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WHAT IS THE IMPACT OF 'LIMING' LAKES ON THE ABUNDANCE AND DIVERSITY OF LAKE BIOTA?

Systematic Review

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Abstract

Background

Liming, adding of limestone or dolomite, has been conducted for many years in order to mitigate the acidification of lakes caused by 'acid rain'. However, despite the long-term application of liming in many countries, there is still debate on the impact of liming on lake biota. Hence, we conducted a systematic review and meta-analysis in order to answer the question: What is the impact of 'liming' lakes on the abundance and diversity of lake biota?

Methods

A systematic search was conducted for all relevant papers using search terms for liming combined with terms for lakes and the biota. All articles found were included if they report on a study containing a relevant population (the biota in freshwater lakes), intervention (liming), and outcome (change in abundance/density or richness/diversity of biota).

Results

The search found 143 relevant articles. The available evidence suggests that on average liming increases the diversity of fish, zooplankton and phytoplankton, whereas the diversity of benthic organisms is not increased. The diversity of zooplankton and phytoplankton is estimated to decrease in some lakes but only in a small minority. The meta-analysis on the abundance of zooplankton, phytoplankton, and benthic invertebrates indicates they do not increase with liming. The impact of liming on fish abundance is less clear cut. The largest fish study suggests fish may increase in abundance with liming. However, there is a lack of studies with both baseline and control sites, making it hard to be certain whether the changes observed were due to liming. Liming has also been used to restore fish abundances by providing conditions for survival of stocked fish. The liming appears to have enabled the restocking of fish in some instances. However, many studies did not actually test if fish would have survived before liming or stock fish in control sites.

Conclusions

Increasing and preserving the diversity of organisms present in an ecosystem can (but may not always) represent a favourable ecological outcome, especially if achieved across a broad spectrum of the ecosystem and of acid sensitive species that were previously absent due to acidification. In this regard liming of lakes can be considered, in some circumstances, an effective conservation measure. However, in a minority of lakes diversity decreased with liming. The evidence base is insufficient to explore reasons for variation in effectiveness and more powerful study designs are required to enable prediction of when extremes of impact may occur.

Keywords:

Liming, lakes, biota, zooplankton, phytoplankton, benthic organisms, macrophytes, fish.

1. Background

"Acid rain" and the associated acidification of waterways first became a widespread environmental concern in the 1970's (Menz and Seip 2004). Since the industrial revolution humans have been releasing sulphur and nitrogen oxides into the atmosphere from industrial emissions, causing a decrease in the pH of rainfall in many areas, and hence of associated water bodies.

Acidification frequently causes changes in freshwater ecosystems including in the fish and invertebrates (Schindler et al 1985, Moiseenko 2005). In order to mitigate the problems of 'acid rain', including acidification of water bodies, considerable efforts have been made to reduce industrial emissions since the 1980's. The emissions of sulphur have been successfully reduced through various legislations, both in Europe and North America, reducing the acidity of rainfall (Evans et al 2001). However, recovery is not uniform, there are still areas where the acid load outstrips the soils neutralizing capacity, nitrogen oxide emissions have not decreased to the same extent as sulphur emissions and the recovery of areas with decreased deposition is not uniform. Thus, there are still many areas that suffer from acidic surface waters and it may be many years before all surface waters recover, if ever (Evans et al 2001).

In order to protect lakes and fish stocks until the emissions can be reduced other methods have had to be implemented. One of the most widespread mitigation techniques is adding of calcium carbonate in order to raise the pH; commonly termed liming (Henrikson and Brodin 1995, Clair and Hindar 2005). Liming of lakes has occurred for centuries in order to support aquaculture and control the pH for aquaculture production. Liming has also been used to increase the productivity of lakes (Waters and Bell 1957). In addition, in more recent years, liming has been used to control eutrophication (Prepas et al 2001) and remove radioactive material (including fallout from Chernobyl, Håkanson and Andersson 1992).

In terms of acidification, liming to mitigate acidification of waters has been implemented in North America and many European countries but the largest liming programs are in Norway and Sweden. Sweden has invested 3.8 billion SEK (approximately €0.4 billion) on liming between 1983 and 2006 (Bostedt et al 2010).

The biological responses to liming of acidified surface waters are equivocal (Angeler and Goedkoop 2010). For organisations managing acidified lakes, an evidence base to aid prediction of the impact and/or effectiveness of liming interventions might usefully inform decision making. Therefore this systematic review aims to find and summarise the best available evidence on the impact of liming on invertebrates, fish, phytoplankton and macrophytes (a sister review has been conducted on rivers and streams (Mant et al. 2011; the reviews were divided as different parts of an ecosystem may react differently to liming).

2. Objective of the Review

Primary question:
What is the impact of 'liming' lakes on the abundance and diversity of lake biota?

The question contains the following components:

> **Population:** Biota in freshwater lakes (of all sizes, although size will be included in later analysis)
> **Intervention:** Liming of lakes to ameliorate the effects of acidification (including the indirect liming of lakes by liming of catchment areas and tributaries).
> **Outcome:** Change in abundance and diversity of lake biota; fish, invertebrates, zooplankton, phytoplankton and macrophytes.
> **Comparator:** No intervention or before the intervention or both

3. Methods

The review was conducted according to the *a priori* methods set out in Mant and Pullin (2011).

3.1 Search

The search was conducted using terms for the intervention, population in terms of habitat and population in terms of biota. A comprehensive list of terms was used for each component, in an attempt to find all literature of relevance. These were combined in the most efficient way possible depending upon the database being searched. For example, Web of Science allowed combinations of all terms in one search. * denotes wildcard.

The terms used were:
Population (habitat): Lake, Catchment, watershed, loch, pond, llyn, mere, tarn
Population (biota): Fish* (includes fishes, fishery etc.), Salmo*, Trout, Roach, rutilus, charr, salvelinus, Perch, Pike, Invert*, Macroinvertebrate*, macrofauna, meiofauna, zooplankton, crustacea*, microcrustacea*, daphnia, insect*, Ephemeroptera, Plecoptera, Trichoptera, coleopteran, chironomid, Mollus*, bivalve*, gastropod, phytoplankton, diatom*, cyanobacteria, Macrophyte*
Intervention: Liming, lime*, chalk*, calcium carbonate, dolomite.

Terms within categories were linked with the Boolean operator 'OR'. Terms between categories were linked with the Boolean operator 'AND'. All combinations of terms were covered by the search. The final search string for each database is in Appendix A.

The search was conducted within the following online databases which cover the breadth and depth of available literature on the topic:

1) ISI Web of Knowledge
2) Science Direct
3) Directory of Open Access Journals
4) Copac
5) Index to Theses Online
6) CAB Abstracts
7) ConservationEvidence.com
8) CSA Illumina
9) Agricola

No time or document type restrictions were applied. However, due to time and resource limitations data was only extracted from English language papers (in contrast to the proposals original aim). However, a complete list of non-English language papers is provided in Appendix B.

An Internet search was also performed using the following meta-search engines and recommended sites:

http://www.alltheweb.com
http://scholar.google.com
http://www.Scirus
http://www.dogpile.com
http://www.google.com
http://data.esa.org/

The first 50 hits were examined for appropriate data (Section 3.2.).

Websites of relevant specialist organisations, listed below, were also searched (including using the term 'liming', and the term for 'liming' in the native language of the organisation). Bibliographies of included material were searched for relevant references.

Alterra	National Parks
Anglers trust	Natural Resources Canada
British Ecological Society	Norwegian Directorate for Nature Management
Centre for Ecology and Hydrology	Norwegian Institute of Water Research (NIVA)
Countryside Council for Wales	Norwegian Institute of Air Research (NILU)
Danish EPA	Norwegian Institute of Natur Research (NINA)
Department for the Environment, Food and Rural Affairs	Ontario Ministry of Environment
Dŵr Cymru / Welsh Water	Research Councils UK
Environment Agency	Salmon and Trout Association
Environment Canada	Severn Trent Water
Environment Protection Agency Ireland	Scottish Agricultural College
Northern Ireland Environment Agency	Scottish Executive
European Commission Joint Research Centre	Scottish Environment Protection Agency
European Environment Agency	Scottish Natural Heritage
Finnish Environment institute (SYKE)	Society for Ecological Restoration
International Union for Conservation of Nature	Swedish Environmental Protection Agency
Joint Nature Conservation Committee	United States Environment Protection Agency
Macaulay Land Use Research Institute	United Utilities
Natural England	Welsh Assembly Government

3.2 Article screening and study inclusion

All articles retrieved were entered into an Endnote database and duplicates removed. Articles relevant to the specific question being asked were identified in three stages using specific inclusion criteria (listed below), in order to systematically remove articles which did not contain relevant data. At each stage, if there was insufficient information to exclude a study it was retained.

