

Akademisk avhandling för filosofie doktorsexamen
Thesis for the Degree of Doctor of Philosophy

Paleolimnological Reconstructions of Fish Population Changes in Acidified Lakes

Fredrik Palm

University of Gothenburg
Faculty of Science



UNIVERSITY OF GOTHENBURG

Department of Zoology
Animal Ecology
Box 463, SE-405 30 Gothenburg
SWEDEN

The oral defense of this thesis will take place at 10:00 am on Friday March 25th 2011 at the Department of Zoology, Medicinargatan 18, Göteborg, Sweden. The opponent is Professor John P. Smol from Queen's University, Department of Biology, Kingston, Ontario, Canada.

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Fredrik Palm

Department of Zoology, Animal Ecology

University of Gothenburg

Box 463, SE-40530 Göteborg

Sweden

E-mail: fredrik.palm@zool.gu.se

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Cover picture: Sunrise in sediment core, by Fredrik Palm

ISBN 978-91-628-8238-9

This thesis is available online at: <http://hdl.handle.net/2077/24148>

Printed by GESON SVERIGE AB, Göteborg, Sweden



The wonder of the world,
The beauty and the power,
The shapes of things,
Their colours, lights and shades,
These I saw.

Look ye also while life lasts.

/ Denys Watkins-Pitchford

In memory of
Jan A. E. Stenson (06/03/1942 – 23/10/2006)

Paleolimnological Reconstructions of Fish Population Changes in Acidified Lakes

Fredrik Palm, 2011

ABSTRACT

This thesis deals with the potential of using subfossil *Chaoborus* spp. and *Daphnia* spp. remains preserved in lake sediments to reveal past fish community alterations related to progressive acidification. Sediment cores were collected from acidified lakes with known present fish population status and well-documented fish community developments in southwest Sweden. Sediment analyses were conducted on comparatively large sediment samples from sediment cores that were sectioned for a high temporal resolution (5-mm intervals).

Subfossil *Chaoborus* assemblages were related to fish population developments in the study lakes. Mandibles of *Chaoborus flavicans* were common in most sediment samples, and this species is known to co-exist with fish. However, *C. obscuripes* mandibles were exclusively recovered and identified in more recent sediments from fish-free lake periods, as judged from known historical fish population changes in the study lakes. These findings suggest that past fish extirpations can be revealed by the appearance and successive presence of *C. obscuripes* mandibles in sediment records. Progressive acidification was additionally confirmed by the loss of acid-sensitive daphnid zooplankton, as indicated by developments in sedimentary assemblages of *Daphnia ephippia* (resting eggs).

In this thesis I describe and test the first ever paleo-method for revealing periods of cyprinid presence in lake histories, without including fish fossils in the analyses. Cyprinid fish species are characterized by pharyngeal teeth that are used to crush and fragment food items. Cyprinid fish, in this thesis represented by roach (*Rutilus rutilus* L.), were shown to evacuate significantly higher proportions of fragmented *Chaoborus* mandibles than non-cyprinid perch individuals (*Perca fluviatilis* L.), when fed live *Chaoborus* larvae in the laboratory. Similar and significant differences in proportions of fragmented subfossil *Chaoborus* mandibles were also shown for three independent paleolimnological approaches; (1) a comparative study of surface sediments from lakes with and without cyprinid fish, (2) a stratigraphical analysis of a dated sediment core from a lake with a known period of roach presence, and (3) a comparison between two dated sediment cores from lakes that differed in historical presence of roach.

These findings have important implications for future freshwater management by providing tools for assessing past fish population changes in acidified lakes with uncertain developmental histories.

Keywords: Acidification, *Chaoborus*, *C. flavicans*, *C. obscuripes*, cyprinids, *Daphnia*, fish loss, fish community composition, ephippia, fragmentation, mandibles, perch, roach, sediments, ²¹⁰Pb-chronology

ISBN 978-91-628-8238-9. ELECTRONIC VERSION: <http://hdl.handle.net/2077/24148>

Sedimentära rekonstruktioner av förändrade fisksamhällen i försurade sjöar

Fredrik Palm, 2011

POPULÄRVETENSKAPLIG SAMMANFATTNING

Fisk utgör en viktig del av det biologiska livet i våra sjöar och vattendrag. Vi vet idag att många fisksamhällen har förändrats drastiskt till följd av den svåra sjöförsurning som uppmärksammades som ett allvarligt miljöproblem under senare delen av 1960-talet. Eftersom många sjöar inte provfiskades förrän efter det att försurningen var ett faktum (om de provfiskats alls), så har vi i dagsläget dålig kännedom om hur deras fisksamhällen har förändrats under försurningsutvecklingen.

Denna avhandling utreder möjligheten att använda sedimentära kvarlevor från tofsmygglarver (*Chaoborus* spp.) och vissa hinnkräftor (*Daphnia* spp.) för att påvisa försurningsutveckling och förändringar i sjöars fisksamhällen. För att kunna kontrollera hur väl dessa indikatorer fungerar så har sedimentprover samlats in från ett antal försurade sjöar där vi har god kännedom om fisksamhällenas nuvarande status och deras historiska förändringar.

Analys av mandibler (mundelar) från tofsmygglarver visade att dessa kan användas för att påvisa fiskförkluster i kraftigt försurade sjöar. Mandibler från arten *Chaoborus flavicans* var vanligt förekommande i sedimenten. Denna art kan finnas både i fisktomma sjöar och tillsammans med fisk. Däremot påträffades mandibler från arten *C. obscuripes* bara i mer nutida sediment från fiskfria perioder. Mandibler från *C. obscuripes* kan därför användas till att visa om en sjö har förlorat all fisk till följd av svår sjöförsurning och om man analyserar äldre bestämda sediment finns även möjligheten att få reda på när fiskdöden inträffade. Försurningsutvecklingen var även relaterad till en successivt minskad förekomst av vilägg (ephippior) från försurningskänsliga hinnkräftor av släktet *Daphnia*. Dessa sedimentära fynd försvann helt från sedimenten efter 1950-talet, vilket stämmer väl med försurningsutvecklingen i de sjöar där detta analyserats.

I denna avhandling beskrivs en helt ny sedimentbaserad metod för att visa om fisksamhällets artsammansättning har förändrats till följd av att försurningskänsliga fiskarter, exempelvis karpfiskar (cyprinider), har försvunnit. Cyprinider har svalgbenständer som krossar och fragmenterar födan och laboratorieförsök visade att mört (*Rutilus rutilus* L.), en vanligt förekommande cyprinid, fragmenterade *Chaoborus* mandibler i högre utsträckning än abborre (*Perca fluviatilis* L.). Andelen fragmenterade *Chaoborus* mandibler var också högre i sedimentprover som härrörde från sjöar och sjöperioder där mörten förekommit. Denna metod gör det därför möjligt att i efterhand ta reda på om fisksamhällets artsammansättning har förändrats historiskt utan att behöva leta efter artspecifika och ytterst sällsynta kvarlevor från fiskar i sedimenten.

Denna avhandling uppmärksammar betydelsen av sjöars utvecklingshistoria inom modern vattenvård och bidrar med förslag på unika metoder som kan öka vår kunskap om historiska fiskförändringar i försurade sjöar.

ISBN 978-91-628-8238-9. ELECTRONIC VERSION: <http://hdl.handle.net/2077/24148>

LIST OF PAPERS

This thesis is a summary of the following papers, which are referred to by their roman numerals:

- I Palm, F., Stenson, J. A. E., Lagergren R., 2005. Which paleolimnological zooplankton records can indicate changes in planktivorous fish predation? Verh. Internat. Verein. Limnol. **29**: 661-666.
- II Palm, F., Svensson, J-E., 2010. Subfossil *Chaoborus* mandibles confirm historical fish decline in two acidified lakes, SW Sweden. Journal of Fundamental and Applied Limnology **177**: 313-320.
- III Palm, F., El-Daoushy, F., Svensson, J-E., 2011. Fragmented subfossil *Chaoborus* mandibles reveal periods of cyprinid presence in lake histories. Journal of Paleolimnology **45**: 101-113.
- IV Palm, F., El-Daoushy, F., Svensson, J-E. Development of subfossil *Daphnia* and *Chaoborus* assemblages in relation to progressive acidification and fish community alterations in SW Sweden. *Manuscript*.

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Paper III by Springer Science+Business Media B.V.

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INTRODUCTION

Change can only be appreciated by comparison between past and more recent conditions. Consequently, there is an inherent dimension of time in all changes. This is also most certainly true in freshwater management. The development of our modern industrial society has invoked many stressors on freshwater resources, and freshwater ecosystems have similarly developed in response to human activities.

This thesis focuses on fish community developments related to anthropogenic acidification. The studies presented show the possibilities of reconstructing certain aspects of fish population changes. These attempts utilize remnant body parts of certain midges and zooplankton stored in sediments, in which sediment depth represents the necessary dimension of time needed to estimate change or development. This thesis should thus not be viewed purely as an account of describing progressive acidification in Scandinavia. More importantly, the presented studies may be interpreted as efforts to derive general methods for revealing past fish extirpations and historical changes in fish community composition, where the sampled acidified lakes serve as the arena for assessing these possibilities.

Acidification history and effects on freshwater fish populations

The atmospheric deposition of acid substances (Box 1.) has led to drastic changes in the Scandinavian fish fauna and, although acidification was not acknowledged as a large scale environmental problem until the 1960's, some of the negative effects following acidification have been known since the beginning of the twentieth century. Scandinavian studies on brown trout populations identified relations between increased acidity and fish mortality (Dahl 1920; 1927), which were later emphasized by observations of successive fish losses in acidified lakes (*e.g.* Hultberg and Stenson 1970; Almer et al. 1972; Jensen and Snekvik 1972). Additional lake inventories confirmed acidification as a major threat to fresh water ecosystems in Sweden (Hörnström et al. 1973, Almer et al. 1974; Almer and Hanson 1980), initiating both liming projects and research on ecological- and biochemical mechanisms in these disturbed environments (*e.g.* Andersson and Olsson 1985).

