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Associations between water chemistry and fish community composition: a comparison between isolated and connected lakes in northern Sweden

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SUMMARY

1. The correlation between water chemistry, physical variables and fish community composition was examined in 40 small (≤30 ha) coastal lakes in northern Sweden. Twenty of the 40 lakes were isolated from other water bodies and 20 were connected to the Baltic Sea. Lakes were fished in summer, using three different methods. Water chemistry was sampled in late winter prior to ice-out and pH was measured additionally in summer.
2. Our central question was whether water chemistry plays a greater role in the composition of fish communities in isolated lakes than in connected lakes, as isolated lakes cannot be recolonised once a species has become extinct.
3. Results indicate that winter anoxia affects community composition only in isolated lakes, whereas acidity is of importance in both connected and isolated lakes. Methane (indicating anoxia), was significantly correlated with variation in fish community composition in isolated lakes, and a group of variables that indicate anoxia (CH_4, pCO_2, inorganic carbon and dissolved oxygen) explained 24–34% of the variation. pH alone explained 12% of the variation in community composition for connected lakes and a group of variables indicating acidity (summer and winter pH and ANC) explained 10–20% of the variation in isolated lakes. Lake area was the most important physical variable, being significantly correlated with the variation in fish community composition in connected lakes.
4. In isolated lakes, the presence of pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) was associated with low CH_4. The occurrence of crucian carp (*Carassius carassius*) and roach was positively correlated with pH, and the crucian carp was apparently also affected by predation by pike and perch. In connected lakes the effect of anoxia was low, probably due to the possibility of recolonisation and pockets of oxygenated water, allowing pike and perch to persist and thereby limiting the distribution of crucian carp.

Keywords: acidity, anoxia and water chemistry, fish, isolation

Introduction

The formation of lakes because of land upheaval is an ongoing process on the Baltic coast of northern Sweden. Lakes formed in this way may be isolated, in the sense that connecting streams are lacking, or they may be connected to other water bodies via streams that can be used by fish as routes for colonisation. If environmental conditions in an isolated lake become too harsh for fish reproduction or survival, species may be lost for very long times, whereas a connected lake could be rapidly recolo-

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nised. Therefore, we expect harsh chemical conditions to have a larger impact on variation in fish community composition in isolated than in connected lakes.

Local factors known to influence fish community composition in lakes include anoxia, low pH and predation (Appelberg *et al.*, 1993; Magnuson *et al.*, 1998; Jackson, Peres-Neto & Olden, 2001). Fish death because of oxygen deficiency was recognised as a problem in Swedish lakes as early as the eighteenth century, when land owners were obliged to open up holes in the ice to aerate their lakes (Hargeby, 1985). Winter depletion of oxygen under the ice in this dark and cold habitat is caused by oxidation of methane (Liikanen *et al.*, 2002) and other organic substances (Fang & Stefan, 2000), in combination with reduced photosynthesis and sometimes an input of oxygen-depleted ground water (Chambers, Scrimgeour & Pietroniro, 1997; Kalff, 2002). Late winter is therefore a period when conditions critical for fish survival might be reached (Chambers *et al.*, 1997; Fang & Stefan, 2000). There is large variation between species in the tolerance to anoxic conditions and it is often found that large piscivores are less tolerant than smaller species (Tonn & Magnuson, 1982). Thus it has been proposed that an interaction between predation and anoxia controls fish communities in lakes with prolonged ice cover. Anoxia excludes large piscivores from shallow lakes, thereby allowing the persistence of species that are sensitive to predation but tolerant to anoxia (Tonn & Paszkowski, 1986).

Acidity is another chemical factor structuring fish populations in many regions (Keller, Gunn & Conroy, 1980; Bergquist, 1991). In northern Sweden, acidity is due primarily to natural processes (Laudon *et al.*, 2001). Low weathering rates result in a low buffering capacity in soils and waters, and boreal lakes are often surrounded by wetlands which contribute to acidity through organic acids. Sulphuric soils also contribute to the natural acidity of some lakes. When these soils are oxidised, downstream waters can be affected by acidification and an increase in metals (MacDonald *et al.*, 2004). Acidity affects fish negatively in various ways, due both directly to high H^+ concentrations and also indirectly to the mobilisation of toxic forms of metals, in particular aluminium (Gensemer & Playle, 1999). The most important physiological mechanisms of acid toxicity to fish are related to gill function, including precipitation of mucus which in turn causes suffocation (Alabaster & Lloyd, 1982; Poléo, 1995) and ionoregulatory effects causing salt loss (reviewed in Gensemer & Playle, 1999). The sensitivity of fish to acidity varies between species and young fish are typically more sensitive than older life stages (Almer *et al.*, 1978; Keinänen *et al.*, 2004). In moderately acidic waters, tolerant species such as perch (*Perca fluviatilis* L.) can increase in abundance, but overall species richness and species diversity decline progressively as acidity increases (reviewed in Degerman *et al.*, 1995).