At the first stage only the titles of the articles were assessed and irrelevant articles removed. Articles remaining after this first stage were filtered on viewing the abstract and then the full text (see Appendix B for a full list of articles excluded at this stage).

Study inclusion criteria:

- **Relevant subject(s):** Biota in freshwater lakes (of any size).

- **Types of intervention:** Direct or indirect addition of lime to ameliorate the effects of acidification; including directly adding lime to lakes as well as, indirectly adding lime by liming tributaries or catchments of lakes. All methods of liming were included.

- **Types of comparator:** No intervention or before after comparisons or both (Before after control impact studies – BACI).

- **Types of outcome:** Change in abundance/density or richness/diversity of biota (e.g. fish, invertebrate, zooplankton, phytoplankton or macrophyte groups).

- **Types of study:** Any primary study comparing limed and un-limed subjects whose outcomes fit the above. Review articles do not normally contain primary data but were searched for the primary studies they cite. No geographic restriction was applied to this review.

In order to assess the effects of between-reviewer differences in determining relevance, two reviewers applied the inclusion criteria to 20% of articles at the start of the abstract filtering stage and the kappa statistic (Edwards et al. 2002) was calculated. The kappa statistic was 0.76, which shows a high level of agreement between the reviewers.

3.3 Potential Effect Modifiers

The impact of liming could be modified by a large number of different variables including; characteristics of the subject (e.g. lake depth, lake volume, water retention time[1], level of management of lake, water temperature, level of afforestation in catchment, drainage pattern, soil type, geology, age of forest, age of catchment, fish condition, age structure of fish, barriers), chemical characteristics (e.g. Calcium, Aluminium, Sulphur (SO_4, SO_2), Nitrogen (NO_X), dissolved organic carbon, Iron, pH, hardness, alkalinity, Phosphorus (SRP and TP)), methodological variables (e.g. liming method, type of lime, dose of lime applied, time since intervention, longevity of intervention, outcome measure used (Shannon, Margalef, richness etc.), invertebrate sample method, fish sample method, method of analysis) and general study variables (e.g. latitude and longitude of study site, altitude, mean annual temperature, mean annual precipitation, timing of snow melt, presence or absence of acid sensitive taxa,

[1] The time it takes for a changeover (inflow/ outflow) of water equivalent to the volume of the lake.

additional interventions such as fish stocking, North Atlantic Oscillation Index, presence of sea salt episodes (periods of increased salinity)).

As a limited number of studies were found not all of these reasons for variation could be investigated. The effect of the pH after liming was investigated where possible; however, fewer factors could be investigated than had been planned in the protocol.

3.4 Study quality assessment

Study quality was assessed along with data extraction (section 3.5) in order to ascertain the reliability of and potential bias in the effects reported. Well-conducted studies of high quality have less potential for bias than their poorer counterparts. Studies were assessed as to whether they had before intervention data, independent control sites or both.

Studies with baseline data and control sites (Before, After, Control, Impact; BACI studies) are generally more reliable than studies with only before and after data from limed lakes (Before After, BA studies) or studies with no baseline data (Control, Impact, CI studies). Apart from the requirement to have a comparator (baseline data or a control site) no study was excluded from the review due to study design. The number of replicate samples within a lake and the number of lakes included within the study was also recorded. Studies with more replicates will provide a more reliable estimate of the mean.

Additionally, other potential sources of bias were assessed. This included differences in the measurement of the before/after or control/impact data, changes in the lake other than liming over the period of the study i.e. the presence of stocking of fish and differences between control and treatment lakes other than the treatment (i.e. how were they selected).

3.5 Data extraction and data synthesis

Data on the impact of liming on the total abundance and taxonomic richness of fish, zooplankton, phytoplankton, benthic invertebrates and macrophytes were extracted wherever possible. The data on the abundance and taxonomic richness was used to calculate an effect size, a single estimate of the size and direction of the effect of liming, for each group of organisms in each study, wherever the data allowed. The effect size used was the log ratio of the limed compared to the control sites or years. Calculating the log ratio allowed the impact to be compared across studies that used different units of measure to record the outcome (i.e. richness or abundance).

Data on the nature of the study were also collected, including details of the liming and the impact of the liming in terms of pH. Unfortunately, despite acid neutralizing capacity (ANC) being considered a better measure of lake acidity in recent years, as many of the studies were relatively old, pH was more commonly reported.

The impact of liming on zooplankton, phytoplankton and benthic invertebrates was analysed using meta-analysis. There was insufficient data for meta-analysis to be conducted on the fish or macrophyte data. The meta-analysis was carried out, using the DerSimonian Liard random effects model, in order to calculate a combined mean effect across studies. The studies were weighted by inverse variance and where no variance could be calculated a variance of two times the maximum measured variance was used. The analysis was carried out using the package 'metafor' version 1.6-0 within the statistical program R version 2.14.1. The impact of study design and susceptibility to bias were also investigated by comparing the effect sizes found using different study designs.

Where the data did not permit the use of meta-analysis the results were tabulated, along with the study design so that they could be compared between studies. Unfortunately, insufficient data were found to be able to carry out an analysis of the factors causing the variability between studies, including the impact of different liming techniques, as was planned in the protocol.

4. Results

4.1 Studies found

The initial search conducted in May 2011 retrieved 4245 articles, however after assessment at the title and abstract stages only 643 were identified as potentially relevant to the review (Figure 1). Assessment at full text identified 143 articles relevant to the review question (see Appendix B for a full list of excluded studies). A further 43 were excluded from the review as they could not be retrieved (listed in Appendix B), and 36 articles were excluded due to being in a language other than English (listed in Appendix B) and the resources not being available to translate them.

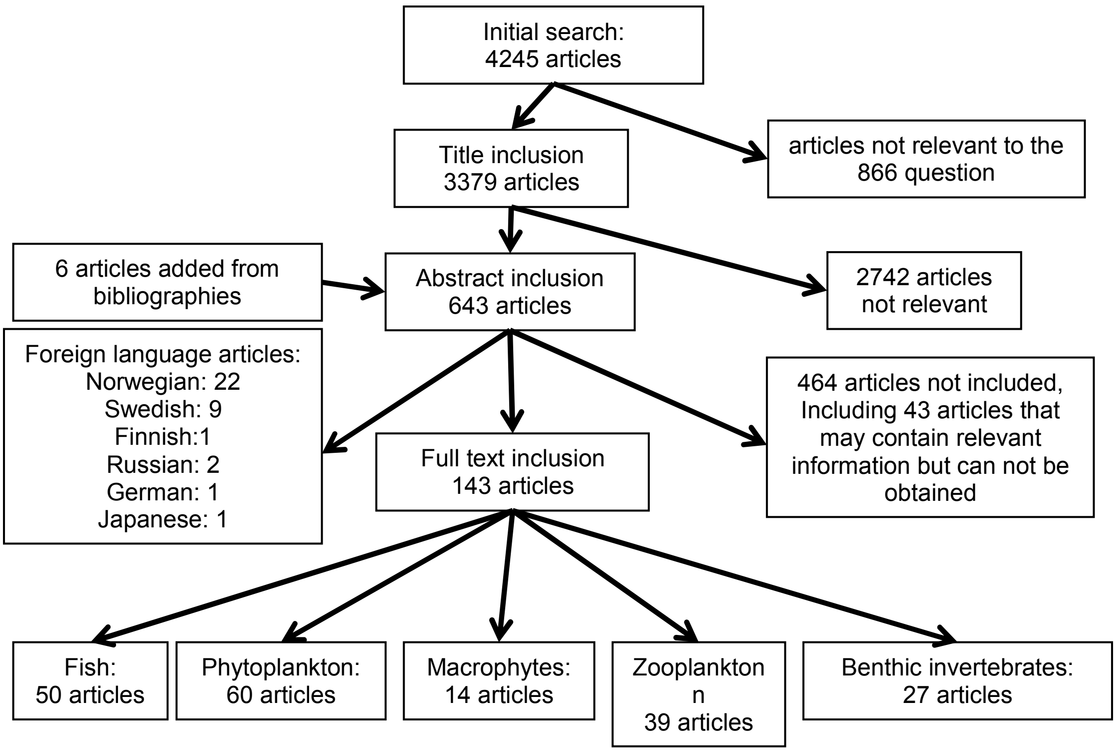

Figure 1: The number of articles found and included at each stage of selection.