Acidic conditions can influence different life-stages in fish, with important consequences for intra- and interspecific dynamics. The main effects of acidification

Box 1. Acid Deposition

Anthropogenic acidification (increased acidity of waters due to human activities) is mainly caused by emissions from combustion of fossil fuels and other industrial processes. These emissions reach land and water through atmospheric acid deposition (acid rain). The main chemical precursors for acidic conditions are atmospheric concentrations of sulphur dioxide (SO₂) and nitrogen oxides (NO_x), that in reaction with water, oxygen, carbon dioxide, and sunlight, form sulphuric (H₂SO₄) and nitric acids (HNO₃) – the primary agents of acid deposition. The effects of acid deposition on lake biota is further influenced by land-use activities (e.g. forestry) and despite decreases in emissions (especially in SO₂), a large number of lakes in southwest Sweden are still severely acidified.

on fish is reproductive impairment, resulting in reduced recruitment (Johansson and Milbrink 1976; Runn et al. 1977), while juvenile fish might experience gill dysfunction in relation to elevated levels of soluble aluminium and other metals (Norrgrén et al. 1991; Norrgrén and Degerman 1993; Gensemer and Playle 1999). Acid sensitive fish populations thus consist mainly of older fish individuals even at early stages of progressive acidification, and as old individuals die, these species are lost. One such acid sensitive fish species is roach (*Rutilus rutilus* L.), which commonly displays loss of juveniles earlier than more acid tolerant species, e.g. northern pike (*Esox lucius* L.) and Eurasian perch (*Perca fluviatilis* L., Almer et al. 1974). The loss of acid sensitive juvenile fish can release older cohorts from intraspecific competition, whereas the successional loss of sensitive fish species can induce interspecific shifts in competition, favouring tolerant fish species that may display improved recruitment, abundance, and specific growth rate (Muniz 1984; Appelberg et al. 1992). However, even the most tolerant fish species suffer reduced recruitment and metal toxicity during severe acidification. Thus, in severely acidified lakes all fish are extirpated – resulting in fishless lakes.

Countermeasures and lack of historical controls

Liming is nowadays the most prevalent action to mitigate lake acidification nowadays. The Swedish liming programme was initiated in 1976. This programme included more than 7000 lakes during the 1990s, wherein approximately 200 million tonnes of lime were spread each year (Svenson et al. 1995). Several positive effects can follow lime treatment, especially regarding the survival of acid sensitive species

and life stages, as many chemical properties are rapidly improved and may remain adequate by repeated treatments. Such conditions have also been achieved in many lakes (Skjelkvåle et al. 2003; SEPA 2007). However, chemical restoration does not necessarily equal biological restoration (Henrikson and Brodin 1995). An immediate return of biological communities in close resemblance with pre-acidification status implies that affected species are still present to repopulate limed lakes. If not present, extirpated species need to recolonize lime treated habitats. Recolonization will probably be quite unproblematic for zooplankton with dormant eggs and invertebrates with airborne adult stages (Degerman et al. 1995), but the re-establishment of some species might be stalled by developed functional shifts in competition and predation (Stenson and Svensson 1994). Fish in Sweden do not fly, and they will instead depend upon existing adjacent populations for possible reinvasions of limed lakes (Ragnarsson 2008).

Although successive loss of fish species is regarded a typical consequence of severe lake acidification (Appelberg et al. 1992), detailed information on past fish populations is very limited in terms of where and when fish became extirpated. It is likely that many acidified lakes have lost acid-sensitive fish species while retaining viable populations of more tolerant species. Furthermore, some severely acidified lakes may have lost all fish, while other lakes historically never harboured fish. Hence, there is a need for methods that can be used to describe past fish communities and identify presently fishless lakes, especially as restoration goals preferably should relate to past conditions (Norberg et al. 2008).

Since lake histories are archived in lake sediments (Smol 2008), paleolimnological approaches, *i.e.* methods based on investigations of sedimentary deposits, could prove prosperous in revealing these historical insights.

Paleolimnology – *the limnologist's time machine*

Lake sediments accumulate every day - all year round - and are made up of autochthonous matter (produced within the lake), as well as allochthonous materials and substances (derived from the surrounding catchment and atmosphere). Lake sediments can be viewed as a potential historical record of overall lake development, as they continuously integrate a substantial part of everything produced within (or distributed to) the lake catchment during accumulation (Smol 1992). Since sediments form by accumulating onto already existing sediments, intact sediment archives will

include an inherited time-line of events, consisting of old sediments in deeper parts with progressively younger sediments towards the sediment surface (Fig. 1).

Multidisciplinary studies of physical, chemical, and biological information preserved in lake sediments form the scientific field of paleolimnology, where *limnology* is freshwater ecology, with the prefix 'paleo' originating from the Greek word *palaios* meaning *old* or *ancient*. The physical structure and chemical properties of lake sediments can provide information on factors influencing sedimentation, different sources of materials, and past geochemical conditions (Frey, 1964; Last and Smol 2001). The biological information is usually derived through analysis of algal- or animal remains (see Box 2., Smol 2001a; b), but certain chemical properties in sediments can also be used to trace biological changes (e.g. nitrogen isotope concentrations related to salmon population dynamics, Gregory- Eaves et al. 2009).

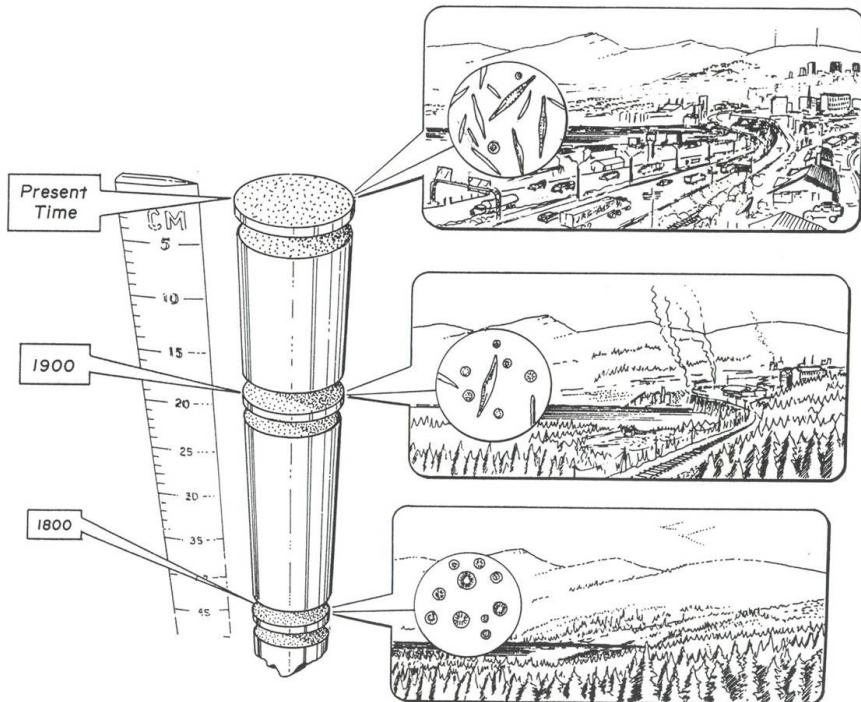


Figure 1. The representation of different subfossil assemblages (e.g. diatoms) at various sediment depths in relation to the development of the modern industrial society. From Smol (2008); used with kind permission from Wiley-Blackwell.

Box 2. Animal Remains in Sediments

Animal remains preserved in lake sediments are fairly diverse and constitute a major part of the functional groups occurring in inland waters. These remains are not truly fossilized and commonly referred to as subfossil remains. In some cases most of the external animal morphology is preserved, but often only parts of the original animal can be recovered. Common subfossil remains of zooplankton are carapaces and head shields of *Bosmina* spp. and resting eggs (ephippia) of *Daphnia* and *Ceriodaphnia* spp., while large predatory zooplankton, such as *Leptodora* and *Bythotrephes* spp., can be detected by subfossil caudal spines. Midge larvae are represented by chironomid head capsules, whereas mouthparts (mandibles) of phantom midge larvae (*Chaoborus* spp.) and other invertebrate larvae (e.g. ephemeropterans) can be common in sediments. Less common are remains from fishes which can potentially be identified by sedimentary scales, vertebrae, teeth, and otoliths. Examples of different subfossil remains are shown in Fig. 5.

In other words, paleolimnology has the potential to address a wide range of crucial questions concerning the whole complex of lake ecological interrelationships and to test hypotheses regarding environmental impacts in freshwater ecosystems, due to the unique temporal perspective offered by sediment archives (Birks 1998; Battarbee 1999).

Sediments normally accumulate quite steadily, influenced mainly by seasonal changes in production and water currents. However, formed sediments do not generally rest passively at the lake bottom disconnected from the water column. Instead, sediments constitute an important component of the total lake production, including several important chemical and biological processes that can affect sediment characteristics after sedimentation (Svensson and Leonardson 1996). Also processes of bioturbation, e.g. the activities of burrowing invertebrate larval stages and foraging fish, may distort and mix sediment after accumulation and thus influence the temporal accuracy of sediment archives. These effects can be reduced, but never totally avoided, by collecting sediments in areas with minimal sediment disturbances, such as profundal sediments in the deepest part of lakes.

Since all sediment archives are more or less disturbed, it is important that we try to isolate possible discrepancies. Fortunately, this can be assessed when constructing sediment chronologies through the process of dating sediments.

Sediment dating

Establishing an adequate sediment chronology is often of major importance when interpreting sediment archives. By doing so, the inferred sedimentary changes can be chronologically aligned to lake development and enables researchers to check if known events in lake histories are represented by related changes in sediment profiles.