Our aim was to investigate the relationship between chemical factors and fish community composition in small coastal lakes that have or lack streams connecting them to the Baltic Sea. The brackish Baltic Sea has a high diversity of freshwater fish species and is the principal colonisation source for coastal lakes in the region. Specifically, we test the hypothesis that chemical factors are more important in isolated than in connected lakes. Thus we examined variation in fish community composition and water chemistry in 20 isolated lakes that lack inlets and outlets, and 20 lakes that are connected to the Baltic Sea by streams that fish could use for recolonisation.

Methods

Study lakes

The 40 lakes studied are located close to the coast of northern Sweden, at a latitude of 63°25′ to 64°15′N and longitude of 19°10′ to 21°15′E. The lakes are situated in a boreal landscape where the terrain is rather flat. The average temperature is around −10 °C in January and 15 °C in July. Average annual precipitation is 700 mm and most lakes are ice- and snow-covered for approximately 6 months each year. All lakes are small (range 1–30 ha), shallow (maximum depth 0.9–5.5 m), situated at a low altitude (2–35 m) and close to the Baltic Sea (0.1–13.5 km), and many are surrounded by wetlands. Land upheaval because of isostatic rebound is presently 0.8–0.9 cm year^{-1} and the age of the lakes ranges from 200 to 3500 years. Twenty of the lakes have permanent streams connecting them to the Baltic Sea (connected lakes). The streams have low gradients and lack barriers that can hinder fish migration, and fish can colonise the connected lakes either from the sea or from surrounding streams. The other 20 lakes have no perennial connecting streams and field visits confirmed that they are isolated during typical spring flood conditions. We refer to these lakes as 'isolated', but note that they may not be isolated in an absolute sense. We cannot absolutely exclude the possibility that fish could colonise during rare extreme flood events or could be transported to these lakes by humans or birds. Lakes were excluded if interviews with fishery rights owners revealed that water chemistry or species compositions had been altered by liming, rotenone treatment or species introductions.

The lakes were chosen to minimise the variation in physical variables between the two groups. Because large isolated lakes could not be found in the region, however, the lake groups differed in maximum depth, catchment area and lake area (Table 2). The difference was significant for lake depth and catchment area ($t = 2.17$, $P < 0.05$ and $t = 3.46$, $P < 0.01$, respectively), but not for lake area ($P > 0.05$).

Physical variables and water sampling

Lake area, altitude, catchment area and length of the stream connecting to the sea were measured from maps. For isolated lakes we measured the distance to the sea along the most likely colonisation route at extreme flooding conditions, also referred to as 'stream length'. Maximum lake depth was measured in summer with a sounding line or echo sounder (Eagle Fish ID 128).

The lakes were sampled for full water chemistry (see below) in late winter and for pH also in summer. The winter sampling period was between 24 March and 14 April 2004 when the lakes were still covered with ice. Water samples were taken at or near the deepest part of the lake (typically >75% of known max depth) and field measurements were performed in the same location. After a hole was drilled in the ice, a sample was first taken for dissolved gas (CO_2 and CH_4) analysis. A 15-mL air-bubble-free water sample was collected from approximately 0.4-m depth using a syringe and subsequently transferred to a sealed 60-mL glass serum bottle containing N_2 gas at atmospheric pressure, for headspace analysis. Dissolved oxygen concentration (DO), conductivity and temperature were measured in the field, using instruments from Wissenschaftlich-Technische Werkstätten (WTW), calibrated daily. Water samples were collected using a Ruttner water sampler and then transferred with multiple rinses to acid-washed 250 mL high-density polyethylene bottles. In lakes with an established thermocline, field measurements were performed and water samples collected both above ('shallow' samples) and below ('deep' samples) the thermocline. The shallow sample was in most cases taken at 1-m depth and the deep sample was taken 0.2–0.5 m from the bottom. In very shallow lakes only one sample was taken, at 0.7-m depth.

The summer sampling period was from 12 June to 16 August 2004. Samples for pH measurements were collected using a 7-m pole from the shore. An acid-washed 250-mL HDPE plastic sampling bottle was attached to one end of the pole and samples were filled near the centre of the water column.

Chemical analyses

Upon returning from the field, water samples were subsampled for analyses of various solutes. pH was measured using a Ross 8102 combination electrode (ThermoOrion; Thermo Electron Co., Waltham, MA, U.S.A.). Samples for absorbance and dissolved organic carbon (DOC) analyses were filtered using 0.45-μm MCE membrane filters. Samples for DOC were frozen until analysis, while absorbance samples were refrigerated. Absorbance was measured at 254 nm in a 1-cm quartz cuvette using a HP-DAD 8453 E UV-VIS spectrophotometer (Hewlett Packard Company, Palo Alto, CA, U.S.A.). DOC was analysed using a Shimadzu TOC-V_{CH} analyser (Shimadzu Co., Kyoto, Japan) after acidification and sparging to remove inorganic carbon (IC).

