

Effects of multiple abiotic stressors on the species and genetic biodiversity of littoral Cladocera in two types of acidic habitats in Germany: hard-water mining lakes and soft-water bog lakes

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by

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Berichte aus der Biologie

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Abstract

Ecological research on acidic lakes has historically focused on soft-waters, whereas biota in natural and man-made acidic hard-water lakes remains poorly studied. My thesis deals with the effects of multiple abiotic stressors on species and genetic biodiversity of littoral Cladocera in two types of acidic waters in Germany: hard-water mining and soft-water bog lakes. I found that these two types of acidic lakes supported highly distinct cladoceran communities, both with respect to their species diversity and composition, what led to the rejection of my null hypothesis that pH was the only abiotic factor, determining species diversity patterns. Further analyses revealed that, apart from pH, TDS-related parameters and trophic significantly affected species composition of littoral Cladocera in the studied lakes. Particularly the role of TDS and its constituents for colonization of acidic lakes was virtually neglected by previous studies, based on soft-water lakes' data sets. My study on the genetic diversity of a generalist cladoceran *Chydorus sphaericus* provided evidence for genetic erosion, i.e. negative effects of abiotic stress in the acidic hard-water mining lakes, but not in the acidic soft-water bog lakes. I conclude that the observed genetic erosion in acidic mining lakes may have negative long-term consequences for population persistence even in broadly tolerant species. In general, biodiversity at both species and genetic levels was much more impaired in the acidic hard-water mining lakes, compared to the naturally acidic soft-water bog lakes. This was possibly due to both ecological (higher abiotic stress) and evolutionary (insufficient time for long-term adaptations to evolve) mechanisms. My results suggest that colonization of acidic hard- and soft-water lakes in each case should require specific physiological adaptations at both species and population levels. The practical relevance of my studies is that they provide the scientific basis for monitoring of acidic hard-water mining lakes based on littoral Cladocera. Especially genetic diversity proved a valuable indicator of water quality in acidic mining lakes, as it provided unique information on population-level responses, being complementary to species diversity assessment. Overall, my results demonstrate that inclusion of acidic hard-water lakes into the research framework as well as comparisons among communities in chemically diverse acidic lakes can result in important new insights, concerning the community organization and adaptations in acidic environments.

Keywords: extreme environments, acidic mining lakes, bio-indication, evolutionary toxicology, Chydoridae, *Chydorus sphaericus*

Zusammenfassung

In der Vergangenheit konzentrierte sich die ökologische Forschung über saure Seen auf Weichwasserseen, während Lebensgemeinschaften in natürlichen oder künstlichen sauren Hartwasserseen kaum untersucht wurden. Meine Dissertation behandelt die Effekte multipler abiotischer Stressoren auf die Arten- und genetische Diversität litoraler Cladoceren in zwei unterschiedlichen Typen saurer Gewässer: Hartwasser-Tagebauseen und Weichwasser-Moorseen. Meine Ergebnisse haben gezeigt, dass die beiden sauren Gewässertypen sehr unterschiedliche Cladoceren-Gemeinschaften beherbergen. Deswegen musste die Null-Hypothese, dass der pH-Wert als einziger abiotischer Faktor die Arten-Diversität in sauren Seen bestimmt, abgelehnt werden. Weiterhin zeigte sich, dass neben dem pH-Wert TDS (total dissolved solids)-bezogene Parameter und die Trophie signifikant die Artenzusammensetzung der litoralen Cladoceren beeinflussten. Da sich die Untersuchungen bisher auf Weichwasserseen konzentrierten, wurde insbesondere die Rolle der TDS für die Besiedlung von sauren Gewässern allgemein nicht angemessen berücksichtigt. Meine Untersuchung zur genetischen Diversität eines Generalisten, der Cladocere *Chydorus sphaericus*, ergab Hinweise auf genetische Erosion als Folge von starkem abiotischen Stress in sauren Hartwasser-Tagebauseen, während ähnliches für saure Weichwasser-Moorseen nicht festgestellt werden konnte. Durch genetische Erosion könnte es langfristig selbst bei sehr toleranten Arten zu einem Aussterben der betroffenen Populationen kommen. Insgesamt war die Biodiversität sowohl auf Arten- als auch auf genetischer Ebene in sauren Hartwasser-Tagebauseen viel stärker beeinträchtigt als in sauren Weichwasser-Moorseen. Dafür waren wahrscheinlich neben ökologischen Faktoren, auf Grund stärkerem abiotischen Stress, auch Evolutionsmechanismen, wegen einer zu kurzen Zeit, um Adaptationen in den jungen Tagebauseen zu entwickeln, verantwortlich. Anhand meiner Ergebnisse kann vermutet werden, dass jeweils spezielle physiologische Anpassungen auf Populations- und Art-Ebene für die Besiedlung von sauren Hart- und Weichwasserseen erforderlich sind. Durch diese neuen wissenschaftlichen Grundlagen könnte das Monitoring von sauren Hartwasser-Tagebauseen mithilfe litoraler Cladoceren verbessert werden. Insbesondere die genetische Diversität wäre in Ergänzung zur Artendiversität ein wichtiger Indikator für die Wasserqualität von sauren Hartwasser-Tagebauseen, weil nur sie auch Informationen über Reaktionen auf Populationsebene liefert. Zusammenfassend zeigen meine Ergebnisse, dass wichtige neue Erkenntnisse über Besiedlungsmechanismen und Anpassungen an saure Lebensräume durch die Berücksichtigung von sauren Hartwasserseen und den Vergleich von Lebensgemeinschaften in chemisch unterschiedlichen sauren Seentypen erzielt werden können.

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List of papers

This thesis is based on the following papers, which are referred to in the text by their Chapter numbers:

BIODIVERSITY AND ECOLOGY OF ZOOPLANKTON IN ACIDIC MINING LAKES

- I. Belyaeva, M. & R. Deneke, 2013. The biology and ecosystem of acidic pit lakes: Zooplankton. In W. Geller, M. Schulze, R. Kleinmann & C. Wolkersdorfer (Eds), *Acidic Pit Lakes—the Legacy of Coal and Metal Surface Mines*. Environmental Science Series, Springer-Verlag, Berlin: 117–125. ([Chapter 2](#))

SPECIES DIVERSITY OF LITTORAL CLADOCERA IN ACIDIC HARD- AND SOFT-WATER LAKES

- II. Belyaeva M. & R. Deneke, submitted. Multiple stressors for biodiversity in acidic lakes: Evidence from comparison of littoral cladoceran communities from hard- and soft-water lakes. ([Chapter 3](#))
- III. Belyaeva M. & R. Deneke, 2007. Colonization of acidic mining lakes: *Chydorus sphaericus* and other Cladocera within a dynamic horizontal pH gradient (pH 3–7) in Lake Senftenberger See (Germany). *Hydrobiologia* 594: 97-108. ([Chapter 4](#))

GENETIC DIVERSITY OF A TOLERANT CLADOCERAN SPECIES, CHYDORUS SPHAERICUS, IN ACIDIC HARD- AND SOFT-WATER LAKES

- IV. Belyaeva M. & D. J. Taylor, 2009. Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology. *Molecular Phylogenetics and Evolution* 50: 534-546. ([Chapter 5](#))

- V. Belyaeva M. & D. J. Taylor, submitted. Genetic erosion in a littoral cladoceran species, colonizing extreme acidic mining lakes, and the colonization sources for the acid tolerant populations. (Chapter 6)

Authors' contributions

Papers III and IV are reproduced with kind permissions from the publishers.

The original ideas of all studies belongs to me, and I also have carried out all of the practical work (unless otherwise stated), and have written all papers. Dr. Rainer Deneke helped with sampling, experiments and with a part of the statistical analysis (regression curves) for the Chapters 3 and 4, and he also discussed with me the content of Chapters 2, 3, and 4. Dr. Derek Taylor provided the working place and materials and discussed the ideas presented in Chapters 5 and 6 with me.

Chapter 1

General introduction

Biological responses to acidification have been extensively studied for continental soft-waters, whose biodiversity was severely damaged by atmospheric industrial pollution since 1970s (e.g. Schindler, 1988; Havas & Rosseland, 1995; Nierzwicki-Bauer et al., 2010). Consequently, the current knowledge on the effects of low pH on freshwater communities, especially with regard to lentic waters, is largely based on the prevalent data on soft-water bodies. However, acidic waters on Earth cover a much broader range of abiotic conditions (Geller & Schultze, 2009). In particular, investigations on so far poorly studied acidic hard-water lakes may provide valuable insights into the community organisation and ecological adaptations in acidic environments. Whereas earlier studies considered pH to be the major abiotic factor limiting colonization in acidic waters, a multiple stressor view is currently emerging (Yan et al., 2008). There is accumulating evidence that other environmental gradients, apart from pH, may determine diversity patterns in acidic waters (Christensen et al., 2006; Yan et al., 2008; Keller; 2009; Ormerod et al., 2010; Valois et al., 2011). Despite these recent works, a detailed understanding of the roles of multiple abiotic factors for colonization of acidic lakes has not yet been achieved. Studies on acidic streams have demonstrated that comparing communities in chemically diverse acidic habitats can be fruitful for disentangling the effects of multiple abiotic factors (Petrin et al., 2008), yet, such comparative studies on acidic lakes are virtually lacking. Therefore, in my thesis I compared biodiversity patterns of littoral Cladocera in so far poorly studied acidic hard-water mining lakes and in acidic soft-water bog lakes in Germany (Fig. 1.1), aiming to elucidate the effects of multiple abiotic stressors.