The articles were sorted according to which part of the biota they investigated. There were 50 articles on the impact of liming on fish, 60 on the impact on phytoplankton, 14 on macrophytes, 39 on zooplankton and 27 on benthic invertebrates (the total adds up to more than the total number of included papers as several papers looked at more than one part of the biota).

4.2 Fish

Fifty articles on the impact of liming on fish populations were found. However, the findings from 14 of the articles were excluded from the analysis to avoid pseudo-replication as they covered the same studies (and hence lakes) as other articles, leaving 36 studies. The vast majority of lakes studied came from Scandinavia (Sweden 16 studies, over 100 lakes; Norway 5 studies, 5 lakes; Finland 2 studies, 2 lakes), although there were also studies of UK (2 studies, 2 lakes) and North American lakes (USA 5 studies, 7 lakes; Canada 5 studies, 5 lakes) (see appendix C details of all of the fish articles).

Only a minority of studies, twelve articles, had quantitative data that could be extracted for the change in abundance or number of species of fish with liming. In 14 out of the 36 studies no fish were present in the lakes before liming, fish were then stocked after liming and the stocked fish population was monitored. Hence, it is not possible to use these studies to show how liming changed resident populations abundance or diversity. In the remaining twenty two studies fish were present before liming. However, in three of the lakes the impact of liming could not be assessed (due to the control population crashing (Rask et al 1996), variable stocking that the author states is likely to have impacted on results (Tervet and Harriman 1988) and no before data presented (Nyberg 1995)). In a further seven studies statements about the impact of liming were made but no numerical data could be extracted (Table C.2 in Appendix C). Leaving only eleven studies which presented data on changes in abundance and four of which presented data on diversity.

The eleven studies containing quantitative data on the impact of liming on fish abundance (Table 1) included two from North America and nine from Scandinavia. The fish species covered included: perch (*Perca fluviatilis*), pike (*Esox lucius*), whitefish (*Coregonus spp*.), roach (*Rutilus rutilus*), char (*Salvelinus alpines*), brown trout (*Salmo trutta*), ruffe (*Gymnocephalus cernuus*), cisco (*Coregonus albula*), brook trout, *Salvelinus fontinalis),* fathead minnow (*Pimephales promelas*), pearl dace (*Margariscus margarita)* and yellow perch (*Perca flavescens*). As only four lakes presented changes for all species combined, the studies varied greatly in size (from one to 103 lakes) and it is unclear whether there is overlap between the large study and the smaller studies, no meta-analysis was carried out. One study covered more lakes than all of the other studies combined, Degerman et al 1992 (103 lakes). It found an increase in overall fish abundance with liming, as did the second largest study (26 lakes, Nyberg et al 1986). However, neither of these studies contained control data, they only studied limed lakes over time, from before and after liming (i.e. were of BA study design). There were only two studies that contained control sites. One, Eriksson and Tengelin (1997), had both baseline data and control sites (was of a BACI study design) but only looked at changes in perch, *Perca fluviatilis*, populations. It found an increase in perch with liming. The only study that looked at all species and had control data, Holmgren (2001), found a lower abundance of fish in the limed lakes (although this study had no baseline data so it was not possible to check whether there were differences in the lakes before liming). Additionally the potential for the presence of stocking is not mentioned or taken into account in the large 103 lake study, and may have impacted on the recorded abundance.

Table 1: Change in fish abundance recorded within different studies.

Study	Design	All species	pearch	pike	whitefish	roach	char	brown trout	ruffe	cisco/ vendace	pH change, liming method
Eriksson and Tengelin 1987	BACI, 8 limed, 6 control lakes		1.01 (SE 0.54)								Limed by direct liming to lakes pH in limed lakes before (4.6-5.7) after 6.2-7.1) controls: before 4.5-6.3, after 4.5-6.2
Holmgren 2001	CI benthic fish, 6 limed, 2 acid lakes	-0.33									Limed according to the Swedish national liming program pH limed site: 7 (0.2) control: 5.9 (0.0)
Degerman, et al. 1992	BA, 103 lakes	0.58 p<0.001	0.57 p<0.001	0.19 p=0.46	0.16 p=0.78	0.65 p<0.01				1.01 p = 0.07	Liming by direct lake liming or catchment liming pH mean before 5; after 5.8
Nyberg, et al. 1986	BA, 26 lakes	1.04 (0.05)									Details of liming not given pH before liming <5.5, after liming >5.5
Iivonen, et al. 1995	BA, 1 lake,	-0.02 (0.10)	1.13 (0.30)	-0.53 (0.96)	-1.17 (0.81)	-0.51 (0.25)			-0.81 (0.23)		Limed directly to lake pH before 5.8-6.2, after 6.5-7.3
Appelberg 1995sh	BA, 1 lake		- 0.04 (SE 0.41)	0.36 (SE 0.77)		4.57 (SE 60.31)				0.17 (SE 0.52)	Lake limed directly, and upstream lakes Alkalinity rose from 0 to 0.16
Saksgard and Hesthagen 1995	BA, 1 lake,		-1.43 (SE 0.26)		-1.01 (0.35)		-0.32 (SE 0.73)				Details of liming methods not given pH early 1970s 5.2 to 6.0, after liming pH 5.9- 7.4
Appelberg 1995g	BA, 1 lake		-0.61 (SE 0.21)								Lime applied directly to lake pH before 4.8-6, after 5.6-7.2
Andersen and Vollestad 1996	BA, 1 lake,						1.55 (SE 6.37)	1.00 (SE 2.06)			Large scale liming of lake pH before monthly mean pH 4.9-5.0, after 5.6-6.0
		brook trout	Fathead minnow	Pearl Dace	yellow perch						
Popp, et al. 1996	BA, 1 lake	0.84	2.37	0.45							Limed directly into lake pH increased after liming
Gunn, et al. 1988	BA, 1 lake	3.38			-1.52	(bullhead, white sucker, chub, pike, mud minnow, small mouth bass also recorded)					Liming directly into lake pH before liming yearly mean 5.6 – 5.8 after liming 6.3 – 6.7

The four studies that looked at the impact of liming on fish species richness (Table 2) all came from Sweden. They covered 103 (Degerman et al 1992)87(Bergquist 1991), 26 (Nyberg et al 1986) and 14 limed lakes (Appelberg 1998), however it is unclear whether there is overlap in the Swedish lakes studied as not all of the lakes surveyed were named. In the largest study on 103 lakes, Degerman et al (1992), diversity increased. The temperature and number of nets correlated with number of species, however, "taking the effects of temperature and number of nets into account, the average number of species caught in the lakes at the test fishing increased from 3.2 before liming to 3.8 fish species after liming (ANOVA, $p<0.05$)." The pH of the limed lakes was very variable before liming but the average did increase after liming from 5 to 5.8. The study only looked at the change in diversity over time, there were no control sites recorded. As the study does not contain control data it is not possible to determine whether the increase in species over the time period that liming occurred was due simply to liming or other changes occurring over time, such as decreasing acidic depositions. In the other two studies with no control data, one found a non-significant increase (Nyberg et al 1986) and the other found an increase from 3.6 to 4 species (Bergquist 1991).

