pH_{opt} and A_{hpH} was smaller than the difference for low-pH communities and vice versa for the difference between pH_{opt} and A_{lpH}. This means that lowering the pH in low-pH waters was more toxic than increasing pH, whereas in high-pH waters, increasing the pH was more toxic than decreasing the pH. This is also evident from the graphs of the individual waters (Fig. 1).

Partly similar results could be inferred from comparison of B_{hpH} and B_{lpH}, which is a slope parameter with higher values, when growth decreases rapidly when altering pH. A higher value of the slope is indicative of more extensive toxicity due to changing pH. Both the slope for increasing pH and the slope for decreasing pH varied with pH_{opt}. The slope was significantly steeper, especially in the low-pH waters, when lowering pH than when increasing pH (Fig. 3B), suggesting that lowering pH is more toxic (cf. the individual samples in Fig. 1). For a pH_{opt} of >6.5, the differences in the slope for different waters were small, showing that increasing or decreasing pH had similar negative effects on growth, resulting in symmetrical tolerance responses of the communities. In waters with the highest pH (Fig. 1V), however, a steeper slope when in-



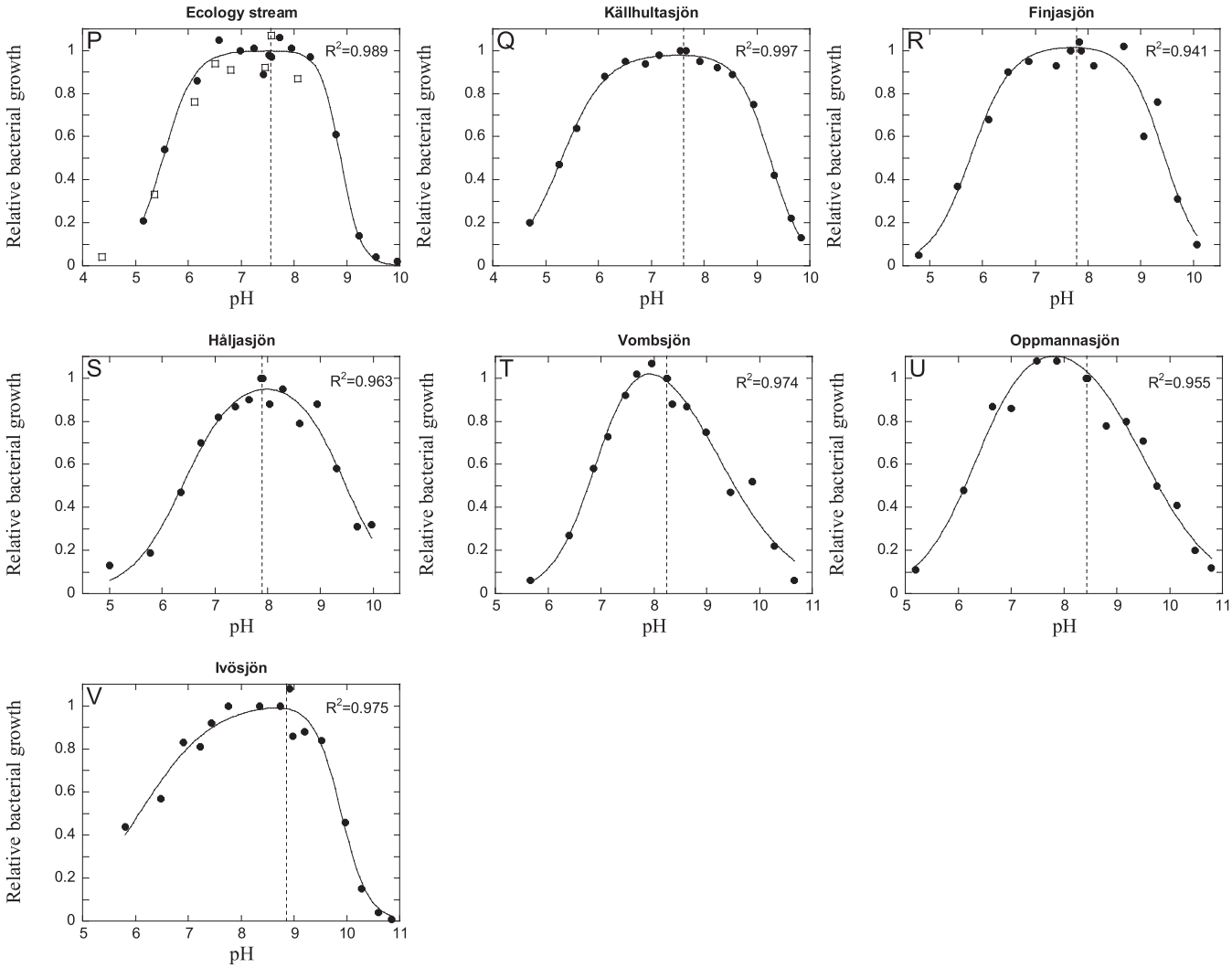


FIG 1 Bacterial community tolerance to pH in 22 lakes and streams varying in pH from 4 to 8.9 measured using H_2SO_4/KOH to change pH (ordered from low to high *in situ* pH; note that the *x*-axis scale is altered accordingly). The lines were fitted to the closed symbols using the double logistic equation, except for panels E and F, where only a single logistic equation to decreasing pH was used. The stippled vertical line indicates *in situ* pH. Open squares in panels B, H, and P denote data when pH was altered by buffers (see Materials and Methods).

creasing the pH suggested this to be more toxic than when decreasing the pH.