There are several more or less indirect ways to construct sediment chronologies, where specific event-horizons can be dated by detecting representative changes in sediment profiles. For example, combustion of fossil fuels is known to have resulted in distinguishable increases of carbonaceous fly-ash particles and the resulting appearance and/or peak concentrations of sedimentary soot particles may thus identify the sediment depths corresponding to certain periods in the development of fossil fuel burning (Renberg and Wik 1984; 1985). Another example is the analysis of sedimentary pollen grains, in which known land-use changes (*e.g.* agricultural settlements) can be traced by species-specific changes in sedimentary pollen occurrences (Blais et al. 1995; Schmidt et al. 2002). However, many of these chronologies are quite crude and are not likely to yield sufficient resolution on shorter time scales.

The most common dating procedures of sub-recent lake histories (~ 150 years) are instead based on radio-isotopic techniques utilizing activity measurements of lead-210 (^{210}Pb) and other radionuclides (Appleby 2001; El-Daoushy et al. 2001). ^{210}Pb is part of the uranium-238 (^{238}U) decay series, with a half-life of 22.26 years. It is readily incorporated in organic materials by chelation reactions or scavenged by particulate matter through adsorption in lakes (Oldfield and Appleby 1984; El-Daoushy 1988). ^{210}Pb in aquatic deposits may originate from external inputs as atmospheric fallout or in-wash from lake catchments and is then referred to as unsupported ^{210}Pb . In sediment dating, it is the activity of the unsupported ^{210}Pb that is of major interest, since this activity will solely be a function of the age of the sediment, the amount initially present and its half-life. Hence, the down-core activity of unsupported ^{210}Pb can be used for age determinations that are based on model scenarios yielding estimates of the time elapsed since respective sediment intervals were formed. However, ^{210}Pb can also be derived from the *in-situ* decay of radium-226 (^{226}Ra). This part constitutes the supported ^{210}Pb , which will usually exist in radioactive equilibrium with ^{226}Ra . The unsupported ^{210}Pb can not be measured directly, and is instead derived by subtracting the supported ^{210}Pb activity (based on ^{226}Ra

measurements) from the total ^{210}Pb activity (El-Daoushy 1986; Appleby 2001). This is also what limits how far back in time sediments can be dated, since unsupported ^{210}Pb will be zero as soon as the total ^{210}Pb reaches levels of the supported ^{210}Pb activity. The sediment interval in which this happens constitutes the *dating horizon*. The age of sediment intervals below this sediment depth must be extrapolated from the ^{210}Pb -chronology or dated with some additional method (see illustration in Fig. 2).

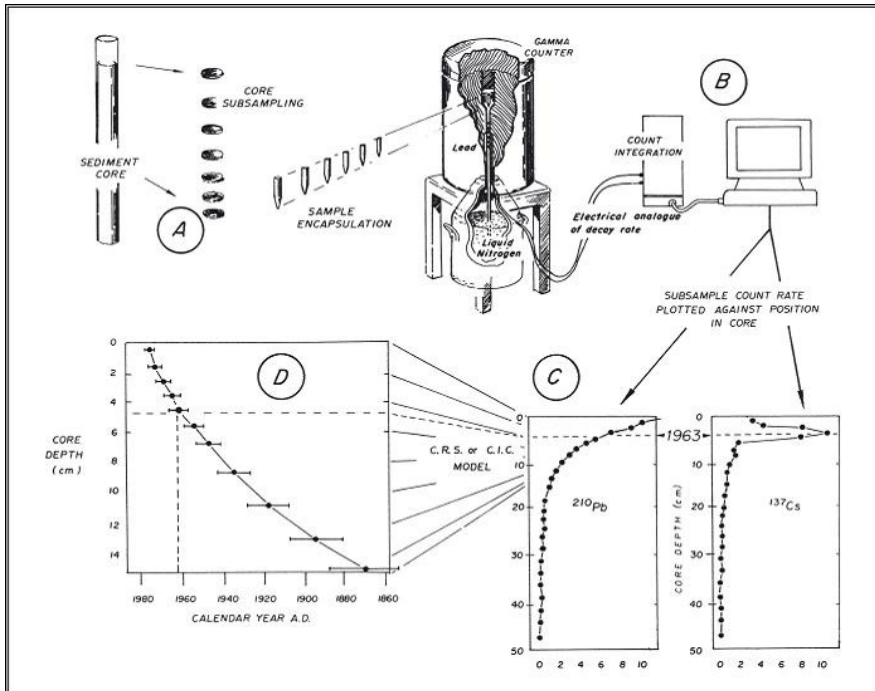


Figure 2. A schematic illustration of the major steps involved in establishing ^{210}Pb -chronologies for lake sediments (hypothetical example). (A) Collected sediment core sectioned in adequate intervals (e.g. 1 cm sections) for the study in question. (B) ^{210}Pb and additional radioisotopes are counted using a low-background gamma counter. (C) The activities of ^{210}Pb and ^{137}Cs plotted against depth in core. (D) The resulting age-depth relationship for the sediment core is usually based on calculations using either a Constant Rate of Supply (CRS) model, or a model based on Constant Initial Concentration (CIC). Age determinations cannot be made accurately for depths below the level of detection for ^{210}Pb (dating horizon) – in this example, for sediments below 15 cm depth. The age of deeper sediments need to be extrapolated. The dotted line shows the placing of the ^{137}Cs peak on the ^{210}Pb -chronology, revealing the match with the ^{210}Pb -estimated date of 1963. Error bars are standard deviations. From Smol (2008); used with kind permission from Wiley-Blackwell.

There are two common models for calculating sediment dates from the profile of unsupported ^{210}Pb ; the CRS (Constant Rate of ^{210}Pb Supply) model, and the CIC (Constant Initial Concentration) model (Robbins 1978). The CRS-model is the most widely used and has been shown to provide adequate chronologies in good agreement with annually laminated sediments (Appleby et al. 1979) and well preserved peat deposits (El-Daoushy et al. 1982).

It is important to remember, however, that all chronological estimates are modelled, and as all models, their reliability needs to be validated. Such model validation is usually based on the agreement between modelled chronology estimates and some other independently derived dates (Fig. 2). Here, the anthropogenic radionuclides of cesium-137 (^{137}Cs) and americium-241 (^{241}Am) may provide independent dates for model validation (Appleby 2001; El-Daoushy et al. 2001). ^{137}Cs and ^{241}Am have been spread worldwide following the atmospheric testing of nuclear weapons in early 1960s, and ^{137}Cs was additionally released in high concentrations during the Chernobyl accident in 1986. Since both of these historical events represent relatively short-termed episodes, sediments representing the years of 1961/63 could potentially be indicated by peak concentrations in both ^{137}Cs and ^{241}Am , while sediments corresponding to the Chernobyl accident in 1986 would be indicated by a more recent second peak in ^{137}Cs concentrations. In addition, some studies suggest that ^{241}Am may be less mobile in sediments (e.g. Appleby et al. 1991; El-Daoushy et al. 2001), and could thus prove important means of ^{210}Pb model validation if ^{137}Cs detections should fail (e.g. Davis et al. 1984).

Tracking past fish developments in acidified lakes

The most obvious way to trace alterations in past fish communities would be by analyzing remains of fishes in sedimentary deposits. This strategy is, however, of limited use since preserved remains of fishes are usually too rare for adequate inferences of past fish alterations (Frey 1964; Patterson and Smith 2001; Davidson et al. 2003). Instead, past fish developments are usually indirectly revealed by related changes in subfossil assemblages of invertebrate prey organisms (reviewed and discussed in paper I). Nevertheless, many indirect reconstructions could suffer additional predicaments when applied to acidified systems, as invertebrate prey species may themselves be acid-sensitive and disappear from sediment records during progressive acidification. One group of organisms that tolerate acid lake

conditions and deposit identifiable subfossil remains in lake sediments are aquatic larvae of phantom midges (Frey 1964; Nilssen 1974). These species not only endure severe acid stress but their abundance and species structure is usually also intimately related to fish dynamics (Pope et al. 1973; Stenson 1978). Subfossil remains of phantom midge larvae thus appear particularly suitable for studying fish community alterations in acidified lakes.

Ecology of phantom midges, *Chaoborus* spp. (Diptera: Chaoboridae)

In this thesis, *Chaoborus* refers to a genus of phantom midges, while a particular species is presented with an additional species-specific epithet (e.g. *Chaoborus flavicans*; or *C. flavicans*). This also applies to cladocerans of the genus *Daphnia*.

Larvae of phantom midges are common inhabitants of most freshwater systems. It is only the larval stages that are aquatic, as all Dipterans (*Di* = two, *ptera* = wing) have a flying adult life-stage in which reproduction occurs. Oviposition occurs soon after emergence and eggs may be deposited on littoral vegetation, filamentous algae or directly on the water surface, depending on species. *Chaoborus flavicans*, the most common species in Swedish lakes, produce a jelly that surrounds the eggs and prevent them from sinking, whereas some species produce sinking eggs (e.g. *C. punctipennis*). Embryonic development is faster in warmer water but larvae usually hatch within a few days (Saether 1997). Chaoborid larvae pass through four developmental stages (instars), where each instar is separated by moulting of the chitinous exoskeleton which allows individual growth. The first and second instars are relatively short (i.e. few days/weeks), but instar duration is progressively longer, and the third instar may last for months depending on water temperature and food availability (Snell and Hazeltine 1963; Yan et al. 1982; Moore 1986). By late autumn *Chaoborus* larvae reach the final larval stage and spend the winter as instar IV, until pupation and adult emergence in late spring or early summer. All instars are omnivorous and tactile ambush-predators attacking prey that come within striking distance. As such, chaoborid larvae have been shown to attack prey in relation to the relative abundance of prey species and prey swimming speeds (Swift and Fedorenko 1975; Moore 1988). However, predation by *Chaoborus* larvae is also limited by prey handling abilities, which likely explains some of the suggested prey preferences (Sprules 1972; Fedorenko 1975a, b; Swift and Fedorenko 1975). Early instars feed primarily on rotifers, copepod nauplii, and large flagellated phytoplankton (Moore 1988; Moore et al. 1994), while third and fourth instar larvae are less size-limited and

consume a wider variety of prey, consisting of large rotifers, copepods, daphnids and other smaller cladocerans (Federenko 1975b; Pastorok 1981; Elser et al. 1987; Yan et al. 1991).