Only one of the four studies contained control sites (Appelberg 1998). In that study the limed sites had higher diversity than the control sites after liming, however, they also had higher diversity before liming. Several species were introduced post liming into the limed sites, stocking occurred in five out of the 14 monitored lakes, potentially raising the diversity. Although there was natural recolonisation after liming in three out of the 14 lakes.

Table 2: Details of the studies that presented quantitative results on the impact of liming on fish species richness.

Study	Liming/ pH change	Results
Degerman et al 1992 103 lakes, BA study design	Liming by direct lake liming or catchment liming pH mean before 5 (range 3.8-6.1), mean after 5.8	3.2 to 3.8 spp. ($p<0.05$)
Bergquist 1991 87 lakes, BA study design	Limed according to the Swedish national program. pH before liming was below 6 in most lakes, after liming pH increased and the average was approximately 6.5	3.6 to 4 spp. ($p<0.001$)
Nyberg etal 1986 26 lakes, BA study design	Details of liming not given pH before liming <5.5, after liming >5.5	2.5 to 2.8 spp. (NS)
Appelberg 1998 14 lakes, CI study design	Limed according to the Swedish national program by lake, wetland, doser or upstream of the lake. pH before liming lake averages' 4.3 to 5.7, after liming average 5.8 to 6.6, control average 4.4 to 5.5	3.3 (SD1.9) to 3.9 (1.6) ($p<0.05$)

The studies that only provided statements of the impact of liming showed similar results to the studies which provided quantitative data (Appendix C). In five out of six of the studies with only before and after data (no control sites) the liming is reported to have been 'successful', however in the one study with control sites the abundance of fish was lower in the limed sites than in the controls (Angeler and Goedkoop 2010).

Stocking
In the studies where fish were not present prior to liming, the liming was carried out in order to allow the reintroduction of fish. Hence they can provide data on whether liming allows the reintroduction of extinct species and therefore helps recovery. However, in the majority of studies it was not tested whether the fish could have survived stocking without liming.

In North America the fish species stocked was the (generally native) brook trout (*Salvelinus fontinalis*). There were six studies (12 lakes) covering such stocking. In three out of the 12 lakes studied all of the fish died (Middle lake, Yan et al 1996; 2 lakes reported in Gloss et al 1989). Yan et al (1996) state that "the remaining Cu and perhaps other trace metals prevented [the stocked fish's] survival". In the other 9 lakes at least some of the stocked fish survived and in 3 of the lakes the fish reproduced as well (Appendix C, Table C.3).

Despite brook trout being invasive to Europe (DAISIE 2007) there is also one study of brook trout being stocked in Norway alongside brown trout (Traaen et al 1997), as well as a study of on the non-native rainbow trout being stocked (Svensson et al. 1995).

The main species for which stocking has been studied in Europe, in relation to liming of acidified lakes, are brown trout (in four lakes), roach and bream together (1 lake), perch (1 lake) and char (1 lake) (Appendix C, table C.4). Details of the levels and success of stocking are not recorded for the large multi-lake studies. Brown trout survived and reproduced in two out of the four lakes they were introduced to, in one only after repeated liming (Barlaup et al 1994). In two of the lakes no reproduction occurred, however, in both lakes trout may never have reproduced even historically (Svensson, et al. 1995, Traaen et al 1997). Perch, Char and Roach and bream were all introduced into one lake and they all survived and reproduced, the populations grew. However, the study on perch also introduced perch to three acidic lakes and in one of the three acidic lakes the perch also survived and reproduced, although to a lesser extent. The studies on char and roach and bream did not check whether stocked fish would survive before liming, or stock fish into unlimed control lakes. Hence, it is not possible to be certain whether the liming allowed the stocked fish to survive or they would have done anyway.

4.3 Zooplankton
Forty articles were found on the impact of liming on zooplankton. Sixteen of the articles presented data from the same study as another article or no data could be extracted from them (Appendix D, table D.3). A further nine studies made statements about the changes in overall zooplankton abundance or diversity but did not present the primary data (Appendix D, table D.2), leaving 14 articles from which quantitative data could be extracted and could be included within a meta-analysis (see appendix D.1 for details of each of the papers).

The studies from which data could be extracted covered from 1 to 14 lakes. Overall there was a significant increase in species richness with liming (mean effect (log ratio): 0.32 (SE 0.08), confidence interval of the mean 0.16 – 0.48, Figure 2) but no change in the abundance (mean effect (log ratio): 0.24 (SE 0.23), confidence interval of the mean -0.22 – 0.70, Figure 3).

The change in species richness varied significantly between studies (test for heterogeneity within the meta-analysis of zooplankton species richness: Q (df=10)=54.5, $p<0.0001$), and one study had a negative effect size (Blomqvist et al 1995). This variability suggests that zooplankton richness will decrease with liming in a minority of lakes (prediction interval, in which 95% of true lake effects are predicted to be overlaps zero: interval= -0.06 – 0.71). The pH after liming was not a significant predictor of the variability between lakes (pH as a moderator within the meta-analysis: QM (df=1)=0.49, p=0.48).

There was also variability in the impact of liming on zooplankton abundance, with abundance increasing in some lakes and decreasing in others. The pH after liming was not a significant predictor of the variability between lakes nor was the type of study (meta-analysis with pH and study design as moderators pH: z= -0.13, p=0.90; design: z= -1.0, p=0.31).

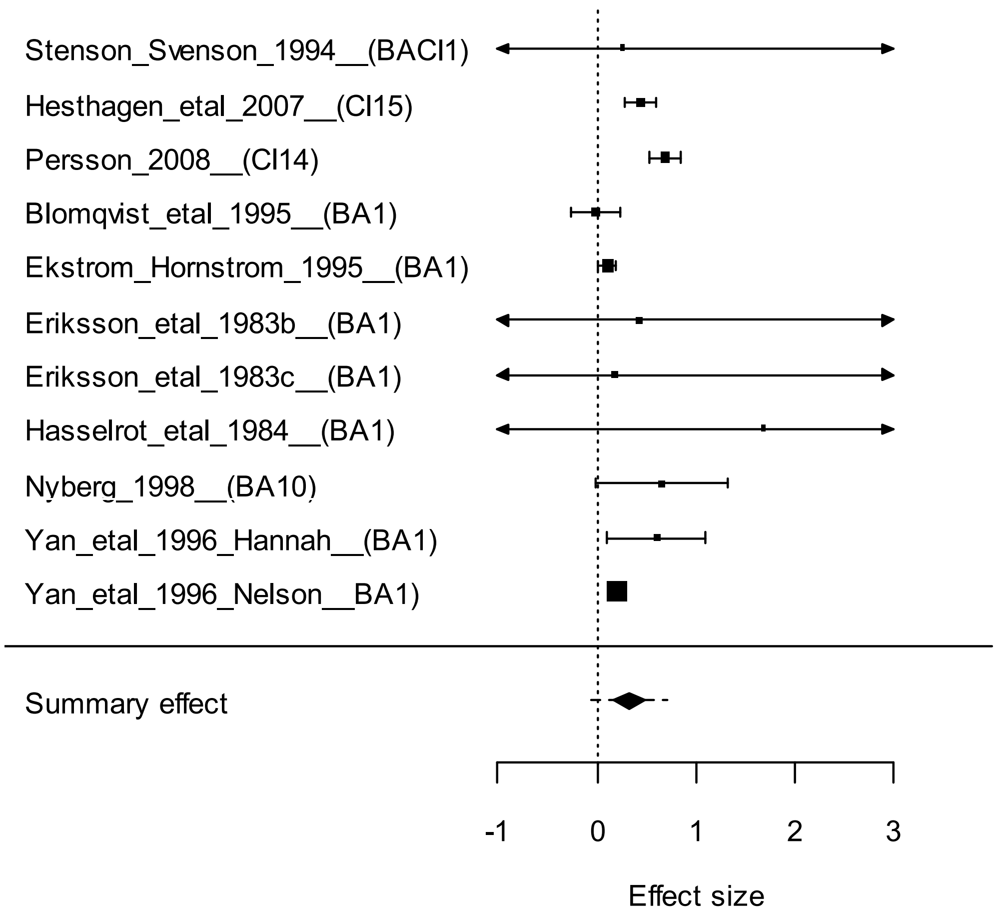

Figure 2: Forest plot of the zooplankton taxa richness. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean, and dotted line: the prediction interval were 95% of true effects are predicted to lie.