Figure 1.1 Typical appearance of the two types of acidic lakes, occurring in Germany: a hard-water mining lake Bärwalder See (left) and a soft-water bog lake Große Fuchskuhle (right), surveyed in this thesis. Note the typical reddish colour of water of the acidic mining lake due to iron compounds and the typical brown water in the bog lake due to high cDOC concentration. Photos: left—Mike Hemm, right—original

1.1 Living conditions in acidic hard- and soft-water lakes: Multiple abiotic stressors for biodiversity

Acidic hard-water lakes are extreme freshwater environments, characterized by rather peculiar abiotic conditions (Geller & Schultze, 2009). Such lakes of natural origin are rare, mostly occurring in the regions, influenced by volcanic activity; however, man-made acidic hard-water lakes have recently become numerous all over the world due to closures of open-cast coal mines (Geller et al., 1998). The water chemistry of acidic mining lakes closely resembles that of naturally acidic volcanic lakes. In particular, oxidation of pyrites and other sulphur-containing minerals leads to high inputs of sulphuric acid as well as iron and aluminium ions (so-called acid rock drainage in the case of natural acidification or acid mine drainage (AMD) in the case of anthropogenic acidification), the metal ions Fe and Al serving as pH buffers within the range of 2 to 4 (Geller & Schultze, 2009). Acidic hard-water mining lakes are oligo- to mesotrophic, and concentrations of dissolved organic carbon (DOC) tend to be low here—mostly well below 10 mg l^{-1} (Friese et al., 2013), what is attributed to photochemical degradation coupled to formation of ferrous iron (Friese et al., 2002). In contrast, acidic soft-water lakes can cover a broad range of trophic conditions and they may also exhibit moderate to very high concentrations of DOC (humic bog lakes). Coloured DOC (cDOC) can mitigate metal toxicity in soft-water lakes, but at high concentrations it may itself be toxic for organisms (Steinberg et al., 2006). In acidic hard-water lakes the effects of DOC are negligible, instead, high water hardness due to calcium and magnesium ions may exhibit

a protective effect (Yim et al., 2006), whereas high total dissolved solids (TDS) concentrations in these lakes may be harmful for biota (Kennedy et al., 2005). Contrarily, in soft-waters organisms may experience osmotic stress due to low TDS (Aladin & Potts, 1995) and they may be further limited by low calcium concentrations (Jeziorski et al., 2008).

Apparently, rather distinct combinations of multiple abiotic stressors and toxicity mitigators act in acidic soft- and hard-water lakes (see the summary presented on Fig. 1.2). As the effects of these stressors on organisms are often interactive (Soucek et al., 2001), and the physiological thresholds to particular chemicals are likely to differ among taxa, the resulting biological impact cannot be easily predicted from lake water chemistry. Field surveys can provide integrative assessment of diverse direct and indirect effects of water chemistry parameters on communities, yet, field data on the biota in acidic hard-water lakes and in mining lakes in particular are very scarce (see Chapters 1.2.2 and 2), despite the world-wide commonness and potential economical value of these waters.

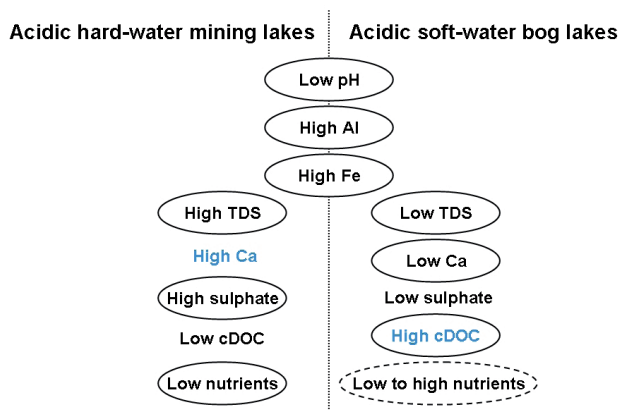


Figure 1.2 Multiple stressors for biodiversity in acidic hard- and soft-water lakes. Factors reaching values, which are stressful for biota in acidic lakes, are enclosed with ellipses. Common stressors for the two types of acidic lakes are in the middle position; unique stressors for each lake type are on the left and right side, respectively. Chemical parameters, mitigating the toxicity of protons and metal ions are shown in blue. Note that high concentration of cDOC can be a stressor and a mitigator at the same time. It should be also noted that nutrients' level may be stressful for organisms at its extremes (oligotrophic or eutrophic state) in acidic soft-water lakes, yet, intermediate values, which are not stressful, may also occur

1.2 Biodiversity of littoral Cladocera in acidic lakes

It is common knowledge that acidification negatively impacts biodiversity in freshwaters (e.g. Brakke et al., 1994). Yet, the magnitude of this impact may vary greatly, depending on the particular taxonomic group and the ambient environmental conditions. This picture is further complicated by that abiotic stressors may affect different levels of biodiversity (Fig. 1.3). The Convention on Biological Diversity, adopted in Rio de Janeiro in 1992, defined three levels of biodiversity: intraspecific genetic diversity, species diversity and diversity of ecosystems. Most studies on the biological effects of acidification have so far focused on the species and ecosystem levels of biodiversity, whereas acidification consequences on the genetic level have been rarely addressed (but see Derry et al., 2009). Yet, the impact of the intraspecific genetic diversity on the ecological processes in ecosystems may be comparable with that of species diversity (Hughes et al., 2008). Furthermore, the genetic level of biodiversity is a basis for microevolutionary processes, which may eventually result in adaptations of populations and species to novel stressors, e.g. those of anthropogenic origin, and thus ensure long-term persistence of species and populations (Bagley et al., 2002; Hoffmann & Willi, 2008). Integrating data on stress effects at different biodiversity levels, although rarely done, can provide a more holistic understanding of ecological processes and help to find appropriate measures to counteract the anthropogenic threats to freshwater ecosystems (Geist, 2011). In my thesis I assessed acidification effects on littoral Cladocera at two biodiversity levels: species diversity (Chapters 2–4) and intraspecific genetic diversity of a tolerant cladoceran species (Chapters 5 and 7.4).

1.2.1 Littoral Cladocera as acidity indicators

Littoral Cladocera represent one of the major taxonomic groups colonizing freshwaters, both in terms of their species diversity and secondary production (Walseng et al., 2006; Lemke & Benke, 2009). Albeit the boundary between littoral and pelagic zooplankton communities is in fact not very sharp (Walseng et al., 2006), here I used the term ‘littoral Cladocera’ to refer to the assemblages sampled in the littoral zone of lakes, including both substrate-associated and near-shore open-water species. Littoral Cladocera were found to be sensitive indicators of environmental changes, including acidification (Jeppesen et al., 2001; Walseng et al., 2001, 2003; De Eyto et

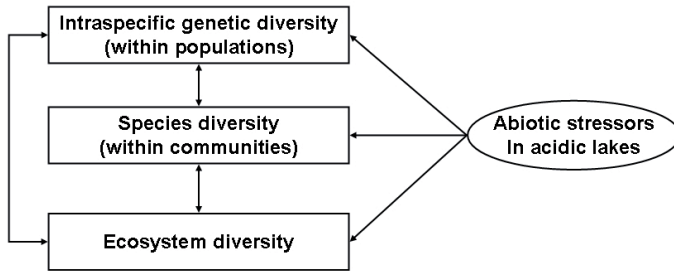


Figure 1.3 Biological response levels to abiotic stressors in acidic lakes. Each of the three biodiversity levels (genetic, species and ecosystem diversity) is affected by abiotic stressors. Furthermore, reciprocal effects among different biodiversity levels should be common, according to Vellend & Geber (2005) and Hughes et al. (2008), see also Chapter 7

al., 2003). Indeed, cladoceran species vary widely in their acidity tolerance from very sensitive species, disappearing at $\text{pH} < 6$, to extraordinary tolerant ones, thriving at $\text{pH} 3$ (Bērziņš & Bertilsson, 1990; Deneke, 2000). While the pelagic zooplankton species have not been recorded in acidic hard-water lakes at pH below 4.5, several littoral taxa can tolerate the harsh abiotic conditions occurring there (Deneke, 2000; Chapter 2). Pilot studies suggest that littoral zooplankton can be valuable bio-indicators in acidic hard-water lakes (Nixdorf et al., 1998, 2005), but no detailed data are yet available on the cladoceran colonization patterns in relation to water chemistry in acidic mining lakes.