Food-web interactions may differ between larval stages mostly due to the successively larger size of older instars. *Chaoborus* larvae are generally too small to be considered preferred prey of size-selective planktivorous fish during the first two instars, but are known to occur in the diet of other predatory invertebrates (Dodson 1974; Williams 1980; Fischer and Moore 1993). However, the third and fourth instars will be eaten if detected by planktivorous fish, and chaoborid larvae have evolved morphological as well as behavioural defences in relation to visually hunting fish (Stenson 1981). In everyday language, chaoborid larvae are referred to as “glass worms”, emphasizing their almost transparent appearance. In addition to this obvious reduction in detectability, some species also exhibit diurnal vertical migration when co-existing with fish (Borkent 1981; Dawidowicz et al. 1990). These species spend the daylight hours in the hypolimnion or sediments to avoid predation by fish, and move upwards in the water column to feed after dusk. At sunrise, larvae will already have descended back into deeper waters. This behaviour is referred to as a ‘lake-adapted’ lifestyle, as opposed to the reduced migration observed in ‘pond-adapted’ chaoborid species that evolved in aquatic habitats devoid of fish (Berendonk et al. 2003). Some pond-adapted species may even avoid ovipositioning in water that smell of fish (Berendonk 1999). Consequently, pond-adapted species are usually not present in lakes with fish (Lamontagne and Schindler 1994; Wissel et al. 2003), but dispersal is quite unrestrained due to their airborne adult stage, and pond-adapted species have been shown to invade lakes following fish extirpations (Stenson 1978; von Ende 1979; Eriksson 1980; Nilssen 1984). Pond-adapted chaoborids with reduced vertical migration patterns may similarly disappear from invertebrate communities if fish populations are re-established, or if fish are introduced to formerly fish-free waters (Northcote et al. 1978; Evans 1989).

Chaoborus in paleolimnology

Since chaoborid larvae are covered by a chitinous and rigid cuticle, they may be represented by remnant body parts in lake sediments, such as antennal segments, premandibular fans, and head capsule fragments (Fig. 5). The most widely used subfossil remains are, however, the easily identifiable and often well preserved chitinous mandibles (Frey 1964). The morphology of *Chaoborus* mandibles are also

generally taxon specific and past species structures are normally retained in subfossil assemblages (e.g. Uutala and Smol 1996). The possibility of identifying mandibles to species level is of vital importance, since *Chaoborus* distributions depend on species-specific differences in susceptibility to fish predation, as described above.

Many acidified areas in North America have suffered distinct alterations in fish fauna similar to those previously described for Scandinavia (Harvey and Lee 1982; Schofield 1982; Magnuson et al. 1984). Such developments have also been inferred from subfossil *Chaoborus* assemblages in some regions. There, the pond-adapted and non-migratory *C. americanus* have been shown to invade acidified lakes following fish extirpations (Johnson et al. 1990; Uutala 1990; Uutala and Smol 1996), and may numerically dominate the developed chaoborid communities. When analyzing subfossil *Chaoborus* mandibles from lakes in the Adirondack Mountains, N.Y., Uutala (1990) inferred a quite diverse array of fish developments. Only mandibles from migratory chaoborids were recovered in sediments from lakes that did not seem to have been severely acidified, as indicated by diatom-inferred pH-reconstructions (Charles et al. 1990). Subfossil *Chaoborus* assemblages from lakes that had become severely acidic during more recent times indicated recent fish losses, as mandibles of *C. americanus* appeared in upper parts of sediment cores. One lake, called the Upper Wallface pond, displayed continuous presence of *C. americanus* mandibles in the sediment profile. Such developments have also been documented in other studies (e.g. Johnson et al. 1990; Uutala and Smol 1996). These particular lakes most probably represent naturally fishless lakes and provide important implications, not least for projects concerning lake restoration, as lakes that are naturally devoid of fish would not be good candidates for fish stocking programmes. Many other North American assessments utilizing subfossil *Chaoborus* assemblages to reveal fish population changes in relation to acidification, fish introductions, and fish poisoning, are further discussed and summarized in Sweetman and Smol (2006). However, the potential of applying similar methods for acidified regions in other parts of the world have hardly been investigated.

The Scandinavian distribution of chaoborids in relation to fish presence is well established. The most common species is *C. flavicans*, which exhibits diurnal vertical migrations and is commonly found in most lake types. Some pond-adapted species have also been described (Borkent 1981; Berendonk 1999), but *C. obscuripes* is the only non-migratory species known to also occur in lakes. However, this species only inhabits fish-free lakes (Nilssen 1974; Stenson 1978; Nilssen 1984; Nyberg 1984).

Similarly to *C. americanus* in North America, *C. obscuripes* has been shown to invade lakes after fish loss (Stenson 1978; Eriksson 1980; Stenson and Oscarson 1985), and this species formed dense aggregations in littoral areas during a fish-free period in Lake Gårdsjön (Henrikson and Oscarson 1984). Due to its susceptibility to fish predation, *C. obscuripes* have also been documented to disappear from lake communities when fish populations are re-established (Nyberg 1984; Stenson and Svensson 1994).

AIMS OF THESIS

The habitat restriction of the non-migratory *C. obscuripes* to fish-free waters, and the fact that mandibles accumulate in the sediments, suggest that subfossil *Chaoborus* mandibles could be used to detect fish extirpations in Sweden. Consequently, the main purpose of this thesis is to describe past chaoborid community structures and to deduce whether subfossil *Chaoborus* assemblages can be used to describe certain aspects of known fish community alterations in acidified lakes located in southwest Sweden.

The specific aims of this thesis were to:

- review the possibilities of using subfossil animal remains in sedimentary deposits to infer fish population changes (Paper I)
- describe and assess historical changes in subfossil *Chaoborus* assemblages in the study lakes (Papers II, III and IV)
- test paleo-methods utilizing subfossil *Chaoborus* mandibles for revealing fish loss following severe lake acidification in southwest Sweden (Papers II, III and IV)
- describe and test a new method for inferring historical alterations in fish community composition based on paleo-studies of subfossil *Chaoborus* mandibles (Papers III and IV)

SITE DESCRIPTION AND HISTORY OF STUDY LAKES

All study lakes are located within or close to the Lake Gårdsjön catchment (58°04' N, 12°01' E) approximately 50 km north of Gothenburg (Fig. 3). Most study lakes are currently acidified and fishless, and situated in an area where lake acidification has been extensively investigated since the 1970s (Andersson and Olsson 1985). Although previously acidified, Lake Lysevatten is the only study lake that has been limed and re-stocked with fish.

The overall study area has many properties that make it particularly suitable for the studies in this thesis. The area has received a substantial atmospheric deposition of sulphur and nitrogen during the twentieth century, and previous investigations provide a good historical documentation of chemical and biological changes in these systems. Furthermore, most lakes in this region are still acidified and the described developments in study lakes may thus be viewed as representative for several lakes in a larger geographic area.

Lake Gaffeln and Lake Lilla Hästevatten (Papers II and IV)

These are quite small forest lakes within the Lake Gårdsjön catchment. Lake Gaffeln is situated approximately 500 m upstream of Lake Lilla Hästevatten, which, in turn, drains into Lake Gårdsjön (Fig. 3). Diatom-inferred pH-trends for Lake Gaffeln showed a period of pH just above 6 for the period 1750-1900, with decreasing pH-trends to about 5.8 until 1950, followed by a sharp decrease to pH 4.7 in the 1970s (Renberg et al. 1993). Historical data from 1971 confirmed the low inferred pH with an average pH of 4.7 in both study lakes, whereas the period of 1977-1980 showed pH 4.75-4.9 in Lake Gaffeln and pH 4.6-4.8 in Lake Lilla Hästevatten. The original fish community in these lakes comprised perch, pike, eel (*Anguilla anguilla* L.), roach and tench (*Tinca tinca* L., Trybom 1903, Hultberg 1985). Roach disappeared from all lakes in the area during the 1950s and 1960s while all other fish species were at least seriously affected or disappeared as well. Net-fishing surveys in 1972 captured 66 adult and one juvenile perch in Lake Gaffeln, while only two perch individuals were caught in Lake Lilla Hästevatten (data collected by H. Hultberg), thus demonstrating seriously impaired reproduction in the remaining single-species fish communities. Before starting studies on fish effects in acidified lakes, all remaining fish were wiped out with rotenone treatments the following year, and hence, these lakes have been effectively fishless since November 1973.

Lake Lysevatten (Paper III)

Lake Lysevatten (58°04' N, 12°02' E) is an oligotrophic clearwater lake in the watershed of Anråseå, and located just north of Lake Gårdsjön (Fig. 3). An inspector of fisheries visited this lake in the year 1900, estimating secchi depth to 6 m at the time. Roach, perch, pike and eel occurred in the lake but the roach population originated from a stocking of approximately 200 individuals 15-20 years before the visit, i.e. during the early 1880s (Trybom 1903). In 1948 the same four fish species were noted in an inventory performed by the Swedish Board of Fisheries, but the roach and pike populations were estimated to be sparse and there were no catches registered for tench, which had been stocked in 1925. The secchi depth was 5 m by then and lake pH was 6.0 (Törnquist 1948/1949). Available data and paleolimnological studies of diatoms showed that the lake became chronically acidic during the 1960s, and pH was below 4.8 throughout the year during the early 1970s (Hultberg 1985; Renberg and Hultberg 1992). The acidification period displayed significant changes in fish fauna assemblages. The only fish caught during net-