The parameter C_{opt} in the double logistic equation is the maximum growth at pH_{opt}. Since the data were initially normalized to that at *in situ* pH, this parameter will by necessity be close to 1. The mean value of C_{opt} was 1.09 with a standard deviation of 0.13. There was no systematic variation in C_{opt} with *in situ* pH ($R^2 = 0.003$). Furthermore, there was also no significant regression between bacterial growth without normalization at pH_{opt} and *in situ* pH ($R^2 = 0.05$).

We found a significant linear regression between the simplified pH tolerance index, the logarithm of the ratio of growth at pH 8/4, and the pH_{opt} derived from the double logistic equation [Fig. 4; $pH_{opt} = 0.59 \times \log(8/4) - 2.96$; $R^2 = 0.83$]. This equation can thus be used to approximately estimate the pH_{opt} for a bacterial community after measuring growth only at pH 4 and 8 (altered with buffer or titration) within the pH range of around 4 to 9.

DISCUSSION

Ecological considerations. Our main result was that pH_{opt} for bacterial community growth in lakes and streams was closely correlated with *in situ* pH (Fig. 2). This has earlier been found for soil bacterial communities (31–33), indicating that pH asserts similar selection pressure on bacterial communities in soil and water. The shape of the pH response curves was also similar in these habitats. For example, the range of ≥90% growth of that at pH_{opt} was around ±1 pH units in our set of waters and ±1.0 and ±0.9 in two data sets from soil (33). Furthermore, the range of 50% growth of that at pH_{opt} of ~4 pH units in the present study (Fig. 3A, indicated by the difference between the two lines for decreasing and increasing pH) was similar to that found by Fernández-Calviño et al. (33).

Previous studies in soil suggested, however, that pH_{opt} at low pH was considerably higher than *in situ* soil pH (33). This was seen as a slope significantly below 1 for the regression of pH_{opt} versus

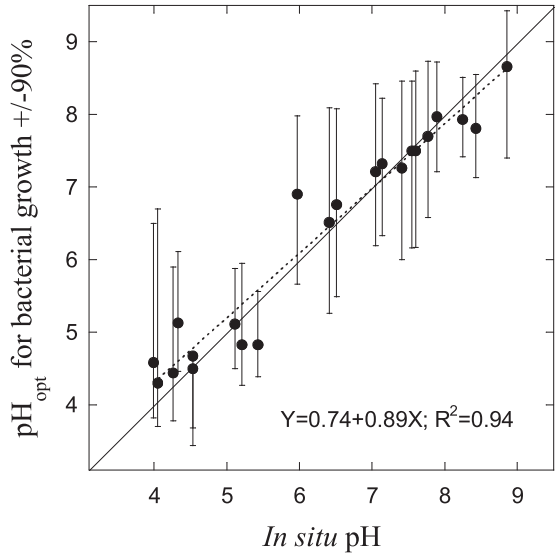


FIG 2 Regression (stippled line) between *in situ* pH and pH_{opt} for bacterial community tolerance determined using the double logistic equation (see Fig. 1). Bars indicate the pH range resulting in 90% of optimal growth. The high 90% for the streams in Fig. 1E and F was omitted due to no decrease with increasing pH.