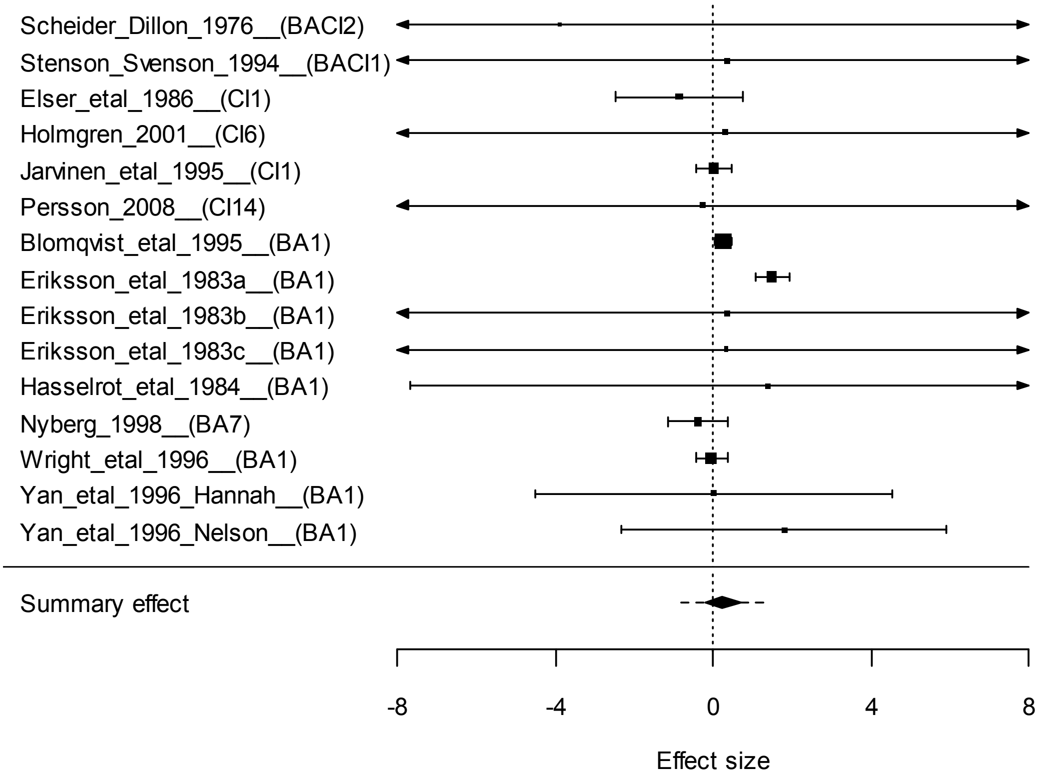

Figure 3: Forest plot of the zooplankton abundance. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean, and dotted line: the prediction interval were 95% of true effects are predicted to lie.

Like the fish data a minority of studies had both baseline and control data, only two out of the fourteen studies were of a BACI design. One of the BACI designed studies, Scheider and Dillon (1976), recorded the largest decrease in abundance of the limed sites compared to the controls (effect size -3.89). However, the other study, Stenson and Svenson (1994), recorded an increase in biomass. Additionally, for 40% of the studies which looked at changes in zooplankton species richness, the error in the effect size observed could not be calculated due to either a lack of replication in the study or a lack of reporting (see Appendix D for details of each study).

It is worth noting that all studies reported an increase in pH with liming. Although, in Hannah and Nelson lakes the level of Cu after liming was still relatively high (>20µg/l), possibly limiting recovery, the result from these lakes are not significantly worse than the other studies. In ten of the studies the pH increased to 7 or over, which may be above the natural pH of the lakes before acidification. In Sweden paleontological studies of lakes within the national liming program, have suggested that several lakes that have been limed in the national program may have been naturally acidic (Norberg et al 2008).

4.4 Phytoplankton

Similarly to the zooplankton only a minority of the studies on phytoplankton contained data that could be extracted. Fifty nine articles were found on the impact of liming but there were 18 articles which reported data from the same study as another article and so were excluded and there were 16 articles from which the impact of liming on the total abundance of phytoplankton or taxonomic richness could not be extracted (Table E.2 in Appendix E). Six studies made a statement about the impact without presenting the numerical findings, leaving 18 studies from which numerical data were extracted (Table E.1 in Appendix E for details of each of the studies). Even within the studies that presented numerical data on taxonomic richness, a majority (eight out of 13), had insufficient replication or presentation of data to accurately estimate the uncertainty in the effect size observed. Hence, primary statistical analysis was carried out alongside the meta-analysis.

As with the zooplankton the species richness of the phytoplankton increased with liming (random effects meta-analysis: mean effect (log ratio) = 0.27 (SE 0.02), prediction interval: 0.24 – 0.31, Figure 4; analysis as primary data: mean=0.31, t-test: t=3.0, df=12, p=0.01), but the abundance did not (random effects meta-analysis: mean effect (log ratio) = 0.15 (SE 0.11), prediction interval -0.06 – 0.37, analysis as primary data, t-test: mean=0.31 (CI -0.16 – 0.78), t=1.40, df=14, p=0.18) (Figure 5).

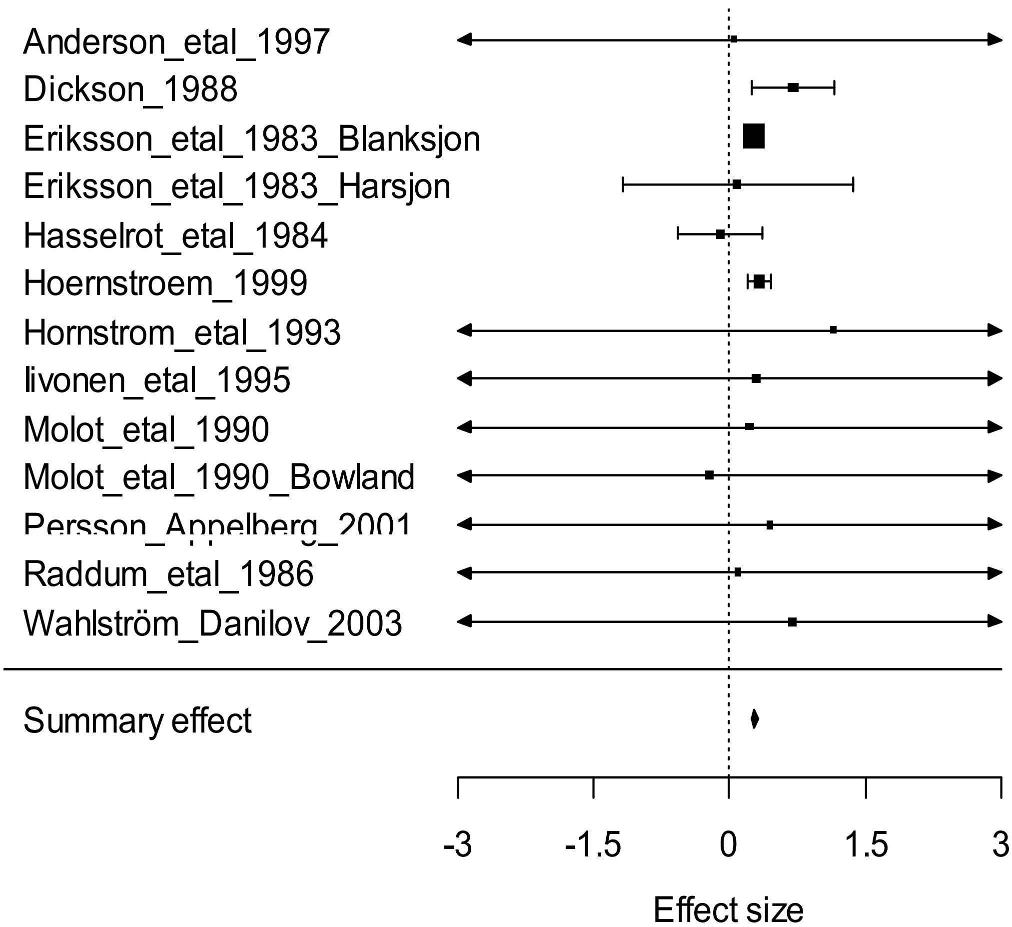

Figure 4: Forest plot of the phytoplankton taxonomic richness. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean.