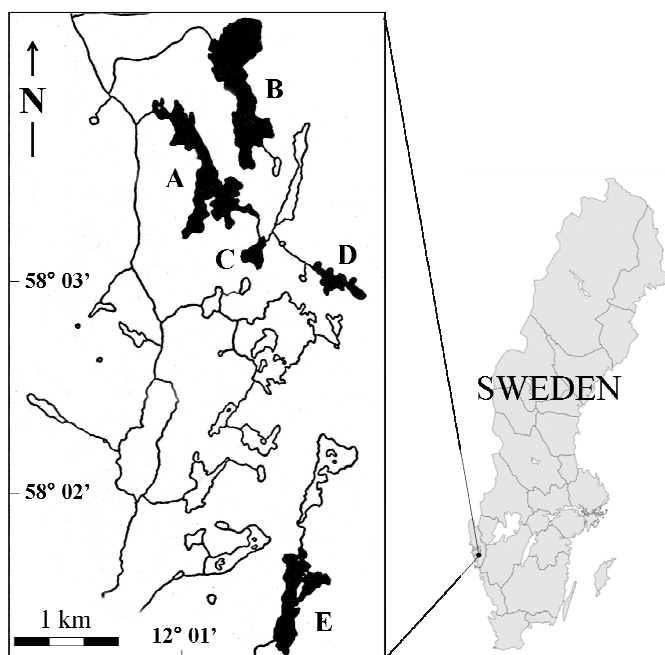


Figure 3. Map of study area and location in southwest Sweden. A) Lake Gårdsjön; B) Lake Lysevatten; C) Lake Lilla Hästevatten; D) Lake Gaffeln; E) Lake Härsvatten

surveys in 1970 to 1973 were perch and catches decreased successively, until a rotenone treatment in November 1973 when the remaining population of only 15 perch individuals was collected (Hultberg 1985). The secchi depth was now between 10-12 m during summer. After rotenone treatment the lake was limed in 1974 and repeatedly stocked with salmonids (*Salvelinus fontinalis* Mitchell and *Salmo trutta* L.). Liming resulted in neutralisation of the lake water, positive alkalinity, and enhanced nutrient conditions, but only for a few years since Lake Lysevatten showed signs of re-acidification already in late 1970s. Lake water pH was constantly below 5.5 during the first half of the 1980s, which resulted in yet another whole-lake liming in autumn 1986 (Andersson and Hultberg 1997). At present only salmonids and pike occur in this lake, and cyprinids have not been recorded since roach was eradicated by acidification.

Lake Härsvatten (Paper IV)

Lake Härsvatten (58°01' N, 12°02' E) is located south of the Lake Gårdsjön catchment (Fig. 3), and is presently included in the ISELAW monitoring programme (Integrated Studies of the Effects of Liming in Acidified Waters), where it is used as an acidified reference lake for unlimed lake conditions (Persson 2008). As such, this lake has been intensively monitored for water chemistry, as well as for lake biota (including *Chaoborus* larvae) since early- and late 1980s, respectively. This lake is historically referred to as an oligohumic forest lake but showed severe acidification during the first documented water samplings in 1971, with summer pH-values of 4.4, negative alkalinity, and 8 m secchi depth (County Agricultural Board of Älvsborgs län 1973). The ISELAW-programme later confirmed Lake Härsvatten as a severely acidified and ultraoligotrophic lake during the 1980s, with yearly means of pH of about 4.5, negative alkalinity, and yearly means of total phosphorus generally below 5 µg l⁻¹. Secchi depth was usually around 10 m, but exceeded 15 m at some samplings during this period. Only minor changes were reported during the 1990s and early 21st century, but pH above 5.0 and increasing levels of total phosphorus have been reported in more recent years (additional information, in Swedish: <http://info1.ma.slu.se/IKEU/>). Lake Härsvatten originally harboured a poor fish community of perch, eel, and northern pike. All fish were completely lost during the 1970s and by that time northern pike had already not been caught for several years (County Agricultural Board of Älvsborgs län 1973). The ISELAW-monitoring

programme performed yearly standardized net fishing in 1994-2001, and also in the years of 2003 and 2007, although no fish has ever been caught during these efforts.

METHODS

All methods described in this part, except for the literature study (Paper I), follows the general paleolimnological methodology described in Fig. 4.

Literature study (Paper I)

The possibilities of inferring past fish population changes were summarized in a literature study. The study discussed published topic-related research articles present in the source data-bases of Science Citation Index, Biological Abstracts, Zoological Records Plus, and the Swedish Library Search Services LIBRIS.

Sediment sampling (Papers II-IV)

Most sediment samples were taken from the ice at the deepest part of study lakes. However, some of the sediment samples included in the comparative study of surface sediments were collected from boat during late summer (Table 1 in Paper III). All sampling areas were located with use of morphometric maps, while the deepest part and actual sampling point was located with an electrical sonar. Sediment cores were collected with a self-closing UWITEC Ballcorer® fitted with core tubes of inner-diameter 58mm (Paper II), or 80mm (Papers III and IV). The retrieved cores were extruded on site in intervals of 5-mm or 1-cm (Paper III), with separate sediment layers sealed in 200-ml polypropylene jars and kept dark at 4°C until treatment. We also used a Ruttner sampler to collect water samples at one metre depth for pH determination.

Sample preparations and analyses (Papers II-IV)

Sediment subsamples of 5–16 ml of wet sediment from each sediment interval were deflocculated in 10% KOH solution. 5-ml subsamples from Lake Lilla Hästevatten core were treated in 100-ml 10% KOH for 30 minutes at 60-70°C (Frey 1986, Paper II), while all other treatments of wet sediment subsamples were performed in 400-ml 10% KOH for 72 hours at room temperature (Uutala and Smol 1996, Papers II-IV). Treated samples were gently rinsed through a 100-µm mesh, with retained material kept dark in de-ionized water at 4°C until analysis. Separate sediment subsamples

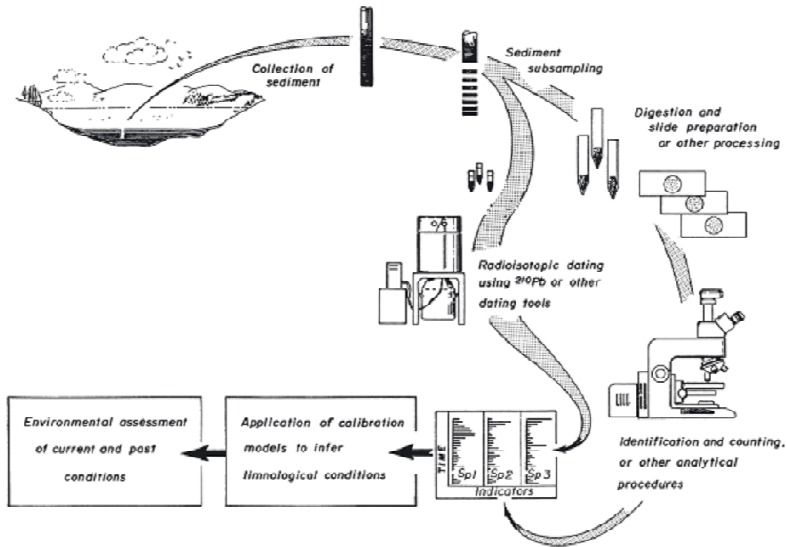


Figure 4. An illustration of the main steps in paleolimnological methodology. From Smol (2008); used with kind permission from Wiley-Blackwell.

were dried for estimation of sediment water content. All master core sediments were freeze-dried at the Department of Earth Sciences, Gothenburg University, except for sediments from Lake Lilla Hästevatten (Paper II) which were dried for seven days in a ventilation cupboard. Moreover, subsamples for the comparative study of surface sediments (Paper III) were dried in a ventilated heating cupboard at 70°C for 72 hours.

Sieved samples were analyzed in a lined counting chamber (Hofmann 1986; Walker 2001), with the addition of surfactant solution Debitol® to remove surface tension. Subfossil *Chaoborus* mandibles and *Daphnia* ephippia (Paper IV) were identified and enumerated at a magnification of 32-160x using a Leitz Diavert inverted microscope. *Chaoborus* mandibles were identified to species according to Sæther (1997), while *Daphnia* ephippia were merely enumerated. All recovered mandibles in Papers III and IV were also measured across the mandibular basal plate (Fig. 2a in Paper III) at 160x magnification, in which micrometer calibration divided graticule scale measurements into 7- μm intervals. Mandibles with broken basal plates were noted as fragmented and only remains carrying the species-specific mandibular teeth were

included in the analyses. Consequently, a mandible was defined as fragmented if material was missing at least along the whole of the anterior edge of the basal plate (Fig. 2b in Paper III), although commonly, much more of the basal plate was missing (Figs. 2c, 2e, 2f in Paper III). Subfossil *Chaoborus* mandibles, *Daphnia* ephippia, and some additional subfossil animal remains recovered during these analyses are shown in Figure 5.

Concentrations of subfossil *Chaoborus* mandibles were presented as number of mandibles per gram dry weight of sediment [mandibles (g dwt)⁻¹, Papers II and IV], mandibles per gram dry weight of organic matter [mandibles (g OM)⁻¹, Papers III and IV], or expressed as mandible accumulation rates [mandibles (cm⁻² year⁻¹), Paper IV], while occurrences of *Daphnia* ephippia were only presented as accumulation rates [*Daphnia* eph. (cm⁻² year⁻¹), Paper IV].

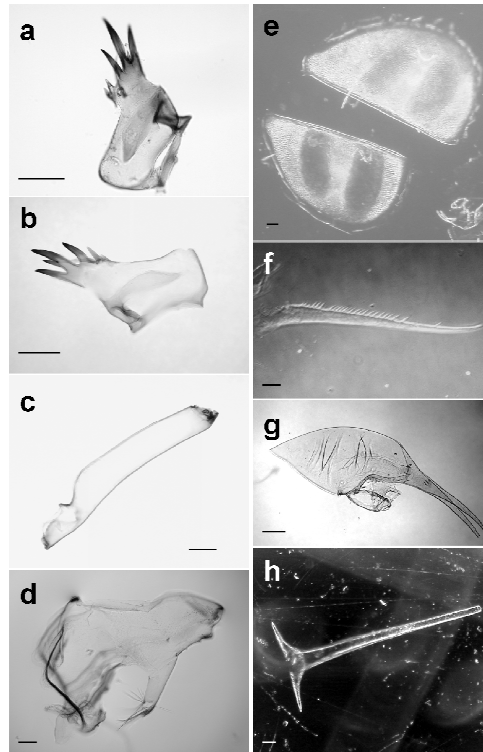


Figure 5. Examples of subfossil remains recovered during analyses. a) *C. flavicans* mandible b) *C. obscuripes* mandible c) *Chaoborus* antennae d) *Chaoborus* head caspule e) *Daphnia* sp. ephippia f) *Leptodora* caudal spine g) *Bosmina* sp. headshield h) *Bythotrephes* caudal spine (fragment). Scale bars represent 200 μm .