1.2.2 How does acidification affect biodiversity of Cladocera at the species and genetic level? The current state of knowledge and hypotheses

Acidification effects on species diversity

Species diversity is a fundamental characteristic of ecosystems, as it determines their functioning and long-term stability (Purvis & Hector, 2000). Although acidification commonly leads to species diversity losses in many taxonomic groups, some tolerant groups do exist, for example, corixid insects, which appear to be unaffected by low pH and high metal concentrations in acidic lakes (Henrikson & Oscarson, 1985; Wollmann et al., 2000). As regards Cladocera, acidification in most cases affects their abundances, biomass, community composition and dominance patterns (Hann & Turner,

2000; Jeppesen et al., 2001; Walseng et al., 2003, 2008; Korosi & Smol, 2012). Yet, the empirical evidence for the acidification impact on a basic diversity parameter, species richness, has been so far equivocal. Whereas some studies found that in this group species richness was significantly correlated with pH (Fryer, 1980; Krause-Dellin & Steinberg, 1986; Walseng et al., 2003), other studies detected no such correlation (Uimonen-Simola & Tolonen, 1987; Arvola et al., 1990; Nilssen & Sandøy, 1990; Hann & Turner, 2000). The reasons for these discrepancies may be methodological (i.e. sampling bias) and/or be due to the fact that different studies surveyed lakes, which were chemically distinct from each other and/or situated in different biogeographical regions. In Chapters 3 and 4 I attempted to tackle both the methodological and water chemistry issues.

Whereas patterns of cladoceran colonization in acidic soft-water lakes are rather well documented, data on acidic hard-water lakes are still very scarce (reviewed in Chapter 2). Those few studies, which are available (Uéno, 1934, 1958; Lackey, 1939; Nixdorf et al., 1998, 2005), do indicate that species diversity in acidic hard-water lakes is likely to be reduced compared to acidic soft-water lakes. Based on the results of these studies on acidic hard-water lakes and analogous to the results of more comprehensive reports on the communities in acidic streams, I can hypothesize that firstly, colonization of acidic soft- and hard-waters would in each case require different physiological adaptations, and secondly, for both ecological and evolutionary reasons, the living conditions in acidic hard-water lakes should be more stressful for organisms, compared to acidic soft-water lakes, resulting in lower biodiversity in the former lakes. The ecological explanation of low species diversity in acidic hard- as well as soft-water lakes involves multiple abiotic stressors. Multiple stressors' paradigm is currently emerging in acidic lakes' research (Yan et al., 2008), and a number of recent publications have considered the effects of additional abiotic factors, apart from pH, for biological colonization (Ormerod et al., 2010; Valois et al., 2011; Korosi & Smol, 2012); however, these effects remain poorly understood up to now. In my work I adopted the comparative approach from the studies on acidic streams, which explicitly compared communities in chemically diverse acidic habitats in order to reveal the effects of other water chemistry parameters, apart from pH (Dangles et al., 2004; Petrin et al., 2007, 2008; Greig et al., 2010). Furthermore, the evolutionary species pool hypothesis (Pither & Aarssen, 2005) predicts that species diversity should increase over time in evolutionary old habitats, such as acidic soft-water lakes, even despite ecological stress, whereas lower diversity is expected in evolutionary

young habitats, where no long-term adaptation could occur, such as acidic hard-water mining lakes.

Acidification effects on genetic diversity

Intraspecific genetic diversity is a key aspect, determining the ability of a species to adapt to various environmental conditions. Anthropogenic pollution, including acidification, can cause seemingly subtle changes at the genetic level of biodiversity, yet these may have large and unpredictable long-term consequences (Bickham, 2011). The genetic erosion hypothesis (van Straalen & Timmermans, 2002) predicts that under exposure to chemical stress selection would remove (numerous) sensitive genotypes, favouring few tolerant genotypes. In the absence of stress, such tolerant genotypes are commonly rare in natural populations, because of the fitness costs, often associated with physiological adaptations to adverse abiotic conditions (Agra et al., 2010). That's why exposure to toxic chemicals would then cause drastic reductions of effective population sizes and genome-wide diversity of local populations, thus, increasing the risk of extinction (van Straalen & Timmermans, 2002), especially when populations are subjected to further stressors (Lopes et al., 2009; Jansen et al., 2011). On the other hand, analogous to the evolutionary species pool hypothesis (see Chapter 3 for details), genotypic diversity in a particular habitat type will possibly increase over time due to adaptation. Thus, I hypothesize that evolutionary old acidic bog lakes should support higher genetic diversity than do evolutionary young acidic mining lakes, unless the latter lakes are colonized from natural acidic hard-water lakes via long-distance dispersal.

Before assessing the effects of abiotic stress on genetic diversity, the patterns of natural genetic variation in the absence of stress should be taken into account (Belfiore & Anderson, 2001). Cyclic parthenogenetic life cycle of Cladocera may have important consequences for their population genetic structure and colonization success in extreme environments. The monopolization hypothesis (De Meester et al., 2002) suggests that animals with this life cycle should have greater potential for rapid adaptation to stressful environmental conditions compared to obligate sexuals. On the other hand, cyclic parthenogens may be particularly sensitive to genetic erosion, as the genetic diversity within local populations is already rather low due to monopolization effects (De Meester et al., 2002). The empirical evidence for the genetic erosion in cyclic parthenogens, colonizing acidic lakes, has been equivocal. To my knowledge, no such data are available on

acidic soft-water lakes, whereas a study on field populations of a pelagic cladoceran species *Daphnia longispina* at sites, only weakly impacted by AMD (pH 6–7, conductivity 272–437 $\mu\text{S cm}^{-1}$, indicating non-elevated TDS concentration), detected no genetic erosion (Martins et al., 2009). Conversely, Lopes et al. (2009) did detect genetic erosion due to AMD in laboratory clonal mixtures of *D. longispina*, however, these results await validation by field studies. It should be noted, that both studies, mentioned above, were able to test for genetic erosion only at weakly acidic conditions, which are not typical for mining lakes (Nixdorf et al., 2003). These studies were further limited in that they used *Daphnia* as a test object, which is a model taxon, yet, it is rather sensitive to low pH and other AMD-related stressors, and in fact this taxon was never recorded from acidic mining lakes at pH < 6 (Chapter 2). Therefore, before my work, it had remained unclear, whether the genetic diversity of tolerant cladoceran species, which do colonize highly acidic mining lakes, is negatively affected by multiple abiotic stressors in these water bodies. In order to test for genetic erosion, I used a tolerant cladoceran species, *C. sphaericus*, which often dominates zooplankton communities in acidic mining lakes at pH 3–6 (Chapter 2).

1.3 Objectives

The general objective of this thesis was to study the biodiversity and colonization patterns of littoral Cladocera in the two types of acidic waters in Germany—man-made hard-water mining lakes and natural soft-water bog lakes in order to address the following question:

What are the consequences of multiple abiotic stress for the genetic and species diversity of littoral Cladocera in acidic hard- and soft-water lakes?

The specific aims and research questions, tackled in my thesis, are listed below.

In Chapter 2 I review the literature on the diversity and ecology of zooplankton (Rotifera, Cladocera and Copepoda) in acidic mining lakes, addressing the following question:

What are the general patterns and processes for the diversity and ecology of zooplankton in acidic hard-water mining lakes?

Besides, in Chapter 2 I identify the knowledge gaps, which are then partly addressed in the subsequent chapters of my thesis.

Chapters 3 and 4 focus on the species level of the biodiversity of littoral Cladocera in acidic hard- and soft- water lakes in Germany. To my knowledge, this is the first detailed study on cladoceran communities in acidic hard-water mining lakes and also the first explicit comparison among the communities in acidic hard- and soft-water lakes, aiming to disentangle the roles of multiple abiotic factors for colonization. In Chapter 3 I tested the null hypothesis that pH was the major abiotic factor, determining cladoceran colonization patterns in acidic lakes. This hypothesis predicts similarity of community compositions among the acidic hard- and soft-water lakes after accounting for the effect of pH. Alternatively, multiple abiotic stressors would cause significant differences among the cladoceran communities in the two types of acidic lakes. After having rejected the null hypothesis, I address the following questions:

What abiotic stressors determine colonization patterns by littoral Cladocera in the two types of acidic lakes?

What are the characteristic features of littoral cladoceran communities colonizing acidic hard- and soft-water lakes?

What are the implications for the ecological quality assessment of acidic mining lakes using littoral Cladocera?

Chapter 4 describes the changes occurring in the littoral cladoceran community along the horizontal pH gradient from 3 to 7 in a mining lake, and it addresses the following questions:

How does mining lake water chemistry affect species-specific pH thresholds of littoral Cladocera?

*Can the supposedly broad pH tolerance of a single cladoceran species (*C. sphaericus*) be shown experimentally?*

Chapters 5 and 6 focus on the genetic diversity of the most acid tolerant cladoceran species—*C. sphaericus*, which often dominates cladoceran com-

munities in acidic hard-water mining lakes. As the genetic diversity of this taxon has never been studied before my thesis, I first establish a phylogenetic framework for the diversity of the *C. sphaericus* species complex, by addressing the following question in Chapter 5:

What are the genetic and morphological diversity patterns in the C. sphaericus species complex at the Holarctic scale?

Based on this background knowledge on the large-scale genetic diversity patterns, I then investigate the effects of abiotic stressors on the neutral genetic diversity of *C. sphaericus* populations in German acidic lakes and I address the following questions in Chapter 6:

How does abiotic stress in acidic hard- and soft-water lakes affect the population genetic structure of C. sphaericus? Is there evidence for 'genetic erosion'?

What are the colonization sources for the acid tolerant populations?

In Chapter 7 I provide a synthesis and a general discussion of the results of my thesis. Future research directions are discussed in Chapter 8.