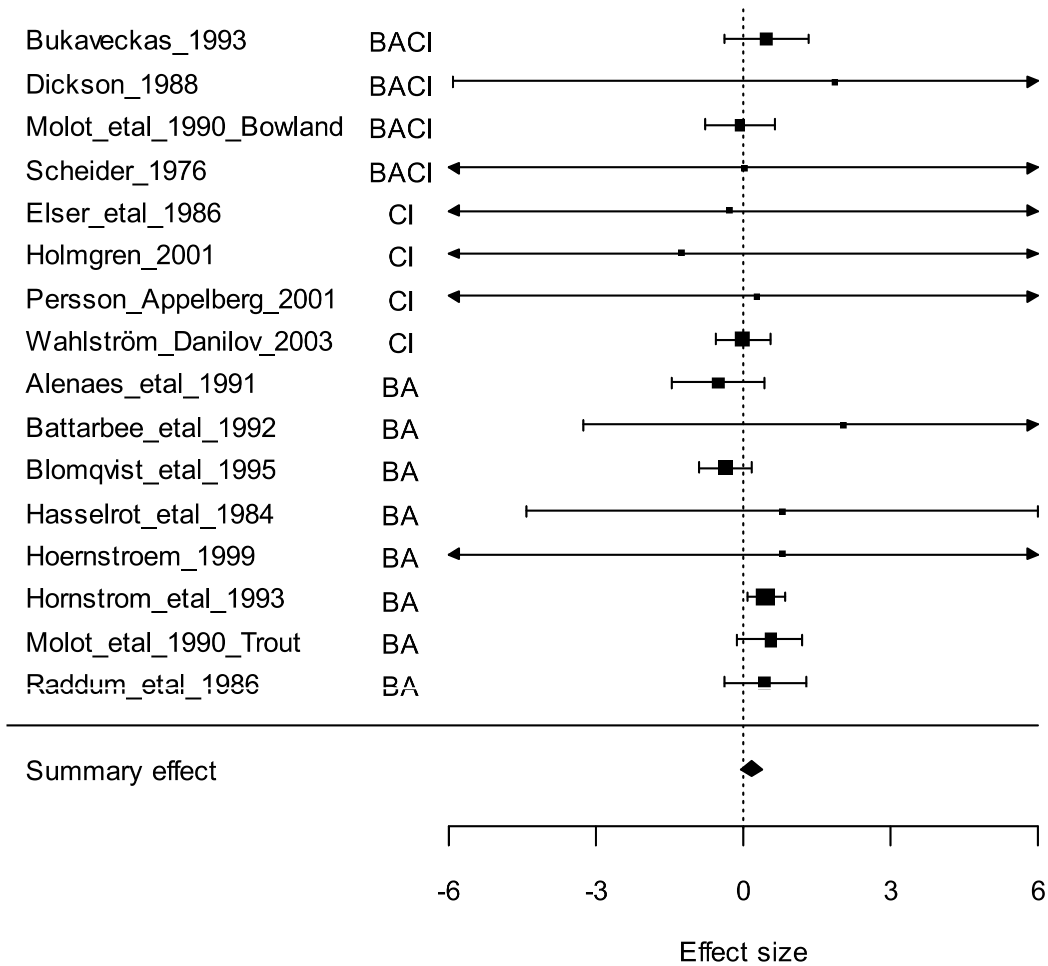

Figure 5: Forest plot of the phytoplankton abundance. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean.

Unlike for zooplankton, the phytoplankton richness did not vary significantly between studies within the meta-analysis. However, the variability between studies is tested in comparison to the within study variability (confidence interval) and it was only possible to calculate the confidence interval in the effect size for a minority of studies. In the other studies the interval was assumed to be larger than any of the calculated intervals. Hence, as the confidence intervals of the effect size could not be accurately calculated, despite the study effect sizes ranging from negative effects to positive effects, it is not possible to ascertain if these differences are due to errors in the estimates of the effect sizes or due to true variation between lakes. The variability between studies was not explained by the pH of the lakes after liming nor the study design (meta-analysis with pH and study design as moderators pH: z= 0.08, p=0.94; design: z= -0.31, p=0.76).

As for phytoplankton richness, the effect size for the abundance of phytoplankton did not vary significantly between studies within the meta-analysis (Q=8.81, df=14, p=0.84). A larger proportion of the confidence intervals of the study effect sizes could be calculated for phytoplankton abundance; more studies had sufficient replication and reporting, although six did not. However, as with the other outcome measure looked at, the majority of studies on phytoplankton did not contain both baseline and control data.

4.5 Benthic invertebrates

Twenty seven articles looked at the impact of liming on benthic invertebrates. Only fourteen of the articles presented numerical data on the impact of liming on the abundance or taxonomic richness of benthic invertebrates, a further six articles presented statements about the impacts (Appendix F). Only in seven articles were no data extractable or they presented data from the same study as another article (Appendix F, Table F.2).

As with zooplankton and phytoplankton there was no consistent positive or negative impact of liming on benthic invertebrate abundance. There was a large range of impacts from negative to positive and the mean impact was not significantly different from zero (meta-analysis with a random effects model: mean= -0.14 (confidence interval -1.25 – 0.88, z=-0.26, p=0.79) (Figure 6). The impact of liming on the taxonomic richness of benthic invertebrates was less variable that the impact on abundance. However, it still ranged from positive to negative. Unlike for zooplankton and phytoplankton the mean species richness did not significantly increase with liming (meta-analysis with a random effects model: mean= 0.05, (confidence interval -0.30 – 0.41), z=0.30, p=0.76) (figure 6).

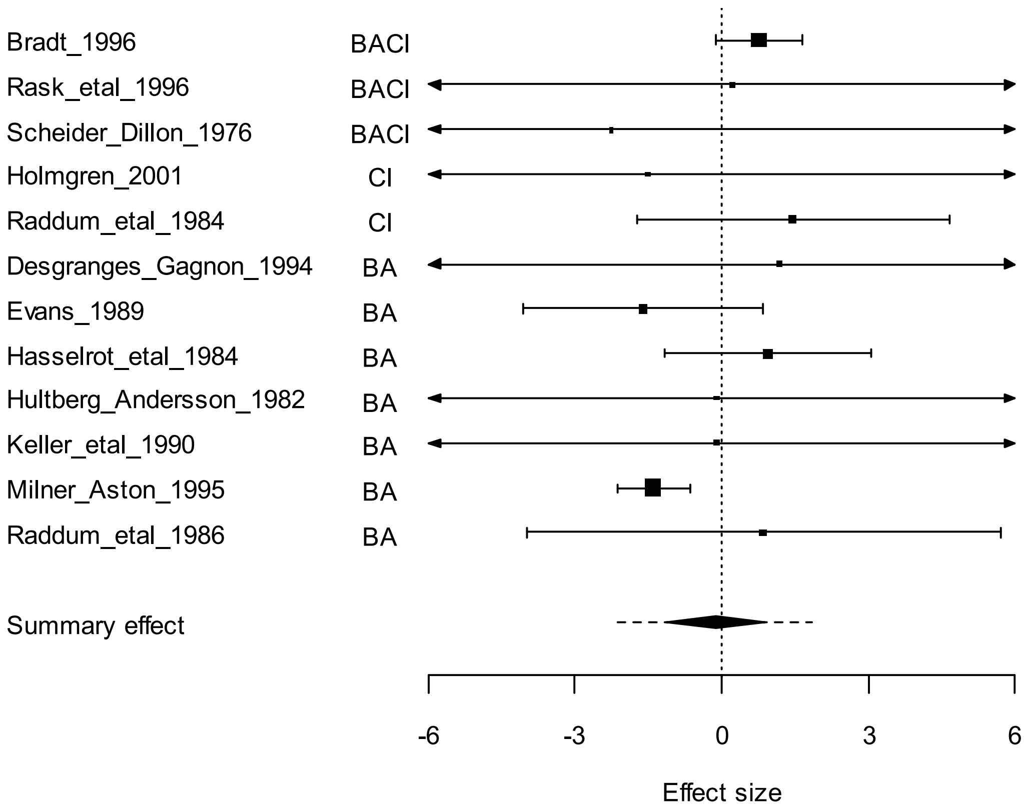

Figure 6: Forest plot of the benthic invertebrate abundance. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean.