Samples for dissolved elemental analyses (Al-tot, Fe, P, Ca, K, Mg, Na) were preserved with ultrapure HNO_3 (1% v/v), stored cool, and analysed using a Varian ICP-OES (inductively-coupled plasma optical emission spectroscopy). Major anions (SO_4^{2-}, Cl^- and NO_3^-) were analysed using a Dionex DX-300 ion chromatograph system (Dionex Co., Sunnyvale, CA, U.S.A.). Base Cation (BC) concentration was calculated as the sum of Ca^{2+}, K^+, Mg^{2+} and Na^+ and concentrations expressed as $\mu eq\ L^{-1}$ of charge. Acid Neutralisation Capacity (ANC) was calculated as the difference between BC and the sum of SO_4^{2-}, Cl^- and NO_3^- expressed as $\mu eq\ L^{-1}$ of charge.

Dissolved gases (CO_2 and CH_4) were analysed using a Varian 3800 Gas Chromatograph (Varian Inc., Palo Alto, CA, U.S.A.) equipped with flame ionisation detector after the method of Klemedtsson *et al.* (1997).

Prior to headspace analysis, the water samples were acidified to pH 2–3 with ultra pure 30% HCl (0.2% v/v) and allowed to equilibrate at 20 °C. Total IC was calculated from the measured headspace CO_2 content of the acidified samples. The partial pressures of CO_2 (pCO_2) and CH_4 in the original sample were calculated using standard carbonate equilibrium equations and Henry's law for gas/liquid equilibria (Stumm & Morgan, 1996).

Fishing

Three different sampling methods were used in order to catch as many fish species as possible. In the summer and early autumn of either 1999 or 2002, each lake was fished with two NORDIC gill-nets; the standard multi-mesh size gill nets used in Sweden (Appelberg *et al.*, 1995). The nets are 1.5-m deep and the upper line is 30-m long. The nets are made up of 12 2.5-m sections, being 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm from knot to knot, respectively. One net was placed near the deepest part of the lake and the other in the littoral zone. The nets were set at the bottom where they were left overnight. In very small, isolated lakes only one net was used.

As northern pike (*Esox lucius* L.) is a passive predator (Eklöv & Diehl, 1994) of low abundance and gill netting is a passive fishing method (Hamley, 1975), it is difficult to determine absence or presence of pike using gill nets only (Olin *et al.*, 2002). Therefore, all lakes were also angled with spinners by two persons for 1 h: connected lakes in August–September of 2002 and isolated lakes in June–August of 2004.

In addition, all lakes were sampled in July–August of 2004 using small detonations. The detonations affect individuals smaller than 10–15 cm within a radius of 2 m. They either die or become unconscious and then often float to the surface. Fishes affected by the explosion were collected using a landing net. Ten explosions were set off in each lake and the detonation spots were chosen to cover as many different habitats as possible. Detonation depth varied from 0.1 to 2.5 m, depending on lake depth. All shots were detonated from shore, using a 7-m fishing rod to reach out. The detonation caps were Nonel®LP, lit using Nonel DynoStart 2, both products from Dyno Nobel, Oslo, Norway.

Statistical analyses

Twenty-three chemical and physical variables were measured, including summer and winter pH (pH S and pH W; Table 2). Prior to statistical analyses, all variables except pH, ANC, Fe and temperature were log transformed in order to approximate normal distributions.

All variables, apart from CH_4, pCO_2 and IC, were measured at two depths. Differences in the concentration of chemical variables between the shallow and deep measurements were small. Due to the risk of contamination of the shallow sample when drilling, statistical analyses were run on deep samples.

Presence, based on all fishing methods, was summed to form an overall species list for each lake. The variation in fish community composition was then correlated with environmental variables for three separate groups of lakes (all 40, only the 20 connected and only the 20 isolated) using canonical correspondence analyses (CCA) (CANOCO for Windows 4.5; Biometris, Wageningen, the Netherlands). Initial examination of the species data with Detrended Correspondence Analysis indicated that CCA was the appropriate multivariate analysis to use as the data were unimodally distributed (gradient length >3). Downweighting of rare species was used for all analyses. The significance level of each environmental variable was first tested individually by comparison with a randomised Monte Carlo simulation with 999 permutations using the routine provided in the CANOCO program. Significant variables ($P < 0.05$) were then included as potential predictors for community composition and forward selection was used to estimate the additional amount of variation explained by each variable. For co-correlated environmental variables, the first variable to be included in the model will explain the shared variation, thus the difference between the importance of the variables will seem larger than it actually is. In order to overcome this limitation, a partial CCA (pCCA) analysis was also performed for each lake group. The pCCA analysis explicitly partitions explained variance between different predictor groups and also calculates variation shared between the groups (Økland & Eilertsen, 1994). In this case the pCCA partitioned the variation explained by indicators of anoxia (DO, CH_4, pCO_2, IC) from that explained by indicators of acidity (pH W, pH S, ANC). The variables were tested by comparison with a randomised Monte Carlo simulation and variables significant in the pCCA model ($P < 0.05$) included using forward selection.

We used stepwise logistic regressions to examine the association between the presence/absence of individual fish species and isolation, acidity and anoxia. Isolation (as a categorical variable), methane (as an indicator of anoxia) and winter pH were included as potential predictors. As response variables, we used presence/absence of the four species found in at least 25% of all lakes; perch (*P. fluviatilis*), pike (*E. lucius*), roach (*Rutilus rutilus*) and crucian carp (*Carassius carassius* L.)