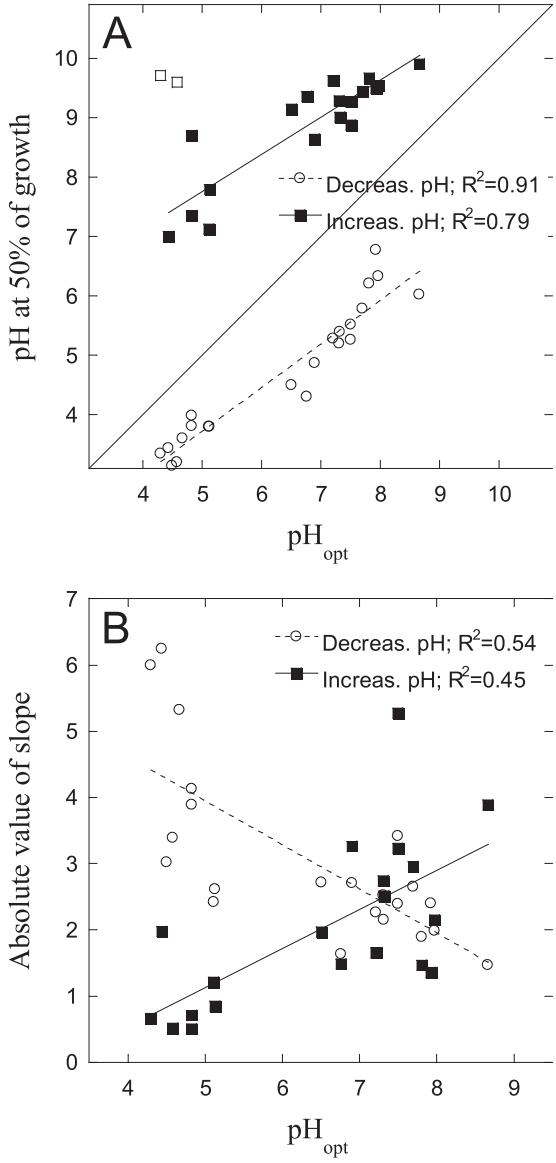


FIG 3 Characteristics of the double logistic equation modeling pH tolerance of the bacterial community as related to pH_{opt}. (A) pH at 50% of maximum growth at pH_{opt}. Decreasing pH (○, A_{lpH}) and increasing pH (■, A_{hpH}). Open squares indicate data that were not included in the regression due to uncertain values for these samples (cf. Fig. 1A and B, increasing pH), and data for increasing pH for panels E and F were omitted due to no measurable decrease. Thin line indicates a 1:1 relationship. (B) The slope of the logistic equations at decreasing pH (○, B_{lpH}) and increasing pH (■, B_{hpH}). The slope for B_{lpH} is negative in the double logistic equation, but absolute values are used to facilitate comparisons between high and low pH.

soil pH. Although this was also the case in the present study (Fig. 2), the difference from a 1:1 relationship was small and mainly due to few low-pH sites with very asymmetric pH tolerance curves, where pH_{opt} was difficult to determine exactly. The main reason for our different results compared to earlier studies is the different models used to calculate pH_{opt}, where especially the second-degree model, but to some extent also the CPM, markedly overestimate pH_{opt} at a low *in situ* pH with asymmetric pH tolerance relationships. Fernández-Calviño et al. (33) used the second-degree model, due to few data points in each pH tolerance curve, in the determination of the community pH tolerance, resulting in a slope of 0.58 to 0.62 for the regression of pH_{opt} on *in situ* soil pH. This is similar to the slope, 0.64, that we found for our 22 waters using the same model, while the more accurate double logistic model resulted in a slope of 0.89, which is not much different from 1. Fernández-Calviño et al. (33) suggested that the fact that pH_{opt} in soil was higher than expected at low *in situ* pH could be at least partly explained by an interaction between the well-known negative effect of low pH on growth of bacteria in soil (40) and the unimodal pH tolerance response. Since in our data set there was no such relationship between growth at pH_{opt} and *in situ* pH, this is most likely not a valid explanation. Summarizing, it is likely that both in soil and water there is a very close correlation between *in situ* pH and the pH_{opt} for growth, even in low-pH environments.

However, at low *in situ* pH ($pH < 5$) the pH_{opt} was less distinct than at higher *in situ* pH. This was due to a pronounced asymmetry in the pH tolerance at low *in situ* pH, where decreasing pH had a much more severe effect on bacterial growth than increasing pH. Increasing pH by >2 pH units in several cases did not affect bacterial growth at all (Fig. 1E and F). Such an asymmetry has earlier been suggested by data for one low-pH soil (see Fig. 2 in reference 32). One reason for the asymmetry could be that more bacterial species are adapted to neutral pH, possibly due to their internal pH homeostatically being kept around pH 7 to 7.5 (41), and in low-pH environments such bacteria may still make up a large part of the bacterial community. Furthermore, a more extreme water pH will be further away from the internal pH, resulting in more energy needed to uphold a stable internal pH. It may also be due to the logarithmic scale of pH, where differences of one pH unit are not the same in actual hydrogen ion concentrations when comparing a pH change at low pH to that at more neutral conditions. Lastly, since most environmental habitats, both aquatic and terrestrial, is within a range from pH 4 to 9, there has been less selection for bacteria to withstand extreme pH conditions outside