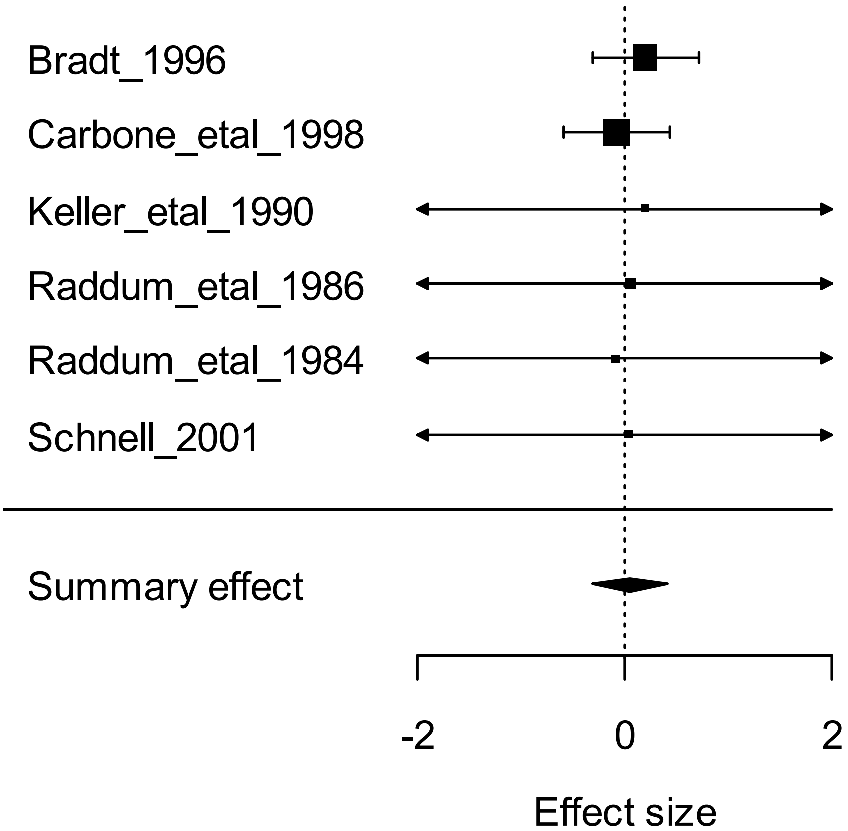

Figure 7: Forest plot of the benthic invertebrate taxonomic richness. Squares: effect size for each study, error bars: confidence interval for each study (arrows indicate where insufficient data were presented in the study to calculate a confidence interval). Summary effect; diamond: weighted mean calculated from the random effects meta-analysis, diamond width: proportional to the estimation in the error of the mean.

4.6 Macrophytes

The impact of liming on macrophytes was looked at in 13 articles (Appendix G); of which two covered data reported in another article and one was on liming in pots not a whole lake, leaving 10 articles (Table 3). The majority of studies described the changes in species and abundances descriptively. The most commonly mentioned macrophytes were *Sphagnum* species and *Juncus bulbosus.* Five out of the seven lakes that reported changes in *Sphagnum* species recorded a decrease after liming. In one article, Roelofs et al (1994), they reported little change but some patches of dead *Sphagnum* developing. However, in the other article, Roelofs et al (2002) the abundance of *Sphagnum* actually increased after liming. Roelofs et al (2002) suggest that this is due to nitrogen-saturated soil and build up of organic matter releasing nutrients after liming and increasing the levels of eutrophication. In all six studies that reported on the changes in *Juncus bulbosus*, abundance increased after liming.

Table 3: Articles that recorded the impact of liming on macrophytes.

Paper	Study design	Results	Location
Alenäs et al 1991	1 lake, before-after liming, description of changes	*Sphagnum* species decreased after liming, Mougeotia disappeared with liming	Lysevatten, Sweden
Battarbee 1992	1 lake, before-after liming, data on percentage frequency with Ekman grab sampler	Number of aquatic macrophyte species increased after liming (from 11 to 20), although the newly established taxa remained a minor component of the community. *Sphagnum auriculatum* decreased in abundance (percentage occurrence before liming 54.2%, 2 yrs after liming: 7.6%). *Juncus bulbosus* increased but not to high levels (0.9 to 3.8%)	Loch Fleet, UK
Brandrud and Roelofs 1995	Control impact study, data from 35 limed lakes and 20 acidic reference lakes	*Juncus bulbosus* more frequent in limed lakes, in 2 of 3 regions surveyed (coverage 63-73% in the limed lakes; 15-18% in the unlimed lakes, in the other area limed lake coverage 26%, acidic lake 19%). Also denser growth *J. bulbosus*, original isoetid vegetation disappeared in areas of dense population. Highest species diversity in long term limed lakes and a (re-)establishment of some acid-intolerant species such as *Myriophyllum alterniflorum* and *Potamogeton spp*.	Multiple lakes all over Norway
Dorland et al 2005	2 lakes, Before, after liming data Map of change in distribution macrophytes	Small positive trends in the number of Red List and characteristic plants species of wet heaths. No significant positive correlation between the number of plant species and years since liming in either site. Abundant growth of *Juncus bulbosus* and several acid tolerant Sphagnum species disappeared following the liming.	Bieze and Schaopedobbe , Netherlands
Grahn 1986	1 lake before and after liming	*Sphagnum* disappeared after liming, *Lobelia dortmanna* and *Isoetes lacustris* increased	Lake Gårdsjön, Sweden
Hagley etal 1996	1 lake, before and after liming	*Sphagnum platyphyllum* was completely eliminated from the lake. The charophyte, *Nitella*, that originally shared dominance in the deep littoral zone with *S. platyphyllum*, decreased in importance. Two vascular plants, *Potamogeton pusillus* and *Najas flexilis*, first found in the lake the year after liming and were abundant for 2 years after liming	Thursh lake, USA
Jackson etal 1990	1 lake, before and after liming	Extensive growths of filamentous algae were essentially eliminated within one year of liming.	Bowland lake, Canada
Roelofs eta 1994	CI study, 6 limed lakes and 5 acidic reference lakes	Limed lakes far high biomass macrophytes (1156 g dry weight per m^2 compared to 84). Largely due *Juncus bulbosus* (biomass of all other macrophytes lower in limed lakes), which dominated limed lakes (except for the most exposed shores of large lakes and the lakes limed after 1990). Acid lakes dominated by isoetid vegetation.	Various, Norawy
Roelofs etal 2002	2 lakes before and after liming	No re-establishment of soft water macrophytes occurred and the dominant Sphagnum species and *J. bulbosus* spread even further. Regular liming of nitrogen-saturated sediments with accumulation of organic matter led to mobilisation of nutrients and eutrophication of the water layer (Bellemakers et al., 1990). Liming after removal of accumulated organic, shows strong decline of *J. bulbosus* and a strong increase of soft water species	Scherpven and Padvindersven, Netherlands
Weiher et al 1994	2 lakes before and after liming	Seven new species were found after calcite addition, one of these, *Potamogeton epihydrus* became the single most abundant species. Sphagnum sp. and Utricularia geminiscapa were extirpated from the lake within 5 years of the initial treatment. Total coverage decreased from 47.62 per cent in 1981 to 22.61 per cent in 1991. The floating-leafed plants and canopy-formers increased in their total coverage and relative abundance	Cranbery pond and woods lake, USA

5. Discussion

5.1 Review limitations

As with all systematic reviews, in order to understand the answer the systematic review provides, it is important to understand the limitations of the review. The review conclusions are highly dependent on the studies found and included within review. Within the search and study selection, 43 articles were excluded as they could not be retrieved and 36 were excluded due to being in a language other than English. Resource limitations meant that more time or resources could not be provided to searching and translation. This may have created a bias within the studies included. However, despite the majority of non-English language papers coming from Scandinavia, the exclusion of Scandinavian language papers did not cause the exclusion of Scandinavian lakes from the study, the majority of lakes covered were still in Scandinavia. The potential for bias within the inclusion of studies may have impacted on the effect size calculated, if the studies that were excluded were systematically different from the included studies.