Results

Fish community composition

Ten fish species were caught in the lakes studied (Table 1), with a maximum of six species coexisting in any one lake. Species richness was on average 3.4 in connected and 1.6 in isolated lakes, including five fishless isolated lakes. In connected lakes, the most common species were perch (*P. fluviatilis*), pike (*E. lucius*) and roach (*R. rutilus*). In isolated lakes, the most common species were crucian carp (*C. carassius*), pike and perch. Ruffe (*Gymnocephalus cernuus* L.), bream (*Abramis brama* L.), bleak (*Alburnus alburnus* L.) and ide (*Leuciscus idus* L.) were found in few lakes, all of them connected, whereas nine-spined stickleback (*Pungitius pungitius* L.) was rare in both isolated and connected lakes. Three-spined stickleback (*Gasterosteus aculeatus* L.) was found only in one isolated lake.

Water chemistry

The lakes studied were in general rich in organic matter (DOC) and absorbance was high. Many had low pH, had high aluminium concentrations and experienced anoxic conditions during winter. Medians and ranges of each environmental variable are presented in Table 2.

Sulphate concentration was significantly higher in connected than in isolated lakes (*t*-test, $t = 2.41$, $P < 0.05$). There was a significant vertical gradient of pH (paired *t*-test, $t = 2.52$, $P < 0.05$) when comparing the deep and shallow measurement, pH increasing with depth, but the mean difference was only 0.14 units. DO decreased with depth ($t = -3.93$, $P = 0.001$), from an average of 2.1–0.7 mg L^{-1}. Temperature increased with depth from an average of 0.7–3.9 °C ($t = 8.81$, $P < 0.001$). No other measured chemical variable differed significantly between connected and isolated lakes or shallow and deep samples.

Fish community composition in relation to water chemistry

In the group of all 40 lakes, 12 variables had a significant correlation with variation in fish community composition when tested individually and were therefore included in the CCA model. Out of the 12 variables, six were physical, three were related to acidity (ANC, summer and winter pH) and three were related to winter depletion of oxygen (CH_4, IC and Fe). The largest portion of the variation in fish community composition (28%) was explained by axis 1 (Table 3), which separated the species into two

Table 1 Presence of fish species in connected and isolated lakes (% of lakes)

Fish species	Incidence in all lakes (%)	Incidence in connected lakes (%)	Incidence in isolated lakes (%)
Perch (*Perca fluviatilis*)	62.5	90	35
Northern Pike (*Esox lucius*)	62.5	85	40
Roach (*Rutilus rutilus*)	35	60	10
Crucian Carp (*Carassius carassius*)	37.5	25	50
Nine-spined Stickleback (*Pungitius pungitius*)	15	15	15
Ruffe (*Gymnocephalus cernuus*)	15	30	0
Bream (*Abramis brama*)	7.5	15	0
Bleak (*Alburnus alburnus*)	5	10	0
Ide (*Leuciscus idus*)	2.5	5	0
Three-spined Stickleback (*Gasterosteus aculeatus*)	2.5	0	5

Table 2 Medians and ranges for deep measurements of chemical and physical variables

	Connected lakes		Isolated lakes	
Variables	Median	Range	Median	Range
Chemical variables				
Absorbance at 254 nm (AU)	0.8	0.3–2.5	1.2	0.1–3.9
Conductivity (μS cm^{-1})	122	57–548	116	39–471
DOC (mg L^{-1})	18	9–62	24	7–105
IC (mg L^{-1})	7.7	3.7–31.2	7.9	2.5–26.5
pCO_2 (atm × 10^{-3})	8.1	2.6–23.1	8.6	2.6–23.7
CH_4 (mg L^{-1})	0.1	0.0–8.7	0.2	0.0–5.0
Oxygen (mg L^{-1})	0.2	0.0–15	0.3	0.1–2.8
Al-tot (mg L^{-1})	0.5	0.2–3.4	0.3	0.1–1.9
Fe (mg L^{-1})	3.3	0.2–9.0	3.5	0.7–9.7
P (mg L^{-1})	BD	BD-0.06	BD	BD-0.05
Base Cations (μeq L^{-1})	963	460–3000	740	194–2090
Cl^- (μeq L^{-1})	239	72–992	172	63–1560
SO_4^{2-} (μeq L^{-1})	288*	110–1478	179	30–891
NO_3^- (μeq L^{-1})	19	0–136	12	0–113
ANC (μeq L^{-1})	256	−32–1160	183	−62–1182
Winter pH	5.9	4.2–6.6	5.8	4.4–6.5
Summer pH	6.3	5.0–6.9	6.0	4.9–6.8
Physical variables				
Temperature (°C)	2.7	0.5–5	2.6	0.1–4.0
Altitude (m)	4	2–19	6	2–35
Stream length (km)	1.1	0.2–5.5	0.6	0.1–13.5
Depth (m)	1.9*	1.0–5.5	1.6	0.9–3.8
Lake area (ha)	11	1–30	4	1–15
Catchment area (ha)	202*	36–7259	31	8–387

Water temperature and all chemical parameters were measured in late winter, with the exception of summer pH. Because of skewed data distributions, all variables (with the exception of pH, ANC, Fe and temperature) were log-transformed prior to statistical analyses to approximate normal distributions ($P > 0.05$, Kolgorov–Smirnov *Z*). Mean values were then compared for the two lake groups, and variables with a significant ($P < 0.05$, two-tailed *t*-test) difference in concentration between isolated and connected lakes are marked with an asterisk.
BD, below detection limit of 0.016 mg P L^{-1}.

groups. Crucian carp, nine-spined stickleback and three-spined stickleback were more common in small, isolated lakes with anoxic conditions while remaining species were more common in larger, deeper and connected lakes (Fig. 1a).