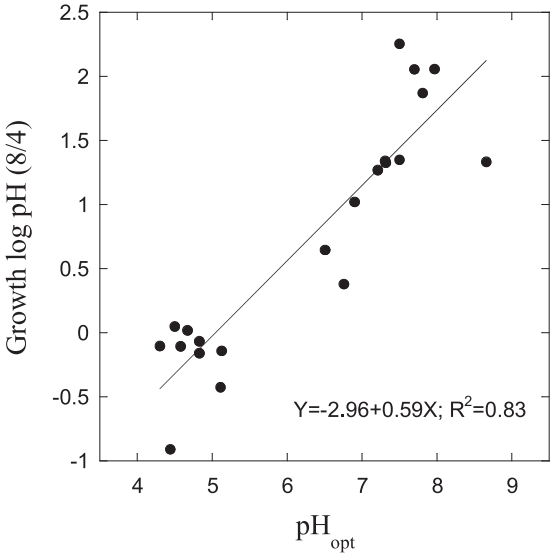


FIG 4 Regression between the logarithmic ratio of bacterial growth at pH 8 of that at pH 4 (log pH 8/4, the simplified protocol with two measurements per water sample) against the pH_{opt} determined using the double logistic equation.

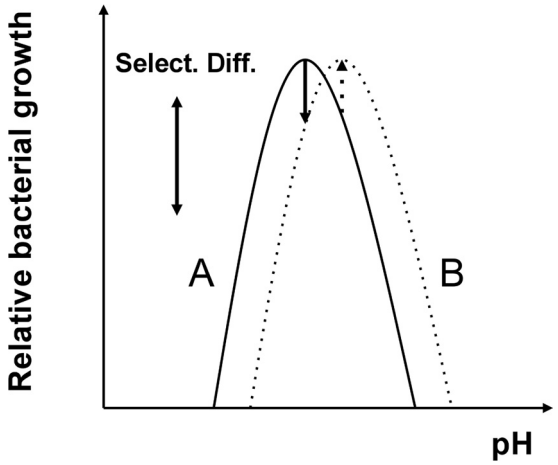


FIG 5 Schematic view of changes in selection pressure (arrows) to altered community composition and absolute growth rates after changing pH for two communities with different community tolerance curves and pH_{opt}. The graph illustrates a change from pH at pH_{opt} for community A to that of community B. Community A, low pH adapted (full line); community B, high pH adapted (stippled line). Select. Diff., the double arrow shows the relative selection pressure when pH is changed from pH_{opt} for one to the other community.

this normal range. The reciprocal asymmetry in the lakes with highest *in situ* pH, where increasing pH was more severe than decreasing pH is in line with several of these explanations. Tank et al. (29) also found that increasing pH from 8 to 10.5, in order to mimic increased pH due to rapid photosynthesis, resulted in almost total inhibition of bacterial growth. The important implication is that changes in pH under extreme pH conditions, both high and low, will be more detrimental than similar changes in a more neutral pH range.

We found variation in the width of the plateau of growth with near optimal growth rates, with 90% of optimal growth varying between 1 and 3 pH units. This variation did, however, not correlate with *in situ* pH. Another possible explanation, not studied here, could be the extent of stability in the environment. One could hypothesize that environments with large temporal fluctuations in pH would have pH tolerance curves with broader optima compared to more stable environments, similar to findings regarding the temperature tolerance of the bacterial community in soil (8). Thus, not only can pH_{opt} be indicative of the environment of the microbial community, but also the actual shape of the tolerance curves may be informative.

Although pH tolerance curves from waters with very different *in situ* pH had little overlap (cf. Fig. 1A and V), this was not the case with pH tolerance curves from waters more close in pH (cf Fig. 1M and S). Still, even pH differences of only one unit have been shown to have a significant effect on the bacterial community (16, 42). One has to be aware that with a unimodal, fairly symmetrical pH response curve, there will be a strong pressure for an altered community even with a minor pH change. Figure 5 illustrates tolerance curves for communities A and B differing only slightly in pH_{opt} and with the same growth rate at pH_{opt}. Changing the pH from that optimal for community A to that of community B will thus not change the overall growth rate. However, community A will grow less well than under its optimum conditions (solid arrow), while at the same time community B will grow better (stippled arrow). The altered "selection pressure" between communities (solid double arrow to the left) will therefore be around two times that of each individual community. Thus, although over all activity will be little affected by a small pH change, the "selection pressure" toward an altered community with altered pH tolerance can be large. This differs from the situation when temperature is changed. At temperatures below optimum growth, decreasing temperature will have a large impact on over all growth rates but a minor effect on "selection pressure" to a more low temperature-adapted community (8).