Chapter 2

Biology and ecosystem of acidic pit lakes: Zooplankton

2.1 Relationship of species occurrence and taxonomic diversity to pH

Species richness of metazoan zooplankton is typically very low in acidic pit lakes, as few species can tolerate their harsh abiotic conditions (Nixdorf et al., 1998). As in other extreme environments, one or very few species dominate, resulting in low community evenness. Regarding the taxonomic composition of zooplankton in acidic pit lakes at pH 2.3–4.5, rotifers clearly outnumber crustaceans. Cladocera are represented by few species, but they can be important colonizers in lakes with pH ≥ 3 , whereas Copepoda are rare (Deneke, 2000).

Despite unfavorable abiotic conditions, even extremely acidic lakes are colonized by zooplankton: at pH 2.3, two rotifer species—*Cephalodella hoodi* and *Rotaria rotatoria*—were observed in the pelagic zone of Lake Plessa 107—a pit lake in northeast Germany (Lessmann et al., 1999). These two species, together with *Elosa worallii*, *Cephalodella gibba*, and *Brachionus sericus* constitute the core zooplankton community in highly acidic pit lakes with pH around 3 (Deneke, 2000). A number of rotifer species from the genera *Lecane*, *Colurella*, *Trichocerca*, *Lepadella*, and *Philodina* are occasionally found in highly acidic pit lakes, but they are never very abundant (reviewed in Deneke, 2000). All of the acid-tolerant rotifer species also occur in less acidic pit lakes (Wollmann et al., 2000), above their species-specific lower

in situ pH thresholds (Table 2.1); however, they disappear from the pelagic zone of neutral and alkaline pit lakes, being replaced by a species assemblage typical of natural non-acidic lakes (Nixdorf et al., 1998). *Chydorus sphaericus* (Cladocera, Chydoridae) is the most acid-tolerant crustacean species that colonizes acidic pit lakes, with an in situ pH threshold of 3.0 (Wollmann et al., 2000). At pH > 3.5, two more crustaceans occur in acidic pit lakes—another cladoceran, *Scapholeberis mucronata*, and the most tolerant cyclopoid copepod, *Diacyclops* sp. (Wollmann et al., 2000). Remarkably, calanoid copepods that often dominate zooplankton communities in soft-water acidic lakes (Brett, 1989) are completely lacking in acidic pit lakes.

The majority of acidic pit lakes fall into the pH range of 2–4.5. At pH 4.5–6, chemical conditions become unstable due to the lack of a buffer system; thus, weakly acidic pit lakes are rare (Nixdorf et al., 2003). Hence, field studies on colonization patterns along the entire pH gradient from acidic to circumneutral conditions in pit lakes are methodologically hampered. The very few pit lakes with a pH range of 4.5–6 are recently or incompletely neutralized lakes, where water chemistry has not yet stabilized. These lakes are valuable sites to study colonization patterns and ecology of colonizing species under weakly acidic pit lakes conditions. For example, littoral Cladocera have been studied within a horizontal pH gradient in Lake Senftenberger See, formed within a channel connecting the acidic (pH 3) and the neutral (pH 7.5) basins of the lake (see Chapter 4). Only two cladoceran species, *C. sphaericus* and *S. mucronata*, were found at pH below 4.9, whereas rather diverse communities occurred at pH \geq 4.9 (Table 2.1). Dramatic changes in cladoceran species composition were observed within a distance of several hundred meters along the pH gradient, despite the lack of any physical barriers that might have limited the dispersal of species. Thus, the loss of species towards the acidic part of the lake can be attributed to the adverse effects of the chemical conditions. To our knowledge, no published studies report on the species composition of Copepoda and Rotifera within the pH range of 4.5–6 in pit lakes, although both groups do occur at these conditions (Belyaeva & Deneke, unpubl.; Wöflf et al., 1998).

Table 2.1 Zooplankton species occurring in acidic pit lakes and the lowest in situ pH at which they were recorded. Classification of lakes is according to Nixdorf et al. (2005), slightly modified. Core species that dominate zooplankton communities in acidic pit lakes are underlined. Lakes are in Germany unless otherwise specified

Taxonomic group	Species	pH	Sampling site	Reference
	Extremely acidic pit lakes			
Rotifera, Monogononta	<i>Cephalodella hoodi</i> (Gosse, 1896)	2.3	Lake Plessa 107	Lessmann et al., 1999
Rotifera, Bdelloidea	<i>Rotaria rotatoria</i> (Pallas, 1766)	2.3	Lake Plessa 107	Lessmann et al., 1999
Rotifera, Monogononta	<i>Cephalodella gibba</i> (Ehrenberg, 1838)	2.5	Pit lakes, Illinois, USA	McConathy & Strahl, 1982
Rotifera, Bdelloidea	<i>Philodina</i> sp.	2.5	Pit lakes, Illinois, USA	McConathy & Strahl, 1982
Rotifera, Monogononta	<i>Elosa worallii</i> Lord, 1891	2.6	Lake Plessa (ML 111)	Wollmann et al., 2000
Rotifera, Monogononta	<i>Lecane</i> (Monostyla) <i>lunaris</i> Ehrenberg 1832	2.6	Pit lakes in Lusatia	Deneke, unpubl.
	Very acidic pit lakes			
Rotifera, Monogononta	<i>Brachionus urceolaris</i> O.F. Müller, 1773*	2.6	Pit lakes, Illinois, USA	McConathy & Strahl, 1982
Rotifera, Monogononta	<i>Coturella</i> sp.	2.7	Pit lakes in Lusatia	Deneke, unpubl.
Rotifera, Monogononta	<i>Trichocerca similis</i> Wierzejski, 1893	2.7	Pit lakes in Lusatia	Deneke, unpubl.
Rotifera, Monogononta	<i>Lecane stichaea</i> Harring, 1913	3.0	Pit lakes in Lusatia	Deneke, unpubl.
Rotifera, Monogononta	<i>Lepadella</i> sp.	3.0	Pit lakes in Lusatia	Deneke, unpubl.
Rotifera, Monogononta	<i>Lecane</i> (Monostyla) <i>bullia</i> (Gosse 1886)	3.0	Pit lakes, Illinois, USA	McConathy & Strahl, 1982
Cladocera, Chydoridae	<i>Chydorus sphaericus</i> (Müller, 1776)	3.0	Grünewalder Lauch (ML 117)	Wollmann et al., 2000
	Moderately acidic pit lakes			
Cladocera, Daphniidae	<i>Scapholeberis mucronata</i> (Müller, 1776)	3.5	Geierswalder See	Belyaeva & Deneke, unpubl.
Copepoda, Cyclopoidae	<i>Diatylops</i> sp.	3.5	Felixsee	Wollmann et al., 2000
	Weakly acidic pit lakes			
Rotifera, Monogononta	<i>Polyarthra</i> sp.	4.7	Gräbendorfer See	Deneke, unpubl.
Rotifera, Monogononta	<i>Keratella cochlearis</i> (Gosse, 1851)	4.7	Gräbendorfer See	Deneke, unpubl.

* Although this species was recorded from North American pit lakes as *Brachionus urceolaris*, it is likely *B. sericans*, which appears to be much more acid-tolerant than the former species. The observed minimum in situ pH of 2.6 for the North American populations differs from that in German lakes, where this species has only been found at a pH of 2.7 and above (Deneke, 2000)

Table 2.1 – (continued)

Taxonomic group	Species	pH	Sampling site	Reference
Rotifera, Monogononta	<i>Keratella testudo</i> (Ehrenberg, 1832)	4.7	Gräbendorfer See	Denéke, unpubl.
Rotifera, Monogononta	<i>Filinia longiseta</i> (Ehrenberg, 1834)	4.7	Gräbendorfer See	Denéke, unpubl.
Cladocera, Daphniidae	<i>Ceriodaphnia</i> sp.	4.7	Gräbendorfer See	Denéke, unpubl.
Cladocera, Bosminidae	<i>Bosmina coregoni</i> Baird, 1857	4.7	Gräbendorfer See	Denéke, unpubl.
Cladocera, Chydoridae	<i>Eurycerus lamellatus</i> (Müller, 1776)	4.9	Senftenberger See	see Chapter 4
Cladocera, Chydoridae	<i>Pleuroxus truncatus</i> (Müller, 1785)	4.9	Senftenberger See	see Chapter 4
Cladocera, Bosminidae	<i>Bosmina longirostris</i> (Müller, 1785)	5.0	Senftenberger See	see Chapter 4
Cladocera, Sidae	<i>Sida crystallina</i> (Müller, 1776)	5.0	Senftenberger See	see Chapter 4
Cladocera, Chydoridae	<i>Alona affinis</i> (Leydig, 1860)	5.0	Senftenberger See	see Chapter 4
Cladocera, Chydoridae	<i>Acroperus harpae</i> (Baird, 1835)	5.7	Senftenberger See	see Chapter 4
Cladocera, Chydoridae	<i>Alona costata</i> Sars, 1862	5.7	Senftenberger See	see Chapter 4
Cladocera, Daphniidae	<i>Ceriodaphnia pulchella</i> Sars, 1862	5.7	Senftenberger See	see Chapter 4
Cladocera, Polyphemidae	<i>Polyphemus pediculus</i> (Linne, 1761)	5.7	Senftenberger See	see Chapter 4
Cladocera, Chydoridae	<i>Alona guttata</i> Sars, 1862	5.7	Senftenberger See	see Chapter 4
Cladocera, Sidae	<i>Diaphanosoma brachyurum</i> (Liévin, 1848)	5.7	Senftenberger See	see Chapter 4
Cladocera, Daphniidae	<i>Simoccephalus vetulus</i> (Müller, 1776)	6.1	Senftenberger See	see Chapter 4