Separate analyses for isolated and connected lakes showed that the species–environment correlations differed between the two types of lakes. In connected lakes, only lake area and pH had a significant correlation, each explaining 12% of the variation (Table 3; Fig. 1b). In isolated lakes, nine variables were significant when tested individually, together explaining 77% of the variation (Table 3; Fig. 1c). CH_4, the variable with the highest explanatory power and thus the first variable to be included in the CCA model, explained 34% of the variation. Analysis by pCCA showed that 10% of this correlation was shared with acidity, so that CH_4 actually explained 24–34% of the variation in fish community composition in isolated lakes. Summer pH, the only significant acidity variable in the pCCA-model, explained 10–20% of the variation. The concentration of DO is known to be tightly correlated to CH_4 (Bastviken, Ejlertsson & Tranvik, 2002; Liikanen *et al.*, 2002), and we found correlations between CH_4 and Fe, IC and pCO_2 (Table 4). Because of the low accuracy of the DO measurements at very low concentrations, we argue that CH_4 may be a better indicator of anoxic conditions in this study (see Discussion). Out of the variables included in the isolated lakes CCA, two were physical (altitude and catchment area), three were related to acidity (ANC, summer and winter pH) and four to oxygen concentration (CH_4, Fe, IC and pCO_2).

Table 3 Results from canonical correspondence analysis

	All lakes	Connected lakes	Isolated lakes
Total inertia	1.585	1.288	1.484
Eigenvalue axis 1	0.449	0.228	0.637
Eigenvalue axis 2	0.171	0.087	0.198
Groups of variables		Level of explanation (%)	
All included variables	54	24	77
Significant variables	26	24	34
Chemical variables (pCCA)	22	12	44
Anoxia alone (pCCA)	11	0	24
Acidity alone (pCCA)	8	12	10
Shared anoxia-acidity (pCCA)	3	0	10
Individual variables			
pH W	1 (12)	12* (2)	3 (9)
pH S	11* (2)		10 (2)
ANC	2 (7)		5 (6)
Fe-tot	2 (9)		4 (8)
CH_4	4 (5)		34* (1)
IC	5 (3)		4 (7)
pCO_2			6 (4)
Lake area	15* (1)	12* (1)	
Catchment area	2 (11)		6 (5)
Altitude	3 (6)		7 (3)
Depth	2 (8)		
Stream length	1 (4)		
Isolation	4 (10)		

All variables significant to species distribution when tested individually are included. Individual variables significant also in the model are marked with an asterisk. Level of explanation is given for all variables, chemical variables, significant indicators of anoxia (CH_4), significant indicators of acidity (pH S, pH W or ANC) and for individual variables. Explained variation shared between anoxia and acidity is not included in level of explanation for each group but is presented separately. Numbers within parentheses denote order of inclusion in the model.

Nine-spined stickleback, bleak and crucian carp were associated with small lakes whereas ide and bream were found mainly in large lakes. Pike and perch had high occurrence at all pH values while all other species were more common in less acidic lakes. Indicators of winter anoxia separated crucian carp, nine- and three-spined stickleback from pike, perch and roach in isolated lakes, as was the case for all 40 lakes (Fig. 1). This pattern is illustrated further in Fig. 2, which shows the distribution of the most common species in relation to CH_4, pH and isolation. Perch and roach were not found in isolated lakes with high CH_4 and pike occurred in only three of the 10 isolated lakes with CH_4 >0.15 mg L^{-1}. The pattern is different in connected lakes, where the distribution of pike, perch and roach seems unrelated to CH_4. In contrast, the occurrence of crucian carp decreased slightly with CH_4 in both connected and isolated lakes. Crucian carp and roach were absent from lakes with pH < 5.3, and occurrence increased with pH.

The significance of these patterns was tested with stepwise logistic regressions utilising all 40 lakes. The occurrences of pike and perch were negatively correlated with methane concentration ($t = -2.42$, $P < 0.05$ and $t = -2.61$, $P < 0.01$ respectively) and isolation ($t = -3.99$, $P < 0.005$ and $t = -2.39$, $P < 0.05$). The occurrence of crucian carp was positively correlated with pH ($t = 2.89$, $P < 0.01$) and isolation ($t = 2.97$, $P < 0.05$). The occurrence of roach was positively correlated with pH ($t = 2.32$, $P < 0.05$) and negatively correlated with both isolation ($t = -2.52$, $P < 0.05$) and methane ($t = -2.18$, $P < 0.05$).