Methodological considerations. Changing the pH by acid/base (titration) or buffer gave similar results (Fig. 1). The former method was also used by Tank et al. (29), when altering pH to mimic pH effects of high photosynthesis. Titration was rapid, with 16 different pH values in one water sample achieved within 30 min. The use of buffer would be even easier. However, one has to test for negative effects of high buffer concentrations (32). This, together with problems in finding suitable buffers in the high-pH range, suggests that the titration procedure will be more generally applicable. However, in a few low-pH samples, pH dropped slightly with time after increasing pH, indicating the need for rapid measurements in those cases.

We used both freshly sampled water and water that had been stored frozen before assessments. We found no systematic bias using frozen water. Even if some bacteria are killed by freezing or thawing, the surviving bacteria will be affected by pH in the same way and thus give meaningful pH tolerance curves. However, in some samples the initial bacterial growth rates immediately after thawing were exceedingly low, making the determination of pH responses uncertain. Incubating the water samples after thawing for a few days resulted in higher growth rates. This preincubation did not change *in situ* pH and thus will not change the selection pressure by pH on the community. Thus, the combination of being able to use samples stored frozen and using a standard temperature (22°C) to measure pH tolerance of the community, circumvent the need to measure directly *in situ*, with the possibility of more versatile experimental designs.

Earlier studies in soil have used models derived from pure culture studies to describe pH tolerance of soil bacteria, including a second-degree model and the CPM (32, 33). Although these models can be used with few data points, they were shown to overestimate pH_{opt} for low-pH-adapted communities and did not properly model tailings of the response curves at extreme pH changes. The double logistic equation, here used for the first time, was more efficient (higher R^2 values), was able to adequately account for asymmetric relationships with tailings, and had less bias. Its basis in common ecotoxicology also makes it the first choice.

There was a good correlation between the simplified pH tolerance index determined using only two pH levels (pH 8 and 4) and the pH_{opt}. A similar pH tolerance index has earlier been shown to correlate well with the pH_{opt} in soil (31, 33). Such a ratio can thus be used as a rapid method to follow changes in pH tolerance of a community after a pH change in water, similar to its use after altering soil pH (34, 35). The use of a similar approach with only two pH levels could be inferred by the work of Tank et al. (29), studying bacterial growth after rapid changes in pH due to high photosynthesis. However, these researchers detected only a small difference in pH in their system: pH 8 to 10.5. In order to accommodate the large variation of *in situ* pH in the set of lakes and streams studied here, we used a much larger span.

Concluding remarks. The pH response curve for bacterial growth is a characteristic of the actively growing community in a habitat. As such, the relationship, including the calculated pH_{opt} for growth, is merely a description of the trait variation of the growing community. However, it can be used to interpret changes in community structure, where an altered community structure due to changing pH should be correlated with changes in pH_{opt}, whereas without such a relationship, other drivers for the community change have to be invoked. However, one has to bear in mind that over longer periods, dormant organisms, not included in the leucine incorporation measurements, may also start growing, leading to a growth rate scenario not necessarily directly related to our measurement of the pH tolerance distribution.

The pH tolerance of a community can also be used to study to what extent the community is optimally adapted to the new conditions in a situation of rapid pH change, e.g., during snow melt, rapid photosynthesis, or anthropogenic pollution. With a pH_{opt} for growth different from the *in situ* pH, we would expect impaired function, as shown experimentally in soil (35) and water (29).

In the present study, we focused on only one environmental factor: pH. A similar methodology has been used to determine trait distributions for temperature, toxicity, and salinity in soil (see the introduction for references). For example, adding heavy metals to soil resulted in increased community tolerance to heavy metals (1). The fact that the metals were added as salts also resulted in a lowered pH and altered osmotic conditions, and it was shown that the community also became more tolerant to salt and low pH. Thus, adding metals not only changed the community trait distribution for metal toxicity but also changed those for pH and osmotic conditions, where all three factors would have exerted a selection pressure affecting the community composition. By combining different trait distribution measurements, it is therefore possible to elucidate the factor(s) responsible for changes in the community structure.

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