Most zooplankton species that are found in acidic pit lakes have been recorded from different continents and appear to be cosmopolitan. However, it should be noted that most data on zooplankton communities come from studies on pit lakes in Germany, while less is known about the biota of these habitats outside Europe. *C. hoodi*, *C. gibba*, and *B. sericus* have been recorded in both European and North American acidic pit lakes (Deneke, 2000). There is a lack of detailed taxonomic studies confirming the co-specificity of geographically distant populations. Particularly, uncertainties exist concerning the taxonomic status of *B. sericus*. Although Rousselet (1907) described *B. sericus* as a separate species, it was often later regarded as a variety of *B. urceolaris* (e.g. Koste, 1978). Hence, some records of *B. sericus*, *B. urceolaris* var. *sericus*, and *B. urceolaris* by different authors may possibly refer to the same taxon. If so, it colonizes acidic hard-water lakes separated by large geographical distances, being also found in Australian billabong ponds affected by mine drainage (Tait et al., 1984), geogenically acidified lakes in Japan (Uéno, 1958), and in the Canadian Arctic ponds acidified by burnt bituminous shales (Havas & Hutchinson, 1983). On the other hand, *Elosa worallii* is very common in acidic hard waters, but only in Europe (Nixdorf et al., 1998; Vallin, 1953), while a detailed study of North American pit lakes by McConathy & Stahl (1982) did not detect this species. *C. sphaericus* has been recorded in hard-water acidic lakes on different continents (Deneke, 2000). Yet, these populations probably belong to distinct, though closely related species (see Chapter 5).

Volcanic and coastal sulphur lakes are natural analogues of acidic pit lakes, with similar water chemistry (Geller et al., 1998); they are also inhabited by similar zooplankton communities (Deneke, 2000). However, there are some differences in species occurrence patterns, e.g. occurrence of copepods at pH around 3 in volcanic lakes in Japan (Uéno, 1958), but not in pit lakes. This may be related to adaptation of species in the regions, where hard-water acidic lakes have existed over evolutionary relevant time scales.

2.2 Factors influencing colonization of acidic pit lakes by zooplankton

Zooplankton species richness and community composition in acidic pit lakes are both highly predictable from the lake pH value (Deneke, 2000). The strong correlation between zooplankton species richness and pH (Fig. 2.1) exists despite considerable variability in lake morphology, mixis regime, and

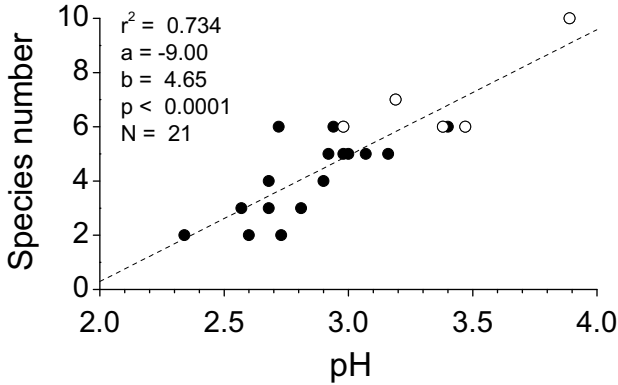


Figure 2.1 Zooplankton species richness along a pH gradient in acidic pit lakes, based on monthly samples from 21 lakes in Lusatia, Germany, over a period of at least one year. Indicated are parameters of linear least square regression fitted to the data. Filled circles indicate that the communities lacked crustaceans; open circles indicate that crustaceans were observed

trophic status of pit lakes. This, however, does not necessarily mean that high concentration of protons alone limits species occurrence. Generally, multiple abiotic stressors act in acidic lakes (Frost et al., 1999). Zooplankton species composition in acidic pit lakes differs greatly from that in soft-water acidic lakes, which indicates that other water chemistry parameters might be crucial for biological colonization. Laboratory tests with cladocerans have shown that aluminum, iron, and other heavy metal ions (Lopes et al., 1999; Soucek et al., 2001) as well as high sulfate content (Kennedy et al., 2005) can contribute to the toxicity of waters affected by acid mine drainage. Metal ions may be elevated in soft-water acidic lakes as well, but their toxicity is mitigated by colored dissolved organic carbon (cDOC; Williamson et al., 1999). As cDOC content is usually very low in acidic pit lakes (Friese et al., 2002), its beneficial effects are diminished. Therefore, abiotic conditions in acidic pit lakes should be more toxic for organisms than those in naturally acidic lakes at the same pH values. Indeed, in our study on littoral Cladocera (see Chapter 4), we reported higher species-specific in situ pH thresholds for most species in a pit lake vs. soft-water acidic lakes (Fig. 2.2). As a result, species diversity in acidic pit lakes is expectedly lower than in naturally acidic soft-water lakes.

Acidity reflects buffering capacity, i.e. the stability of pH conditions. Soft-water lakes are rather poorly buffered, and spatial as well as seasonal

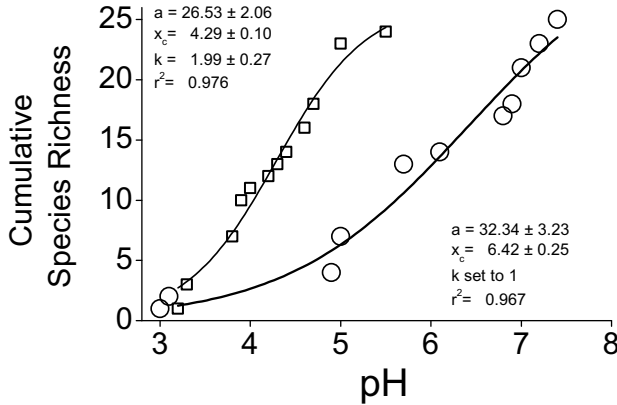


Figure 2.2 Logistic regression curves for the potential cumulative species richness of littoral Cladocera within a horizontal pH gradient from 3 to 7 in a pit lake Senftenberger See (circles) and natural soft-water lakes (squares) based on in situ pH thresholds for individual species. Lower potential species richness predicted for acidic pit lakes, as compared to soft-water lakes, results from higher species-specific pH thresholds (modified from (see Chapter 4), with kind permission from Springer Science + Business Media)

pH fluctuations occur, whereas acidic pit lakes at $\text{pH} < 4.5$ are well-buffered and display very little within-lake variation in pH, with the exception of meromictic pit lakes. At $\text{pH} > 4.5$, soft-water lakes are buffered by cDOC, but in pit lakes, this buffer system is lacking, and so the pH is very unstable (Nixdorf et al., 2003). Precipitation of metal compounds occurs during pH changes, which can adversely affect organisms (see Chapter 4; Weatherley et al., 1991). This may occur, for example, during whole-lake neutralization or within mixing zones, where neutral river water enters a highly acidic pit lake. Thus, the detrimental effect of metal precipitation should be taken into account while planning neutralization measures.

Evidently, specific water chemistry in acidic pit lakes exerts profound effects on the zooplankton species diversity and composition. Secondary production and biomass in acidic lakes seem to be less affected, due to compensating shifts in biomass of tolerant species (Frost et al., 1995). On the other hand, food availability is believed to control zooplankton biomass in acidic lakes (Havens & Carlson, 1998; Nixdorf et al., 1998) as well as within-lake distribution (Weithoff, 2004) and seasonal dynamics of populations, but it is unlikely to limit species occurrence. Another limiting factor is, possibly, availability of micro-habitats in the littoral zone. Many acidic pit lakes are characterized by low macrophyte growth (Pietsch, 1998); hence,

plant-associated zooplankton species may be rare or completely excluded due to a lack of suitable micro-habitats. Biotic factors, such as predation and competition, play an important role in natural lakes. Yet, in acidic lakes, the importance of biotic factors decreases at low pH, where abiotic conditions become increasingly more stressful (Arnott & Vanni, 1993). In the most extremely acidic pit lakes (pH < 2.8), neither predation nor competition seem to be important (Weithoff, 2004), whereas at pH > 2.8, some effects of biotic interactions may be present, although water chemistry is still the major determinant of zooplankton community structure (Wollmann & Deneke, 2004).

Dispersal of zooplankton may be hindered by the young age and/or isolated character of many acidic pit lakes. However, no published data is available on the primary colonization of newly created pit lakes, and it is unknown how long it takes the core zooplankton community to get established. Another interesting question for future studies would be the genetic structure and exchange among populations colonizing acidic pit lakes, i.e. metapopulation dynamics. It is possible that it would be reduced due to isolation of pit lakes, yet, many zooplankton species colonizing these habitats are pioneer species with high dispersal capabilities (see below), which can enhance metapopulation dynamics.