Discussion

Results of this study suggest that different environmental variables affect fish community composition in

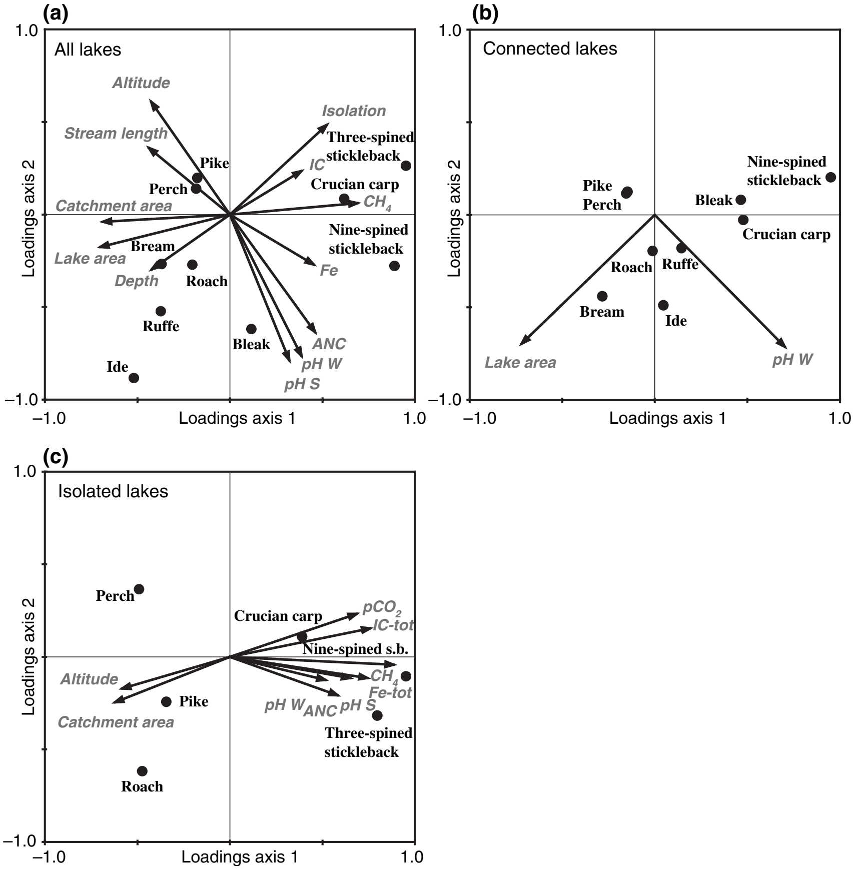

Fig. 1 Canonical correspondence analyses of (a) all lakes, (b) connected lakes and (c) isolated lakes, showing the distribution of fish species in relation to environmental variables. When tested individually 12 variables were significant for all lakes, two for connected and nine for isolated lakes. In the model, lake area and pH were significant in all lakes and in connected lakes, and CH_4 was significant in isolated lakes.

Table 4 Matrix of Pearson correlation coefficients for concentrations of chemical substances related to oxygen concentrations

	$DO_{shallow}$	DO_{deep}	CH_4	pCO_2	IC	$Fe_{shallow}$	Fe_{deep}
$DO_{shallow}$	1						
DO_{deep}	0.75	1					
CH_4	−0.66	−0.34	1				
pCO_2	−0.62	−0.36	0.78	1			
IC	−0.63	−0.34	0.82	0.94	1		
$Fe_{shallow}$	−0.40	−0.13	0.51	0.55	0.60	1	
Fe_{deep}	−0.37	−0.30	0.41	0.50	0.58	0.88	1

isolated and connected lakes. Acidity was associated with fish community composition in all lake groups, explaining 10–20% of the variation in isolated lakes and 12% in connected lakes. This suggests a comparable level of importance for acidity in the two lake groups. In contrast, winter anoxia was correlated with species composition only in isolated lakes, explaining 24–34% of the variation. Thus, we found that winter anoxia explained a larger proportion of the variation in fish community composition in isolated lakes than in connected lakes, which supports our hypothesis that chemical variables are more important in these lakes.

The distributions of pike, perch and roach were negatively correlated with anoxic conditions in isolated lakes, in agreement with prior studies showing that large species, and especially predators, often have a higher oxygen demand than small prey species (Casselman & Harvey, 1975; Tonn & Magnuson, 1982). Crucian carp, nine- and three-spined stickleback were the only species more common in anoxic lakes than in oxic lakes. The crucian carp is well known to be tolerant to oxygen deficiency, but is exceptionally vulnerable to predation (reviewed in Holopainen, Tonn & Paszkowski, 1997; Steinberg, 2003). Small-bodied species are in general more vulnerable to piscivory than large-bodied species (Tonn & Magnuson, 1982; MacRae & Jackson, 2001), often attributed to gape limitation (Tonn, Paszkowski & Holopainen, 1992). Harsh environmental conditions can constitute a refuge from predation, however (Meffe, 1984). Large pike have higher DO require-