Sediment dating (Papers III and IV)

Freeze-dried samples were dated at the Ångström Laboratory, Uppsala University. Sediment chronologies were constructed using alpha and gamma spectrometry of ^{210}Pb , ^{226}Ra and the anthropogenic radioisotopes ^{137}Cs and ^{241}Am (El-Daoushy and Garcia-Tenorio 1995, Hernández and El-Daoushy 2002). These radiometric approaches have been developed, tested and calibrated to give validated and reliable high-resolution chronologies for a wide-range of sedimentation dynamics in aquatic systems (El-Daoushy 1988; El-Daoushy 1990). Assuming a constant rate of supply of the unsupported ^{210}Pb (CRS model) the chronology was calculated from the profile of unsupported ^{210}Pb , which in turn was estimated as the difference between total ^{210}Pb and supported ^{210}Pb . The supported ^{210}Pb was based on direct ^{226}Ra measurements. Low ^{241}Am -activities in the samples and a minor ^{241}Am contamination in the detection system required long counting time to get resolvable information from the ^{241}Am profiles.

Estimation of sediment organic content (Papers III and IV)

Freeze-dried samples were additionally used for estimating sediment organic content as loss-on-ignition (*e.g.* Dean 1974) at 550°C (SIS 1981) at the Erken Laboratory, Uppsala University.

Controlled feeding study (Paper III)

Collection of fish and prey *Chaoborus* larvae

Live roach and perch were collected with NORDIC gillnets in Lake Stora Delsjön near Gothenburg (57°41' N, 12°02' E) in April 2008. Nets were checked and fish removed every third hour until at least 15 fish of both roach and perch had been caught. All undamaged fish were kept in oxygenated 500-L tanks (10°C) at the Zoology Department, University of Gothenburg, Sweden. Fish were fed sparsely with bloodworms every day but were not fed during three days prior to the study (Ethical license nr. 113/2008). Instar IV larvae of *C. obscuripes* were collected in fish-free Lake Härsvatten (study lake in Paper IV) with littoral net tows (50- μm mesh, \varnothing 40 cm) and collected larvae were kept in 30-L buckets of oxygenated lake water at 10°C.

Study set-up and execution

From the stock tanks eight fish per species with similar ranges in size and wet mass (mean \pm SE (min–max), $n = 8$; length_{roach} 154.5 ± 9.7 mm (118–200 mm); length_{perch} 157.6 ± 6.8 mm (133–190 mm); mass_{roach} 35.8 ± 8.3 g (13.1–86.3 g), mass_{perch} 35.9 ± 6.2 g (18.1–72.1 g)) were individually separated in 25-L aquaria (oxygenated water, 10°C). 50 *C. obscuripes* larvae were poured into the aquaria (each aquaria containing one fish individual), and fish were then allowed to feed undisturbed. All aquaria were regularly inspected for prey consumption, and fish were left in the aquaria for 72 h after all prey had been consumed, in order to allow for sufficient gut evacuation time of roach and perch (Hofer et al. 1982; Lessmark 1983).

Sample preparation and analysis

Fish were gently removed by hand, without the use of nets. The aquaria were carefully emptied and all water sieved through a 100- μ m mesh, with all remaining material treated and analyzed according to methods used for sediment samples described above.

Statistical analyses (Papers III and IV)

Non-parametric linear regression was used in Paper III to test if proportions of fragmented mandibles (PFM) were dependent on the size of individual fishes, while the Mann-Whitney U-test was used when testing for differences in mandible concentrations and estimated PFM between treatments of roach and perch presence (Papers III and IV). However, the Median test was used when variances in PFM differed significantly ($p < 0.05$, Squared Ranks test; Conover 1999). Calculations for the Median test were based on the Fisher's exact test for 2x2 contingency tables if any of the expected frequencies were below 5 (Siegel and Castellan 1988). Two-tailed test probabilities are given for statistical tests, except for calculations based on the Fisher's exact test, in which p-values are to be interpreted as the probability of the test case and all more extreme cases (the sum of small p-values). Mann-Whitney U-test and Median test were performed in SPSS 16.0 (Paper III) and 18.0 (Paper IV), while non-parametric linear regressions and Squared Ranks tests for equal variances were performed in StatsDirect 2.7.7.

MAIN FINDINGS AND DISCUSSION

This section provides an overview of the main findings and summarized discussions from papers I to IV included in this thesis.

Inferences of past changes in planktivorous fish predation (Paper I)

This study reviewed the possibilities of inferring past changes in fish communities and verified that fish leave identifiable remains in lake sediments (Lagler and Vallentyne 1956; Vallentyne 1960; Pennington and Frost 1961; Frey 1964). However, these remains (*e.g.* vertebrae, scales, and teeth) are usually quite scarce and of limited use in fish reconstructions (Patterson and Smith 2001; Davidson et al. 2003). Consequently, most fish inferences have been based on the far more common remains of subfossil prey assemblages, originating from a wide variety of invertebrate prey organisms. There are intimate relations between fish populations and their prey communities, and both vertebrate and invertebrate predation has been shown to influence morphology, size, migratory behaviour and ontogenetic characteristics of prey species (Boersma et al. 1998; Tollrian and Dodson 1999). Hence, the existing efforts to qualitatively or quantitatively reconstruct past fish dynamics could be presented according to the different characteristics analyzed for specific prey assemblages (*i.e.* alterations in community structure, size distributions, or morphological features). In this study, however, most qualitative inferences were interpreted as suffering from uncertainties in the somewhat vague historical relation between fish predation pressure and actual fish densities. Since visually hunting fish are light dependent, some qualitative reconstructions were proposed as potentially inferring changes in fish predation pressure perhaps more related to alterations in water transparency than to changes in fish biomass. Moreover, it may generally not be possible to tell whether alterations in subfossil prey assemblages are due to shifts in dominant fish species or related to a more gradual change in fish biomass (*e.g.* Kerfoot 1981).

This literature study further identified larval mandibles from certain species of phantom midges (*Chaoborus* spp.) as being particularly suitable for inferring fish loss in severely acidified lakes. *Chaoborus* larvae can be common invertebrate predators in lakes, as some species are nearly transparent and exhibit diurnal vertical migration as an anti-predatory adaptation to visually hunting fish (Borkent 1981). However, some species are more heavily pigmented and do not display pronounced vertical

migration, and are limited to fish-free habitats (Northcote et al. 1978, Stenson 1978). Some of these non-migratory species have been shown to invade lakes following fish extirpations and, since the mandibles of these larvae are well preserved and easily identified (Frey 1964), their occurrence in sediment profiles may well indicate fish-free periods in lake histories (Johnson et al. 1990; Uutala 1990; Lamontagne and Schindler 1994; Uutala and Smol 1996).

Despite the apparent potential to utilize subfossil *Chaoborus* mandibles for inferring fish loss in acidified lakes, every study describing such applications has actually been limited to the North American continent. This focal conclusion emphasized the importance of executing similar investigations in acidified areas of additional geographical regions (e.g. northern Europe).

Reconstructing altered fish population status (Paper II-IV)

We collected sediment cores from acidified lakes with known fish extirpations in order to test whether the development of subfossil *Chaoborus* assemblages could be related to loss of fish in southwest Sweden. The study lakes are located within or close to the Lake Gårdsjön catchment (Fig. 5), an area that has been subject to intensive acidification research during the last four decades (Andersson and Olsson 1985). The study lakes were known to have lost all fish sometime during the 1970s, and all lakes, except Lake Härsvatten (paper IV), were treated with rotenone during the autumn of 1973 to remove possible remnant fish. Poor fish population status had, however, already been confirmed by extremely scarce catches during test-fishing in previous years (Hultberg 1985, Papers II-IV).

Subfossil *Chaoborus* mandibles proved to be quite common in all study lake cores, although some sediment intervals were devoid of mandibles. Mandibles from the non-migratory *C. obscuripes* were recovered from the upper parts of both sediment profiles in paper II, and their appearance in the sediment records coincided with an increase in concentrations of *C. flavicans* mandibles. These developments were interpreted as an indication of the known fish eliminations in these study lakes. The first appearances of *C. obscuripes* mandibles were recorded in sediments that corresponded to the period of severe lake acidification and fish loss, according to sedimentation rates estimated for previous studies in the Lake Gårdsjön area (Renberg and Wik 1985; Andersson and Renberg 1992; Huser and Rydin 2005). However, mandible counts of *C. obscuripes* were low in both study lake profiles, which could be an effect of a documented littoral preference in this species (Nilssen

1974; Henrikson and Oscarson 1984). The results in paper **II** thus suggest that subfossil *C. obscuripes* mandibles may be used to indicate fish elimination in much the same way as *C. americanus* in North America. However, the overall prospects of using *Chaoborus* mandibles to reconstruct historical fish status may differ between Europe and North America. In eastern North America at least four chaoborid species are commonly present (Sweetman and Smol 2006), while the chaoborid community of acidified lakes in northern Europe is only represented by two species. So despite the close genetic relationship between American and European lake dwelling *C. flavicans* (Dupuis et al. 2008), the interactions with competitive and/or predatory chaoborid species may moderate the effects of fish predation on *C. flavicans* in North America, but not in Europe.

No mandibles of *C. obscuripes* were recovered from the Lake Lysevatten core (paper **III**). However, this lake was only completely fish-free for about one and a half years between the rotenone treatment in November 1973 and the stocking of salmonids (*Salmo trutta* and *Salvelinus fontinalis*) in June 1975 (Andersson and Hultberg 1997), and this time span was probably too short for *C. obscuripes* to form a lake population dense enough to be detected in profundal sediments.