2.3 Ecology of species colonizing acidic pit lakes

Anthropogenically stressed environments, such as acidic pit lakes, are commonly colonized by extremophilic specialists as well as generalist species with broad tolerance ranges to a number of environmental factors. In contrast to bacteria, algae, and protists, which are often represented by acidophiles in acidic pit lakes, the evidence for acidiphily among zooplankton is rather weak (but see Weithoff et al., 2013). Most of the zooplankton species occurring in acidic pit lakes have been also found in natural lakes at acidic and neutral conditions (Deneke, 2000), although they exhibit somewhat different abundance patterns and habitat preferences there (see below). Furthermore, core species from acidic pit lakes—*C. sphaericus*, *B. sericus*, *E. worallii*, and *C. hoodi*—cultured at ambient pH, could be successfully transferred to neutral pH under laboratory conditions (see Chapter 4; Deneke, unpubl.). Hence, zooplankton community in acidic pit lakes largely, if not exclusively, comprises generalist species. In contrast, there are possibly some specialists among zooplankton species colonizing acidic soft-water lakes that

have not been recorded in acidic pit lakes or in neutral hard-water lakes, e.g. the rotifers *Lecane acus*, *Habrotrocha lata*, *Keratella serrulata* (Bērzziņš & Pejler, 1987), and the cladocerans *Acantholeberis curvirostris* and *Streblocerus serricaudatus* (Flöbner, 2000). However, the designation of these species as specialists is derived from field occurrence data alone. It remains unclear whether their physiological optima lie under soft-water acidic conditions, or if they are simply weak competitors that are outcompeted in species-rich neutral lakes. Still, many soft-water acidic 'specialists' have not been found in species-poor acidic pit lakes, which suggests that they are not capable of tolerating the abiotic stresses there. The complete absence of specialists in acidic pit lakes is probably explained by insufficient time for adaptive evolution, since those are young habitats with peculiar abiotic conditions, and their natural counterparts, such as volcanic lakes, are rare.

Zooplankton species found in acidic pit lakes can be characterized as pioneers that also colonize newly created and perturbed water bodies. Pioneer species are r-strategists (meaning they have high growth rates) with efficient dispersal mechanisms, and they are able to use a wide range of food sources. For example, *C. sphaericus* is often the first crustacean species colonizing new sites (Louette et al., 2008); it is capable of using different food sources (De Eyto & Irvine, 2001), tolerates a wide range of abiotic conditions (see Chapter 4), and is often abundant in anthropogenically disturbed environments, e.g. highly eutrophic lakes (De Eyto, 2001). Despite their large potential for colonization, pioneer species are presumably weak competitors. Their mass development is only observed in species-poor communities, such as acidic pit lakes, or in natural lakes, when superior competitors are (temporarily) absent, e.g. *C. sphaericus* developing in the absence of *Daphnia* and copepod nauplii (Matveev, 1986).

Absence of pelagic forms and a simultaneous habitat shift of littoral species to the pelagic zone is a common observation in all kinds of acidic lakes, including soft waters (Nilssen, 1980; Nilssen & Wærvågen, 2003). This is probably due to the lack of competition and predation pressure under acidic conditions (Locke & Sprules, 2000). In European acidic pit lakes, no zooplankton species were found exclusively in benthic or littoral habitats (Deneke, 2000), although rotifers and crustaceans exhibited heterogeneous distributions within the water column (Weithoff, 2004; Wollmann & Deneke, 2004). In North American acidic pit lakes, two species, *Lecane bulla* and *Philodina* sp., were found only in benthic algal clumps, but not in plankton (McConathy & Stahl, 1982). The absence of large pelagic species in acidic pit lakes results in a shift towards species with body size < 500 μm . A

similar shift in size structure has been documented for naturally acidic and atmospherically acidified lakes (Schindler et al., 1985).

It is not quite clear what physiological adaptations of tolerant zooplankton species have led to successful colonization of acidic pit lakes. In general, organisms living at low pH are capable of maintaining a circum-neutral internal pH via passive or active mechanisms (Rothschild & Mancinelli, 2001). Additional challenge for colonization of pit lakes is seemingly provided by the high total ionic content and high metal concentrations typical of these waters. Hence, it is possible that survival of zooplankton in acidic pit lakes is ensured due to different physiological mechanisms than those that are employed in soft-water acidic lakes. There is still a lack of knowledge concerning particular mechanisms of ion regulation in metazoan zooplankton species under acidic hard-water conditions. A study on a rotifer, *C. hoodi*, from extremely acidic pit lakes failed to detect any additional physiological costs or specific ecological adaptations (Weithoff, 2005).

2.4 Ecological characteristics of zooplankton communities in acidic pit lakes

The major features of biological communities in acidic pit lakes are greatly reduced species diversity and extirpation of fish, so that the role of top predators is taken over by invertebrates (Wollmann et al., 2000). This leads to truncated and very simple food webs (Weithoff et al., 2013). In acidic pit lakes, zooplankton community composition is controlled by pH and related water chemistry parameters, whereas zooplankton biomass is possibly controlled by food availability (Wollmann & Deneke, 2004; Weithoff et al., 2013), as in soft-water acidic lakes (Havens & Carlson, 1998). Biotic interactions seem to be rather weak, at least in the most acidic pit lakes (Weithoff, 2004). However, some ecological patterns occurring in natural lake ecosystems, such as niche separation among different species (Weithoff, 2004), seasonal succession, and top-down and bottom-up effects on zooplankton (Wollmann & Deneke, 2004; Wollmann et al., 2000), have been observed in acidic pit lakes as well. On the other hand, zooplankton community composition is rather peculiar and unique in acidic pit lakes, what may result in rather specific biotic interactions.

Nixdorf et al. (2005) proposed a classification scheme for acidic pit lakes based on pH, chemical buffer system, and colonization patterns by phyto- and zooplankton (Table 2.2). The defined lake groups can also be viewed

Table 2.2 Classification of acidic pit lakes based on water chemistry and zooplankton colonization (modified from Nixdorf et al., 2005)

Lake group	pH	Conductivity mS cm ⁻¹	Acidity mmol l ⁻¹	Buffer system	Characteristic zooplankton species
Extremely acidic	< 2.8	> 3	> 15	iron	<i>Cephalodella hoodi</i> , <i>Elosa worallii</i>
Very acidic	2.8–3.5	1.5–3.0	> 1.6–15	iron	<i>Brachionus sericus</i> , <i>Chydorus sphaericus</i>
Moderately acidic	3.5–4.5	< 1.5	0–1.6	aluminium	<i>Diacyclops</i> sp., <i>Scapholeberis mucronata</i>
Weakly acidic	4.5–6.5	< 1.5	ca. 0.5	lacking buffer system	diverse littoral Cladocera

as successive stages of habitat maturation, as acidic lakes should become neutral with time due to natural alkalinity generation by chemical and biological processes. Characteristic core species occur in each group of lakes (Table 2.2), whose presence often has a pronounced effect on the food web structure and seasonal population dynamics (Wollmann & Deneke, 2004). In ‘extremely acidic’ pit lakes (pH < 2.8), the zooplankton community is dominated by two small rotifer species, *C. hoodi* and *E. worallii*, and potential community grazing rates are very low (less than 1 % per day). Hence, the impact on primary producers is negligible; indeed, no top-down effects on phytoplankton have been observed in extremely acidic pit lakes even during metazooplankton abundance peaks (Lessmann et al., 1999; Weithoff, 2004). Heliozoa play the role of top predators in the pelagic food web; however, it appears that they are not capable of exerting any noticeable effect on zooplankton (Weithoff, 2004).

In ‘very acidic’ pit lakes ($2.8 \leq \text{pH} < 3.5$), the appearance of larger zooplankton species with higher clearance rates—*B. sericus* and *C. sphaericus*—leads to top-down effects on phytoplankton (Wollmann & Deneke, 2004). As a result, a seasonal pattern is observed with a break-down of phytoplankton biomass following mass development of both zooplankton species in summer (Wollmann et al., 2000). Corixidae prey on both dominant zooplankton species here, while heliozoans disappear from pit lakes at pH > 2.8. As corixids can feed both on benthos and zooplankton, they enable benthic-pelagic coupling in the food webs (Wollmann et al., 2000).

In ‘moderately acidic’ pit lakes (pH 3.5–4.5), zooplankton communities are more diverse, including several additional rotifer species, a cladoceran *S. mucronata*, and copepods (Wollmann et al., 2000). The community composition is intermediate between the acidic and neutral pit lake communities (Wollmann & Deneke, 2004). Still, acid-tolerant species that are characteristic of very acidic pit lakes also dominate here. Weakly acidic pit lakes with pH > 4.5 are very rare due to the lacking buffer system.

The metazooplankton community can serve as a reliable and simple

biological indicator of water quality in acidic pit lakes (Nixdorf et al., 2005). Colonization patterns mostly reflect physiological thresholds of individual species. Hence, species composition and community structure is intimately related to chemical conditions, whereas variation due to biotic interactions and within-lake habitat heterogeneity is almost absent. Pit lakes with similar water chemistry conditions, though they might be different in their morphology, mixis regime and trophic status, are still remarkably similar with respect to their zooplankton communities. All this makes zooplankton well-suited for bio-indication of ecological quality in acidic pit lakes.