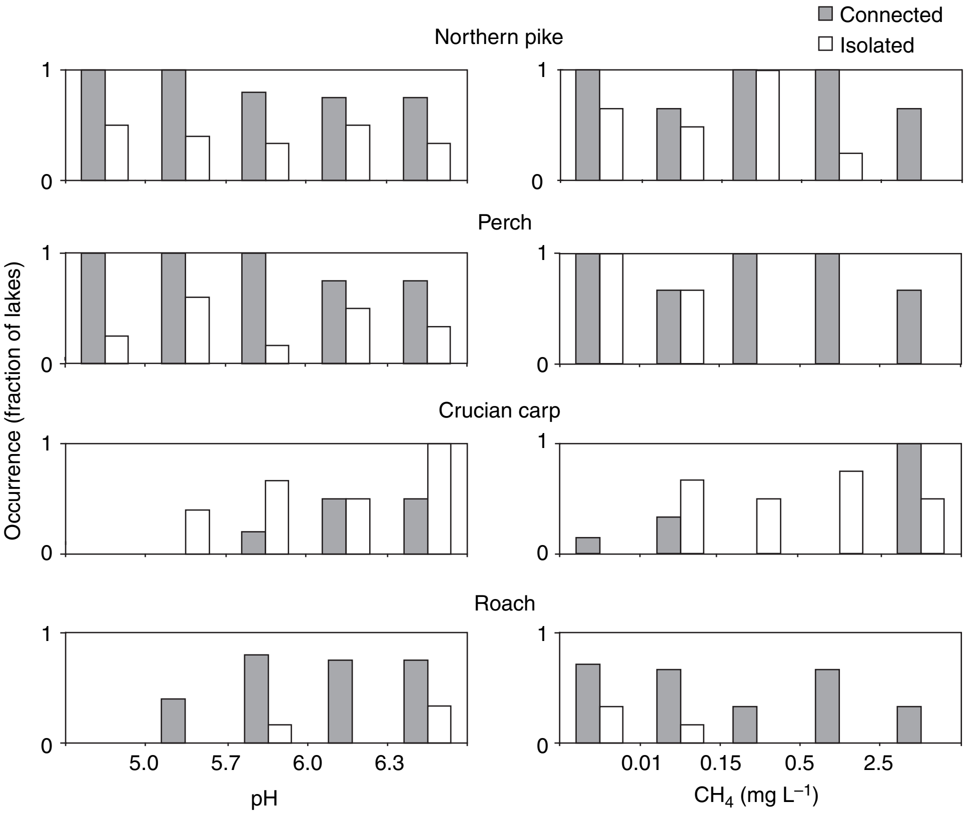

Fig. 2 Incidence of pike, perch, crucian carp and roach in relation to CH_4 and pH. Lakes are divided into five categories based on CH_4 concentration and five based on pH. Presence of fish is shown as fraction of lakes within each category containing a given species.

ments than small individuals (Casselman & Harvey, 1975) and pike, brook stickleback (*Culaea inconstans*, Kirtland) and fathead minnow (*Pimephales promelas*, Rafinesque) may be able to coexist in small lakes because of harsh environmental conditions such as winter anoxia depressing the abundance of pike (Robinson & Tonn, 1989). We suggest that the higher occurrence of crucian carp, nine- and three-spined stickleback in anoxic and isolated lakes is because of oxygen deficiency constituting a refuge from piscivory.

There are several possible explanations for the lack of correlation between variation in fish community composition and anoxia in connected lakes. Our measurement from the centre of the lakes may not fully represent the range of DO environments available to fish in these lakes during winter. Spatial variation in winter chemistry was measured in one of the connected lakes in this study, and it was found that while pH varied relatively little (±0.06 units), DO varied substantially (±1.7 mg L^{-1}) and could be several mg L^{-1} higher near an inlet (J. Öhman, I. Buffam, G. Englund & H. Laudon, unpublished data). Oxygen concentrations are often higher by connecting streams (Hargeby, 1985; Danylchuk & Tonn, 2003) and fish have been found to aggregate around inlets and outlets in lakes with oxygen deficiency (Tonn & Magnuson, 1982). As isolated lakes do not have connecting streams, such lakes should have a more homogenous distribution of DO and the existing fish should have less access to oxygen rich areas. A second possibility is that connected lakes show higher temporal variability in water chemistry. Such variability may weaken the correlation between measurements taken during typical winter conditions and the conditions during more extreme years that probably control the fish communities in many lakes.

A third explanation is that fish can escape connected lakes as they become anoxic and recolonise when conditions have improved. Tonn & Magnuson (1982) suggested that the absence of species such as pike from connected lakes during winter was because of seasonal migration as a consequence of oxygen deficiency. Although the underlying mechanisms have not been investigated, the relationship between anoxia and species composition found only in isolated lakes supports our hypothesis that chemical status is more important in isolated than connected lakes.

Confounding factors are a potential problem in all comparative studies. We found that catchment area and lake depth differed significantly between isolated and connected lakes, and the difference in mean lake size was, although not significant, substantial (Table 2). Catchment area should not be considered as a confounding variable as the absence of connecting streams is a direct consequence of a small catchment area. The difference in lake depth and lake size could explain parts of the association between winter anoxia and variation in species composition, however, as deep and large lakes may have a more heterogeneous distribution of oxygen than small and shallow ones.