Bias within the included studies can also impact on the mean effects calculated in the meta-analysis. Quantitative data could only be extracted from a minority of the relevant studies found and only seven studies included both baseline data and control sites; increasing the chances of bias within the original data. In particular, for fish abundance the difference in the findings of the large study with only before and after data and the one smaller study with control sites makes it particularly uncertain as to whether the differences observed in the fish abundance over time are truly due to liming or other factors changing over time. Studies with only before and after data and no independent controls may have been impacted by decreases in acid rain over the same period as the liming occurred, although this is unlikely for the period covered by the study of Degerman et al. (1993). Isolating the impact of liming from decreasing acidification can be difficult but it is essential. If decreased deposition is causing recovery on its own, liming may not be necessary; liming is likely to have few long term benefits compared with natural recovery (Ormerod and Durance 2009). The lack of any studies on changes in overall fish abundance with both baseline and control data make isolating the impact of liming especially important.

Understanding and predicting the impact of any one liming operation is also limited by the variability between the studies. Despite the diversity of phytoplankton and zooplankton increasing on average, there were lakes in which it decreased. The differences could be due to variability in the nature of the lakes, the liming operations, the experimental designs or chance. If liming operations are to have greatest effect then it is useful to understand when liming will have the differing effects. As there were a limited number of studies, a restricted number of factors could be statistically investigated. Hence, the review can only provide limited information on what causes the observed variations in effect.

The other main limitation of the review was the limited question covered; "What is the impact of 'liming' lakes on the abundance and diversity of lake biota?" A limited question was used as it allowed the rigorous collection of all data on the topic and the statistical analysis of these data. However, the impact of liming on a lake's ecosystem can be wider and more complex than simply changing the abundance or richness of species. Richness and abundance do not provide a complete assessment of an ecosystems health, the functioning of the ecosystem is also important. There is no simple measure of ecosystem function; however, assessing the impact on all different parts of the ecosystem can provide a greater indication of the overall impact of liming on the ecosystem. Additionally, the decision of "should we lime?" will be based on a wider range of implications; including, but not limited to, the impact of liming on non-target habitats (including terrestrial habitats if catchment liming is implemented, Shore and Mackenzie 1993), the cost of liming (Navrud 2001) and the political and social reasons for liming (Clair and Hindar 2005).

6. Reviewers' Conclusions

6.1 Implications for policy

The available studies and meta-analyses suggest that on average liming increases the diversity of fish, zooplankton and phytoplankton, whereas the diversity of benthic organisms is not increased. The diversity of zooplankton and phytoplankton is estimated by the meta-analysis to decrease in some lakes but only in a small minority. The fish diversity in some lakes was increased due to stocking of extinct species, however, naturally re-colonisation occurred as well (Appelberg 1998). Increasing and preserving the diversity of organisms present in an ecosystem is generally considered a favourable ecological outcome, especially if it is across a broad spectrum of the ecosystem. Hence, in this regard liming of lakes can be considered, on average, an effective conservation measure.

The impact of liming on abundance is less clear cut. The largest study of changes in fish abundance (which covered 103 lakes) showed an increase in abundance over time (Degerman, et al. 1992) but the only study looking at all species which had control sites showed a decrease in abundance in limed site in comparison to the controls (Holmgren, 2001). Suggesting that despite fish abundance increasing over time there may be no difference in the abundance of fish in limed lakes compared with the baseline trend due to decrease in acid deposition. The only study with both control and baseline data for fish abundance, only looked at perch (Eriksson and Tengelin 1987). However, it found an increase in limed lakes even when differences in the control lakes were taken into account. Suggesting that the increase in abundance detected in this large scale study may well be due to liming rather than other factors changing over the same time period. However, the lack of studies on other species with both baseline and control sites prevents any generalisation.

The evidence of effect on the abundance of zooplankton, phytoplankton, and benthic invertebrates indicates they do not increase with liming. The effect sizes reported varied from positive to negative and there was no consistent effect across studies. Either liming has no impact on abundance and abundance is just naturally varying, or liming has a very variable impact depending on other factors. The pH of the lake after liming did not significantly explain the variation.

Liming has also been used to restore fish abundances by providing conditions for the survival of stocked fish. In general the liming appears to have enabled the restocking of fish in some instances. However, many studies did not actually test if fish would have survived before liming or stock fish in control sites. Hence, the fish may have been able to survive even without liming. Only one study stocked fish in acid lakes alongside the stocking in the limed lakes (Hesthagen, et al. 2001). Within that study the stocked perch did survive and reproduce within in one of the acid lakes, although to a lesser extent than in the limed lake, suggesting that liming may increase the chances of stocking being successful and restoring fish to a lake. Although stocking only 'restores' lakes if the lakes would naturally contain the stocked fish, in some studies there is a suggestion that this was not the case, as the stocked fish had not previously been recorded as reproducing in the lake (Svensson, et al. 1995, Traaen et al. 1997).

The combination of the abundance of organisms not changing and the species richness increasing suggests that liming could have positive ecological implications. However, as with the stocking, it is still important to assess whether the additional species would have naturally occurred in the lakes prior to anthropogenic acidification. In Europe water bodies are now assessed with reference to the European water framework directive, which requires waterbodies to achieve 'good ecological status' in respect to specified reference conditions (EU 2000). The effectiveness of liming as a policy intervention will depend on whether the observed changes can be interpreted as positive in the context of 'good ecological status'. In the past liming has potentially occurred in lakes that were naturally acidic (Norberg et al 2008). Additionally, the fact that liming and then stocking occurred in lakes which historically may never have had reproducing populations of the stocked fish, suggest that liming has not always been carried out to 'restore' lakes to their 'natural' or historical condition.

In predicting the probable impact of liming on lake biota it is important to consider what would happen without liming or if liming is removed. In recent years acidic depositions have been decreasing (Evans et al 2001) and there are some signs of lake recovery (Kernan et al. 2010). It is suggested that both naturally recovering lakes and limed lakes are converging on the conditions of circum-neutral lakes, in terms of univariant measure (such as species richness or abundance) (Angeler and Goedkoop 2010). Liming may speed up this recovery, although, associations between functional feeding groups indicated less connectivity and food web complexity in limed lakes relative to the other lake types possibly due to disturbance suggesting liming may not actually speed recovery (Angeler and Goedkoop 2010). There are also still areas where the acid load outstrips the soils neutralizing capacity (Matejko et al. 2009). In these areas chemical recovery will not take place on its own and liming may protect the ecosystem until depositions can be reduced further. Although, if acid load is still outstripping the soils neutralising capacity liming would have to be periodically repeated (Claire and Hindar 2005), as a single liming only has an impact for a limited time period, dependent on the retention time of the lake (Donnelly et al. 2003).

6.2 Implication for research

Future study designs need to be more powerful and at a minimum need to contain both baseline and control data. In particular the lack of control data for fish abundance means there is uncertainty in the effect of liming. Future research including both baseline data and controls sites is needed to isolate the effect of liming.

Secondly, the recorded impact of liming was variable depending on the study, and despite the average impact of liming being to increase phytoplankton and zooplankton species richness, in a minority of lakes they are estimated to decline. Hence, more research is needed on the reasons for this variability; there were too few studies with sufficiently powerful designs to fully investigate the reasons for variability in impact.

Thirdly, the abundance and species richness are crude measures of an ecosystem's health. Hence, there needs to be more research using outcome measures more appropriate to policy objectives, especially those linked to the Water Framework Directive, alongside ongoing long term monitoring.

7. Potential Conflicts of Interest and Sources of Support

None identified. The project is funded by the Natural Environment Research Committee UK (NERC).

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9. Appendices

See Supplementary materials