Chapter 3

Multiple stressors for biodiversity in acidic lakes: Evidence from comparison of littoral cladoceran communities from hard- and soft-water lakes

Abstract

1. Acidic hard-water lakes of natural origin are rare, yet, hundreds of such lakes have recently originated all over the world due to open-pit coal mining activities. The majority of mining lakes are extremely acidic (pH 2–4), and these are characterized by hard-water conditions and high total dissolved solids content, the major ions being sulphate, iron, and aluminium.
2. Biota of acidic hard-water lakes have been poorly studied up to now. This is the first study describing communities of littoral Cladocera in several mining lakes of varying pH. In order to determine the relative importance of pH and other abiotic factors for the colonization of hard- and soft-water acidic lakes, we compared the species diversity and composition of littoral Cladocera in acidic mining lakes versus naturally acidic bog lakes and non-acidic reference lakes in north-eastern Germany.
3. Rarefaction curves used to enable rigorous comparison of species richness among the studied lakes proved asymptotic for both types of acidic lakes. Comparison of rarefaction curves and ANCOVA analysis using rarefied species richness values suggested that both pH and lake type significantly affected species richness of lit-

toral Cladocera. Acidic mining lakes exhibited lower species richness and diversity (PIE index) as compared to acidic soft-water lakes, and the latter had lower species richness than did non-acidic lakes. Multivariate ordination clearly separated the communities found in hard- and soft-water lakes, as well as those in acidic and non-acidic lakes. Forward selection in CCA suggested that among the measured environmental variables conductivity, pH and phosphorus had significant effects on the species composition.

4. Our study illustrates that different types of acidic lakes, such as hard- and soft-waters, can be colonized by rather distinct communities, and this cannot be explained by pH alone. We argue that the current concepts on the biological communities in acidic lakes are likely to be biased by the prevalent data on soft-water lakes, and studying acidic hard-water lakes should result in new insights. Comparative studies among communities in different lake types should be performed more often in the future, as they are likely to provide a better understanding of the effects of multiple stressors and important environmental axes for the colonization of acidic waters.

3.1 Introduction

Large-scale acidification of soft-water lakes in the Northern Hemisphere through acid rain fall-out since the 1970s has drawn much attention to the effects of low pH on the biota in those lakes (Schindler, 1988; Havas & Rosseland, 1995; Nierzwicki-Bauer et al., 2010). More recently many publications have documented the ongoing recovery of previously acidified aquatic ecosystems (e.g. Gunn & Keller, 1990; Yan et al., 1996; Stendera & Johnson, 2008). Hence, historically the acidic lakes' ecological research has largely focused on communities in soft-waters, because of their susceptibility to atmospheric acidification. However, acidic lakes on Earth cover a wide range of abiotic conditions. Investigations on some so far poorly studied lake types, such as acidic hard-waters, may provide valuable insights into the community organisation and ecological adaptations in acidic environments.

Despite the fact that pH is probably never the only abiotic factor, which changes with acidification and affects biological communities (Frost et al., 1999), until very recently most studies on communities in acidic lakes had been unifactorial, and interpreted ecological patterns based solely on pH. Furthermore, most studies have compared lakes that differed in their pH values, but were similar with respect to many other water chemistry parameters, e.g. clear oligotrophic soft-water lakes of the Canadian Shield (Sprules, 1975; Roff & Kwiatkowski, 1977; Walseng et al., 2003). This approach tends to underestimate the effects of other abiotic factors apart from

pH, which are more likely to be revealed, when comparing different lake types. Undoubtedly, both direct and indirect effects of low pH are among the strongest determinants of colonization patterns in acidic lakes (Rahel, 1984; Brett, 1989; Arnott & Vanni, 1993; Havens et al., 1993; Havas & Rosseland, 1995). Nevertheless, there is accumulating evidence that other environmental gradients apart from pH may also shape communities under low pH conditions. Nutrients and dissolved organic carbon (DOC) are generally important factors in lake ecosystems (Thienemann, 1931; Williamson et al., 1999), including acidic lakes (Kullberg et al., 1993; Yan et al., 2008). Other abiotic factors have been shown to be important community regulators in acidic waters, such as total dissolved solids concentration (Kappes & Sinsch, 2005; Kennedy et al., 2005; DeSellas et al., 2008), aluminium and other toxic metal ions (Yan & Strus, 1980; Fryer, 1993; Greig et al., 2010) as well as calcium concentration (Jeziorski et al., 2008). Multiple-stressor view of community regulation in acidic lakes is currently emerging, although it is not yet completely established (Yan et al., 2008). We propose that comparisons among communities from chemically diverse lakes, such as hard- and soft-waters, should contribute to a better understanding of the roles of different abiotic factors for colonization by organisms. Here, we provide a first detailed analysis of littoral cladoceran communities from hard-water mining lakes ranging from highly acidic to neutral and draw a comparison to the communities found in naturally acidic soft-water lakes and non-acidic reference lakes in the same region (Germany).

Littoral Cladocera represent one of the major invertebrate groups colonizing freshwater habitats in terms of their species diversity and secondary production (Walseng et al., 2006; Lemke & Benke, 2009). They have been widely used as indicators of environmental change, including acidification (Jeppesen et al., 2001; Walseng et al., 2001; De Eyto et al., 2003; Walseng et al., 2003). Indeed, littoral cladoceran species vary widely in their acidity tolerance from very sensitive species, disappearing at $\text{pH} < 6$, to extraordinary tolerant ones, thriving at $\text{pH} 3$ (Bērziņš & Bertilsson, 1990; Deneke, 2000). Littoral Cladocera are particularly suitable as indicators in acidic hard-water lakes, because some species can still be found at the harsh abiotic conditions occurring there, whereas pelagic cladocerans are completely extirpated from these lakes at pH below 4.5 (Deneke, 2000; see Chapter 2).

3.1.1 Acidic mining lakes—a poorly studied type of aquatic habitats

Geogenically acidified hard-water lakes is a habitat type that only rarely occurs in nature, mainly in areas influenced by volcanic activity (see Geller et al. (1998) for a review). However, numerous artificial acidic mining lakes (AMLs) with similar water chemistry have recently originated due to closures of coal and metal mines in many countries around the world (Banks et al., 1997; Geller et al., 1998). Oxydation of pyrites and other sulphur-containing minerals is the process, underlying geogenic acidification in both natural and anthropogenic acidic hard-water lakes (Geller & Schultze, 2009). Resulting inputs of sulphuric acid into lake water as well as those of Fe and Al ions, serving as acidity buffers within the pH range from 2 to 4, lead to formation of extremely acidic hard-water lakes. Apart from Fe and Al, other potentially toxic metals, such as Cu and Zn, may sometimes, but not necessarily exceed background levels, depending on the mineralogy of the catchment (Banks et al., 1997). We note that in AMLs in north-eastern Germany, sampled in this study, concentrations of toxic metal ions are below the background levels, except for Fe, Al and Zn (Friese et al., 1998), the concentrations of Zn ranging from 0.1 to 0.25 mg l⁻¹ (data from LUGV Brandenburg), which are unlikely to be toxic for Cladocera (Muysen et al., 2005). Hence, we assume that the toxic effects of water chemistry in Lusatian lakes are limited to those of low pH and high concentrations of iron, aluminium and sulphate. DOC levels are generally very low in AMLs (Friese et al., 2002), and thus DOC cannot mitigate toxic effects of Fe and Al ions, what is often the case in acidic humic soft-water lakes. On the other hand, high Ca and Mg concentrations occurring in AMLs (Table 3.1) can possibly counteract the effects of toxic hydrogen and metal ions (Brown, 1983) and sulphate (Davies & Hall, 2007).

The existing data on acidic soft-water lakes can hardly be useful for predicting colonization patterns in AMLs due to rather different combinations of abiotic stressors that are likely to be acting in hard- and soft-water acidic lakes. For example, high iron and sulphate concentrations are unique stressors in acidic hard-water lakes, while low calcium and high DOC may have adverse effects on communities in acidic soft-water lakes (Steinberg et al., 2006; Jeziorski et al., 2008), but never in AMLs. Furthermore, interactive effects among different stressors and mitigating compounds may be complex in acidic lakes and they are likely to affect individual species as well as communities (Havas & Likens, 1985; Havens & Heath, 1989; Havens, 1993;

Whipple & Dunson, 1993; Soucek et al., 2001). Field surveys, integrating over the diverse effects of water chemistry parameters, can provide valuable information on the colonization patterns in different types of acidic lakes. However, in spite of the commonness of AMLs, their biota has been poorly studied up to now (see Chapter 2 for a review on zooplankton). Some studies indicated that species diversity of several taxonomic groups was lower in AMLs as compared to natural water bodies (Lackey, 1939). The only detailed study on the littoral cladoceran community is available for a single AML with a horizontal pH gradient (see Chapter 4). It revealed higher species-specific thresholds to low pH in the investigated AML, as compared to literature data on cladoceran occurrence in soft-water lakes, which resulted in a faster depletion of species richness with decreasing pH. Still, data collected in a single lake, even within a broad pH gradient, might not be representative of various conditions occurring in AMLs. In the present study we intended to fulfil this gap by sampling littoral Cladocera in several AMLs of varying acidity.

The objectives of this study were twofold: (1) to contribute to the knowledge on the biota in poorly studied acidic hard-water lakes by investigating communities of littoral Cladocera in several mining lakes, varying in pH and other abiotic parameters; (2) to compare species diversity and community composition of littoral Cladocera among the two distinct types of acidic waters—artificial hard-water AMLs and naturally acidic humic soft-water lakes, situated in the same region, in order to obtain insights into the relative importance of abiotic factors for colonization of acidic lakes. As previous studies on littoral Cladocera in acidic lakes possibly overestimated the importance of pH vs. other abiotic factors (see above), we intended to test the null hypothesis that pH was the major limiting factor for colonization by Cladocera. The prediction of this hypothesis is similarity of cladoceran communities among acidic soft- and hard-water lakes after accounting for the effect of pH. Alternatively, if other environmental parameters apart from pH had significant effects on the littoral cladoceran communities, the latter should differ considerably among the two types of acidic lakes.