The sediment profile of chaoborid development obtained for Lake Gaffeln in paper **IV** was similar to the one presented in paper **II**. Both profiles displayed high concentrations of subfossil *C. flavicans* mandibles in deeper sediments, a decline in mandible concentrations of this species during progressive acidification, and an increase in *C. flavicans* mandible concentrations following the appearance of *C. obscuripes* mandibles. Furthermore, mandibles of *C. obscuripes* appeared at about the same sediment depth in both profiles. However, *C. obscuripes* mandibles were recovered from additional sediment intervals in paper **IV**, whereas the simultaneous increase in *C. flavicans* mandible concentrations was not as prominent. The chronology of the Lake Gaffeln core in paper **IV** also confirmed that *C. obscuripes* mandibles first appeared in the sediments following fish elimination (as discussed in paper **II**).

Progressive acidification was indicated by the decline and loss of *Daphnia* ephippia from both study lake profiles in paper **IV**. These large-sized cladocerans are sensitive to acidic conditions, including high aluminium concentrations, and are dependent upon a sufficient supply of calcium for growth in between moults (Brett 1989; Jeziorski and Yan 2006). Calcium is normally derived from weathering of catchment bedrocks and soils but these calcium reserves can be depleted during long-lasting

acidification. However, increased weathering during early stages of acid impact could result in relatively high levels of calcium and nutrients being released to the lake at that time (e.g. Smol 2010). Such an initial acid phase may also be inferred from both lake profiles in paper IV, as both lakes display peaks in abundance of *Daphnia ephippia* at about the start of the 20th century.

The Lake Härsvatten core differed from all other sediment profiles as it described recent periods of complete lack of subfossil *Chaoborus* mandibles (paper IV). These developments were supported by lake monitoring of chaoborid larvae, and reasoned to be a consequence of ultra-oligotrophic lake conditions rather than severe acidity *per se* (Neill and Peacock 1980; Yan et al. 1982; Locke 1991). However, when subfossil mandible occurrences returned to detectable concentrations, both *C. flavicans* and *C. obscuripes* mandibles were present in sediment samples. So when favourable lake conditions returned (as indicated by ISELAW monitoring in water chemistry), mandibles of *C. obscuripes* confirmed the present fishless state of Lake Härsvatten, but the appearance of subfossil *C. obscuripes* mandibles could not be temporally related to loss of fish. Instead, a peak in mandible concentrations of *C. flavicans* might indicate fish extirpation in this lake. *C. flavicans* mandible concentrations were found to be high in one sediment layer at about the time of fish loss, and could thus potentially indicate decreased fish predation pressure and/or fish loss, just before mandibles disappeared from the sediment record.

Inferring altered fish community composition (Paper III-IV)

As discussed in paper I, several potential paleo-methods utilizing subfossil prey assemblages can be used to describe past changes in fish populations. However, such indirect approaches do not provide any information concerning whether the inferred trends in past fish predation were caused by changes in fish densities or additionally accompanied by shifts in fish community composition (i.e. loss or invasion of fish species). It is thus generally believed that only actual fish fossils could provide such details (e.g. Davidson et al. 2003).

While analyzing sediment samples, we noted that not all of the recovered subfossil mandibles were intact. Some were broken across the basal plate while others merely consisted of the mandible part carrying the species specific mandibular teeth (see Fig. 2 in paper III). Such damage could be due to fragmentation of mandibles upon ingestion of *Chaoborus* larvae by fish, especially cyprinid fish (e.g. roach). Cyprinid fish have specialized pharyngeal teeth used for crushing food items before ingestion

(Alexander 1981). If this cyprinid feeding-process could be shown to fragment also *Chaoborus* mandibles then high proportions of fragmented mandibles (PFM) in sediment samples could potentially be used as an indication of cyprinid presence in species-poor boreal lakes in southwest Sweden. This hypothesis was tested in four different ways:

- 1) Eight individuals of both roach and perch were individually fed *Chaoborus* larvae in the laboratory, and the water from each fish aquaria was analyzed to estimate the fragmentation of evacuated mandibles (*i.e.* analyses of fish faeces retained in closed aquaria systems, paper **III**).
- 2) Surface sediments were collected in ten lakes with reproducing roach populations and in eight lakes devoid of cyprinid fish and analyzed for differences in fragmentation of subfossil *Chaoborus* mandibles (paper **III**).
- 3) We analyzed a dated sediment core from Lake Lysevatten which had a known period of cyprinid presence. Subfossil *Chaoborus* mandibles were analysed to test for differences in mandible fragmentation between periods of roach presence and absence (paper **III**).
- 4) We also compared estimated fragmentation in dated sediment profiles from Lake Gaffeln and Lake Härsvatten, which differed in historical presence of roach. Since both lakes have lost all fish, only sediment intervals corresponding to the periods of fish presence were used when testing for differences in PFM between lakes (paper **IV**).

Specific test statistics are presented in papers **III** and **IV**, but can be summarized as all tests resulting in a significant difference in PFM between roach presence and absence (two-sided $p < 0.05$). The laboratory feeding study provided evidence for the causal relationship between fragmented mandibles and roach predation on *Chaoborus* larvae, and the analyses of surface sediments proved that PFM differed between lakes containing roach and lakes devoid of cyprinid fish (paper **III**). Furthermore, estimates of PFM from sediment samples identified the period of roach presence in Lake Lysevatten (paper **III**) and also in Lake Gaffeln (paper **IV**), while low estimates of PFM in Lake Härsvatten samples confirmed the historical absence of cyprinid fish in this lake (paper **IV**). The suggested linkage between fish predation and mandible fragmentation was additionally emphasized in paper **IV**, in which low or non-existing fragmentation was documented for the fish-free periods in both sediment

profiles. Hence, the PFM method was proposed as a potential approach for revealing shifts in past fish community composition, without the inclusion of fish fossils.

Other subfossil remains may also display effects of fragmentation caused by fish predation. For example, the long and slender caudal spine of *Bythotrephes* (Fig. 5) may be broken upon ingestion by fish and it has been suggested that high fractions of broken caudal spines in sediments could reflect significant mortality attributable to fish (Hall and Yan 1997; Kerfoot et al. 2000). However, these issues need to be addressed further, as it is still uncertain whether fragmentation of these subfossil remains could also be used for detecting fish species shifts, or merely indicate changes in general fish predation. Nevertheless, it could prove possible to not only include effects of fragmentation in future studies, but also to extend these and similar ideas to analyses of stored subfossil samples that were collected in lakes with known shifts in fish community composition.

Reconstructing larval instar dynamics from sediments (Paper IV)

Since *Chaoborus* larvae develop through four larval stages (instars), subfossil mandibles of different sizes could possibly originate from different larval instars. This was assessed in paper IV, in which all measured mandibles were presented in size-frequency charts for the separate study lake cores (Fig. 7. in paper IV). Both lakes showed quite distinct and similar normal distributed size peaks which suggested that mandibles could be grouped according to instar-specific size classes. Subfossil mandibles were dominated by instar III and IV, but some smaller mandibles, probably originating from instar II, were also recovered. Similar size-frequency distributions have previously been reported by Stahl (1959).

Predation risk has been shown to differ between instars (Stenson 1990; Fischer and Moore 1993; Fischer and Frost 1997). Thus, to test whether fish loss had an effect on mandible instar distributions, we calculated the proportion of instar IV mandibles for individual sediment intervals. We then tested if there had been any changes in proportions of instar IV mandibles following fish loss. This was only done in lake Gaffeln, since mandibles were too few or non-existing in sediments from the fish-free period of Lake Härsvatten. However, there were no significant differences in proportions of instar IV mandibles between periods of fish presence and absence in Lake Gaffeln. This could be due to small sample size of sediment intervals from the fish-free period, or be influenced by increased variances due to low mandible counts

during this period, as the sediment profile suggested a declining trend in relative occurrence of instar IV mandibles in more recent sediments (Fig. 4. in paper IV).

There was also a general dominance of instar IV mandibles in both lake cores, indicating that instar III mandibles do not accumulate in profundal sediments to the same extent as mandibles from the fourth instar. This pattern is not believed to have originated from differences in detection probabilities between mandibles of different instars, since all mandibles were easily recognized in the counting chamber. These distributions are interesting, as there should be as many instar III mandibles as fourth instar mandibles if deposition was random (since all instar IV larvae must have moulted from the third larval stage). This suggests that sedimentation patterns of subfossil mandibles may differ between instars. For example, instar IV larvae are the only larval stage to moult upon pupation, and hence, this moulting phase may be more or less restricted to the epilimnion (Saether 1997). It is therefore possible that instar IV mandibles originating from pupation are differently distributed in sediments compared to mandibles originating from moulting in instar III or mandibles derived from fourth instar larvae consumed and evacuated by fish. This implies that there could be general changes in mandible distributions to sediments also related to fish loss, but such relations obviously need further investigation.

CONCLUSIONS AND FUTURE PERSPECTIVES

The studies described in this thesis show that subfossil *Chaoborus* mandibles can reveal important information concerning altered fish community composition and fish loss in acidified lakes. High proportions of fragmented *C. flavicans* mandibles reveal the historical presence of cyprinid fish, while subfossil mandibles of *C. obscuripes* can indicate where, and sometimes when, all fish have been extirpated. The absence of *C. obscuripes* mandibles in sedimentary deposits is, in itself, not a trustworthy indication of fish presence, however.

The results presented here are derived from analyses of a limited number of lakes and lake types, of which most are confined to a limited geographical area. Fish, as well as invertebrates, may react differently to progressive acidification in other lake types, for instance humic lakes (Almer 1972). In such lakes, inorganic aluminium may adhere to humic particles with potential de-toxifying effects on lake biota. Humic lakes may also support different invertebrate communities, and usually differ from acidic clearwater lakes in terms of physical characteristics, such as water

transparency and temperature profiles. Consequently, it is important to assess the general applicability of chaoborid paleo-methods for other lake types as well.