Our interpretation of CH_4-concentration as an indicator of anoxia is based on a well-established negative relationship between oxygen and methane concentration in lake waters (Bastviken *et al.*, 2002; Liikanen *et al.*, 2002), which occurs because methane is rapidly consumed when oxygen is available (Rudd, Hamilton & Campbell, 1978; Liikanen *et al.*, 2002). IC and pCO_2 increase in winter partly due to the same reasons as DO decreases: low aeration, low photosynthetic activity and respiratory breakdown of organic matter (Chambers *et al.*, 1997; Fang & Stefan, 2000; Kalff, 2002), whereas Fe is released from the sediments because of a shift in its redox-state (Wetzel, 2001). One would expect measurements of DO-concentration to be a more direct and accurate indicator of anoxia, but we suspect that drilling the hole in the ice caused mixing of oxygen-rich snow and ice into the upper part of the water column in some lakes. The addition of oxygen would have a larger immediate effect on DO-concentration than on the concentrations of CH_4, CO_2, IC or Fe. Additionally, at low temperatures and low DO concentration, the oxygen probe may lack the sensitivity to detect small differences in concentration relevant to fish survival. This interpretation is supported by a moderate correlation between DO and CH_4, and the fact that pCO_2, IC and Fe showed a stronger correlation with CH_4 than with DO (Table 4).

In contrast to winter anoxia, acidity was associated with fish community composition in all lake groups, as indicated by significant correlations with pH. An important difference between the effects of winter anoxia and acidity is that lake acidity affects fish species in all seasons. In the present study, there was little difference between the correlation of summer pH and winter pH even though the latter was lower. As winter is a period when oxygen deficiency and low temperature stress fish populations (Jackson *et al.*, 2001), acidity could become an important additional stressor at that time. On the other hand, young life stages, occurring in spring and early summer, are the most sensitive to acidity (Almer *et al.*, 1978; Keinänen *et al.*, 2004). An additional layer of complexity is added by the dynamic nature of acidity, which is amplified in northern Swedish surface waters during rainfall episodes and the spring snowmelt period (Laudon & Bishop, 1999). These periods were not directly measured as a part of this study. Although inorganic aluminium (Al) is known to be the principal toxic agent in many acidic waters (Poléo, 1995), no correlations with aluminium concentrations were found. However, only total aluminium concentrations were measured, and in such highly humic lakes a high and variable proportion of the aluminium is bound by organic matter, reducing its toxicity. Thus, aluminium toxicity cannot be ruled out or confirmed directly in this study.

Pike and perch were present in all but the most acidic lakes, in agreement with Degerman *et al.* (1995) and Appelberg *et al.* (1993). The acid sensitive roach was missing from lakes with $pH < 5.5$ (connected lakes) and $pH < 5.7$ (isolated lakes), concordant with the conclusions of Appelberg *et al.* (1993). It was only present in two isolated lakes, both with high pH and low CH_4 concentration. As the roach is well adjusted to living in small, humic, boreal lakes in a cold climate (Sumari, 1971), the absence of roach from many isolated lakes can probably be attributed to the chemical stress of winter anoxia and/or acidity.

The occurrence of crucian carp was, just as that of roach, significantly correlated with pH and the species was absent below pH 5.3. Reported acid sensitivity levels of crucian carp vary between studies (Holopainen & Oikari, 1992; Degerman & Lingdell, 1993), but the species has been found to survive at pH 5.16 in an artificial environment (Poléo *et al.*, 1995). As pH in this area is negatively correlated with altitude ($r = -0.64$ for these 40 lakes; Fig. 1c) it has been proposed as an alternative hypothesis that a lower abundance of shallow, predator-free lakes at high altitudes may explain why crucian carp, and also nine- and three-spined stickleback, are rare in acidic lakes (Olofsson, 2005). A partial CCA of each lake group (data not shown) revealed that 35–55% of the variation explained by acidity was shared with that explained by altitude.

Our ability to draw conclusions about factors limiting the distribution of rare species is of course limited. However, we note that none of the species present in <25% of the lakes occurred in lakes with pH < 5.6, which may indicate that acid sensitivity contributes to the distribution of these species as well.

Lake area was the only physical variable significant in the full CCA model, with crucian carp, bleak, nine- and three-spined stickleback associated with small lakes. This result contrasts with findings in studies covering larger geographical areas, which include large lakes and under-represent small, coastal lakes (Holmgren & Appelberg, 2000; Rask *et al.*, 2000). There, crucian carp, nine- and three-spined stickleback were found mainly in large lakes (Holmgren & Appelberg, 2000; Rask *et al.*, 2000), suggesting these species have a bimodal distribution with respect to lake area. A possible interpretation is that predatory interactions are more intense in small lakes, where the prey species can only persist if piscivores such as pike and perch are absent or have lowered abundances because of environmental stress, whereas in large lakes greater environmental heterogeneity may promote prey persistence. Lake area was not correlated with species composition in isolated lakes, probably due to the range being so small (1–15 ha).

In conclusion we found that winter anoxia was related to fish community composition only in isolated lakes, whereas acidity was of importance also in connected lakes. An important implication of these results is that lake connectivity should be taken into account when estimating tolerance levels of fish species from patterns of distribution.

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