Estimations of fragmented subfossil *Chaoborus* mandibles proved useful for revealing past changes in fish community structure. The PFM-method is thus suggested as an indirect indication of cyprinid presence, and could be used to describe losses or introductions of cyprinid fish species in lake histories. Periodical lack of fragmented mandibles in sediments may also be regarded as an independent indication of significant declines in general planktivorous fish predation and eventual fish loss. Although the causal relations between fish consumption and fragmentation are robust, the suggested PFM-method needs to further validated and applied to additional lakes with different fish histories. This could actually be done for lakes that have already been sampled, since PFM can be estimated also for stored samples originating from lakes with known historical alterations in cyprinid presence.

The inclusion of *Daphnia* ehippia in sediment analyses provided an indication of progressive acidification, supposedly more related to past nutrient levels than to alterations in fish predation pressure. Multi-indicator approaches are common in paleolimnological research and are often needed to disentangle the effects of multiple stressors in aquatic systems. This could be further developed for *Chaoborus* mandible analyses as well. Some attempts have already been described, such as the inclusion of diatom analysis for pH-reconstruction when assessing past fish alterations in acidified systems (Uutala et al. 1994), or the inclusion of subfossil *Chaoborus* mandibles in chironomid based inference models of past hypolimnetic anoxia (Quinlan and Smol 2010a). Of course, there is no limit to the number of potential subfossil remains that could be combined in multi-indicator approaches, and appropriate indicator combinations need to be decided on a case-to-case basis.

Subfossil *Chaoborus* mandibles have recently been shown to reflect the community composition of contemporary larval assemblages in Canadian Shield lakes (Quinlan and Smol 2010b). This was done by comparing lake-dwelling chaoborid communities with subfossil mandible assemblages in surface sediments. This thesis includes a similar comparison supporting these findings, as monitoring time-series data of larval occurrences could be shown to be intimately related to corresponding developments inferred from subfossil mandible occurrences. Together with the similarities obtained from the two different Lake Gaffeln cores, this suggests fair representativeness as well as potential reproducibility of subfossil mandible

estimates, thus emphasizing the reliability of the single-core approach commonly used in paleolimnological investigations. However, the actual cause for some of the inferred chaoborid developments are still uncertain and remain unresolved. One example is the historical fluctuations in subfossil mandible concentrations documented in deeper parts of sediment cores from Lake Lysevatten and Lake Härsvatten. Forestry and other land-use activities may be involved here, but seem improbable as explanations for the somewhat cyclic pattern of mandible concentrations, especially in Lake Härsvatten sediments (Fig. 6). Instead, perch population cycles, originating from intercohort competition and predation in perch populations (Claessen et al. 2000), may be responsible for the documented developmental patterns. Cyclic trends or fluctuating mandible concentrations have, in fact, only been documented during cyprinid-free lake periods, *i.e.* during perch-dominated planktivory. To shed some light on these uncertainties, possible paleo-signals of perch population cycles need to be further addressed, preferably in lakes

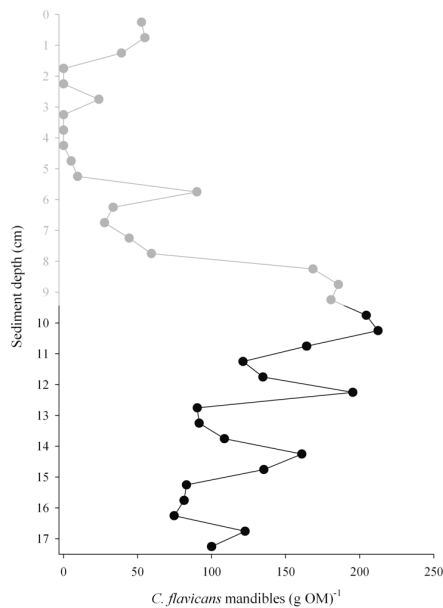


Figure 6. Number of *C. flavicans* mandibles per gram organic matter in the Lake Härsvatten sediment core. Upper half shaded for focus on the lower half of the profile. Modified from paper IV.

with documented historical presence of such perch population dynamics (*e.g.* Persson et al. 2000). Such investigations would probably benefit from the inclusion of measurable subfossil zooplankton remains (*e.g.* *Daphnia ephippia*), in order to additionally estimate possible effects of altered size-selective predation pressure.

Methods for inferring changes in past predation pressure of planktivorous fish are continuously refined (*e.g.* Davidson et al. 2010a; b), and except for the studies included in this thesis, there have also been quite a few fairly recent studies utilizing chaoborid remains to address wide-ranging aspects of freshwater assessment (Luoto and Nevalainen 2009; Kurek et al. 2010; Quinlan and Smol 2010a; b). This is promising, as chaoborid remains in sediments could still be considered a somewhat under-utilized constituent of the sedimentary proxies readily available for paleolimnological investigations.

ACKNOWLEDGEMENTS

Quite a few years have passed since I first entered the zoological institution in Gothenburg. It must have been in the year of 2001, when I attended the basic courses in biology and got more familiarized with the concepts of evolutionary ecology. During those undergraduate studies I took all courses related to freshwater ecology and I remember all of them as almost ridiculously enjoyable, for one obvious reason; Jan Stenson. Professor Jan Stenson was the person who introduced me to the diverse and fascinating world that resides beneath every water surface within our landscape, and he also taught me how to adopt a scientific approach in order to study these environments and learn even more about them. He was the one who encouraged me to apply for this Ph.D.-project in applied limnology, and I got to have him as a truly inspiring supervisor for some very pleasant years. Sadly, he left us all too soon, in October 2006.

- Dear Jan, this thesis would not exist if it weren't for you and all the enthusiasm you so gladly shared. I miss you.

I was later fortunate enough to get Professor Torgny Bohlin as a supervisor. Just like Jan, Torgny always motivated me in my studies (both at undergraduate- and graduate levels), and he always kept his door and ears open to promote ecological and statistical discussions. Thank you Torgny! By the way, you are the grand master of proof-reading and text-condensation as well...

I would also like to thank my assistant supervisor Jan-Erik Svensson for all his efforts during these years. You are as much an inspirational guide as a dear friend, and surely a great conference companion. You have always been there to encourage, discuss, ponder, and question any ecological reasoning I have ever presented, and I am ever so grateful for it!

Multidisciplinary research is built on co-operation between different scientific areas, and all project collaborators are sincerely and highly acknowledged. I especially want to thank Professor Farid El-Daoushy at Uppsala University for providing the tools necessary for opening "the black box" of sediment chronologies, Fredrik Nilsson at the County Administrative Board of Västra Götaland for all contributions concerning regional lake acidification and related field samplings, and Hans Hultberg for discussions on early fish-surveys and for sharing your vast historical insights. Several colleagues and students provided further help, and Ragnar Lagergren, Rasmus Ljungqvist, Omar Prieto Santamarta, Kristian Bergström,

Andreas Jönsson, Jonas Wiklund, and Robert Andersson are all appreciated for their assistance in fieldwork or laboratory.

Less practical, but instead far more philosophical (and sometimes almost theoretical?) contributions were made by all fellow Ph.D.-students and colleagues at the department of Animal Ecology. My deepest gratitude goes to Hans Lord (the second last limnologist) for sharing your life-time experiences, to Jakob Augustin (the office gnome) for never ending presence and support, to Inês Gonçalves (the serotonin-drenched smurfette) for all the positive remarks and cheerful comments, and to Jörgen Sagvik, Rasmus Kaspersson, Maria Prager, Bart Adriaenssens, Gry Sagebakken, Jason Pienaar, Ola Svensson, Sofia Anderholm, Sofia Brockmark, Daniel Isaksson, Peter Waldeck, Joacim Näslund, Jenny Leonardsson, Niklas Franc, Annette Johansson, Nils Lundqvist, Tobias Uller, and Caroline Isaksson for all support, company, and splendid teaching assistance. Good luck in future endeavours! I also want to thank Staffan Andersson for being my examiner, and the rest of the senior research staff consisting of Torgny Bohlin, Ragnar Lagergren, Johan Höjesjö, Jörgen Johnsson, Lotta Kvarnemo, Malte Andersson, Donald Blomqvist, Frank Götmark, Angela Pauliny, Peter Lindberg, Mats Olsson and Johan Wallander for providing a solid scientific foundation to lean upon, and the administrative and technical staff, especially Bernth Carlsson, Lilioth Olsson, Lena Sjöblom, Ann-Sofie Olsson, Mattias Söderpalm, Birgit Lundell, and Erika Hoff for always helping out in the most diverse matters.

But still, the every-day support from friends and family has been (at least) equally important during these years. I am thankful for all friends who share my interest in sport-fishing, especially Emil Hjertberg for being a true companion with a good taste in music and for co-authoring some (iconic?) sport-fishing articles, and Gunnarsson, Ricki, Jon, Johan Lundgren, Dräggo, Rassie, Johan Jansson, and Jonas Janzzon for sharing all those memorable moments by the water side. I guess we have to get out there again quite soon? Lingering in-between fishing and science is the admirable Dr. Henrik Ragnarsson Stabo, who personifies the term 'scepticism'. Please remain more sceptical than ever as "ensam magister i bräcklig farkost nu navigerar i hamn"... I also thank all other friends for sharing beers, concerts, and similar havocs!

Finally, thanks to my parents Lennart and Margareta, and my sister Lena for always believing in me and supporting me whatever path I may wander. And last, but certainly not least, many loving thanks to Jennie - my hero - and my children Isac

and Signe, who all let me experience and appreciate the most important things in life... no words could ever express my gratitude... I owe you the world.

Financial support was provided by Formas, Ebba och Sven Schwarz Stiftelse, Wilhelm och Martina Lundgrens Vetenskapsfond, Helge Ax:son Johnsons Stiftelse, Rådman och fru Ernst Collianders Stiftelse, and the Royal Swedish Academy of Science.

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