Table 3.1 List of the sampling localities and water chemistry parameters (Abbr.—Abbreviations, Cond.—Conductivity, TP—Total Phosphorus, Chl a—Chlorophyll a, DOC—Dissolved Organic Carbon, TFe—Total Iron, TAl—Total Aluminium)

Lake	Abbr.	Latitude	Longitude	Lake group	No. samples	No. habitats	pH	Cond. / $\mu\text{S cm}^{-1}$	TP / mg l^{-1}	Chl a / $\mu\text{g l}^{-1}$	DOC / mg l^{-1}	TFe / mg l^{-1}	TAl / mg l^{-1}	Sulfate / mg l^{-1}	Mg / mg l^{-1}	Ca / mg l^{-1}
Montzietrich	MOR	51°35'22" N	14°34'33" E	acidic mining	5	3	3.0	757	0.01	1.0	2.1	22.34	1.44	402.6	17.0	70.6
Sedlitzer See	SED	51°34'06" N	14°05'21" E	acidic mining	5	3	2.91	1931	0.005	0.8	1.4	20.7	5.34	1010.0	47.9	298.0
Barwälder See	BAR	51°23'38" N	14°33'59" E	acidic mining	5	3	3.21	890	0.009	1.5	2.4	9.5	6.9	439.5	22.1	106.9
Grünevalder Lauch	GLA	51°30'54" N	13°39'45" E	acidic mining	5	3	3.04	726	0.013	3.1	1.72	7.81	0.92	315.3	9.7	71.77
Geierswalder See	GSW	51°29'55" N	14°07'44" E	acidic mining	5	3	3.24	1235	0.004	1.6	0.53	6.49	1.59	599.3	25.0	161.0
Felisee	FEL	51°36'48" N	14°32'48" E	acidic mining	11	6	4.06	393	0.005	0.7	1.1	0.37	1.64	222.0	3.3	53.9
Senftenberger See, very acidic (pH < 4)	SFBa	51°28'53" N	14°00'14" E	acidic mining	10	7	3.37	889	0.018	1.0	1.48	3.34	1.48	256.7	16.3	64.6
Senftenberger See, weakly acidic (4 < pH < 6.5)	SFBw	51°29'08" N	14°01'27" E	neutralized mining	6	5	5.4	645	0.018	1.3	3.43	0.57	0.3	218.5	15.5	66.1
Senftenberger See, neutral (pH > 6.5)	SFBn	51°29'23" N	14°01'49" E	neutralized mining	8	6	6.88	611	0.018	1.5	5.38	0.57	0.07	180.3	14.6	67.6
Dreitweibern	DWB	51°23'56" N	14°24'54" E	neutralized mining	4	4	7.57	590	0.011	1.6	3.7	0.42	0.06	243.5	13.6	72.6
Großer Barschsee	GRB	53°07'01" N	13°00'04" E	acidic bog	8	6	5.2	24	0.054	24.0	48.18	0.29	0.09	4.9	0.2	3.1
Fuchskuhle North-East	FKNE	53°06'22" N	12°59'06" E	acidic bog	7	8	6.09	27	0.025	26.0	12.8	0.09	0.07	4.0	1.0	10.6
Fuchskuhle South-East	FKSE	53°06'21" N	12°59'03" E	acidic bog	7	4	5.61	28	0.025	26.0	13.0	0.09	0.07	4.0	1.0	10.6
Fuchskuhle South-West	FKSW	53°06'19" N	12°59'08" E	acidic bog	7	9	6.1	26	0.025	26.0	12.0	0.09	0.07	4.0	1.0	10.6
Kleiner Milasee	KMIL	52°09'14" N	13°57'26" E	acidic bog	9	5	4.32	43	0.028	22.0	11.8	0.32	0.18	1.0	1.0	6.1
Großer Milasee	GMIL	52°09'05" N	13°56'58" E	neutral bog	7	6	6.97	119	0.009	7.3	10.9	0.05	0.05	33.0	2.2	12.7
Tiefer See	TIE	52°09'12" N	13°59'42" E	neutral reference	9	8	8.26	538	0.019	2.9	7.3	0.05	0.05	111.0	8.4	82.2
Melangee	MEL	52°09'36" N	13°59'18" E	neutral reference	10	7	8.53	459	0.062	84.0	9.3	0.05	0.03	74.0	7.9	81.2
Langer See	LAN	52°14'28" N	13°47'10" E	neutral reference	11	9	8.99	471	0.07	105.4	11.5	0.05	0.2	66.0	7.7	52.1
Wolziger See	WOL	52°15'07" N	13°48'07" E	neutral reference	11	10	8.22	467	0.065	38.3	12.1	0.25	0.27	62.0	7.9	55.8

3.2 Material and Methods

3.2.1 Study sites

We studied littoral cladoceran communities in two types of acidic waters—hard-water mining lakes and soft-water bog lakes—in north-eastern Germany. In order to explore the community change towards the neutral end of the pH gradient we included two neutral mining and two neutral bog lakes into the study. Besides, four circumneutral hard-water lakes, one mesotrophic and three eutrophic ones, were used as reference sites (Table 3.1; Fig. 3.1).

Physico-chemical conditions within two of the studied lakes were highly heterogeneous. Therefore, sampling for Cladocera and recording of environmental parameters in these lakes were conducted for each distinct lake part. Lake Große Fuchkuhle has been artificially divided into four completely isolated basins (Kasprzak, 1993). As a result of structural differences among the catchment areas, the four basins now differ considerably in their water chemistry and planktonic communities (Koschel, 1995; Burkert et al., 2004), and thus they were sampled separately. Lake Senftenberger See exhibits a horizontal chemical gradient with pH from 3 to 7 (see Chapter 4). All samples and measurement data from this lake were pooled into three groups: with $\text{pH} \leq 4$, $4 < \text{pH} < 6.5$ and $\text{pH} \geq 6.5$ (Table 3.1), according to the prevailing buffer system (Nixdorf et al., 2003) and colonization by Cladocera (see Chapter 4). Altogether, a total of 21 lakes/lake compartments have been sampled (Fig. 3.1; Table 3.1).

Sampled bog lakes were typical brown-waters, with high concentrations of cDOC. Unfortunately, measurements were only available for the total DOC, and the cDOC fraction was not determined. Lusatian acidic mining lakes represent typical AMD-affected waters with low pH, high conductivity and high concentrations of iron and aluminium ions (Table 3.1), but no other metals were measured in concentrations toxic for cladocerans (Friese et al., 1998). Mining lakes can be classified into three groups according to the prevailing buffer system: iron-buffered (pH 2.2–3.4), aluminium-buffered (pH 3.8–4.0) and carbonate-buffered (pH > 6) (Nixdorf et al., 2003). We sampled mining lakes from all three groups (Table 3.1). Mining lakes with pH values between 4 and 6 are virtually non-existent, because in the absence of a buffer system chemical conditions become unstable (Nixdorf et al., 2003). Nevertheless, we were able to cover this pH range by sampling the pH gradient from 3 to 7.5, formed in Lake Senftenberger See

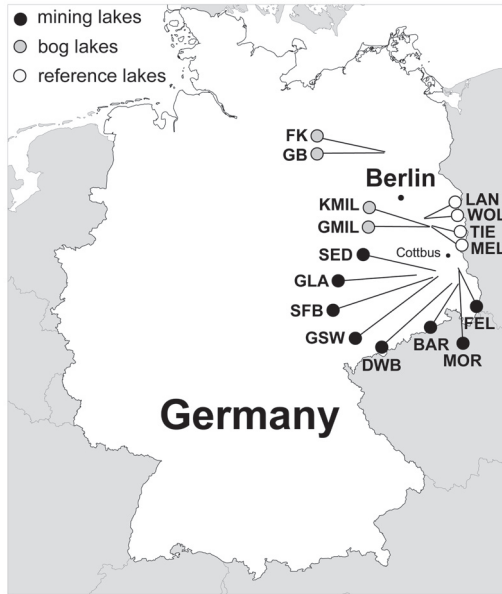


Figure 3.1 Sampling sites. See Table 3.1 for the abbreviations of the lakes' names

(see Chapter 4 for the description of the gradient). Non-acidic mining lakes in our data set (Table 3.1) were neutralized by flooding with river water (Nixdorf et al., 2001). The North Basin of Lake Senftenberger See has been non-acidic since 1979, whereas Lake Dreiweibern reached a circumneutral state in year 2002 (one year before our sampling). The age of the mining lakes was between 5 and 70 years at the time of sampling (Nixdorf et al., 2001). We assumed no effect of lake age on the cladoceran species richness (Dodson et al., 2007).

3.2.2 Sampling and sample examination

Sampling was carried out on two dates, in spring and autumn 2003. Littoral Cladocera are known to display micro-habitat preferences, that is why an attempt was made to sample all distinct littoral habitats found in a lake, in order to achieve as complete species inventory as possible. Habitat types were defined in the field after examination of the entire shoreline and in

accordance with the published data on the habitat specificity of cladoceran species (Rybak et al., 1964; Di Fonzo & Campbell, 1988; Tremel et al., 2000). The following habitat types, which are likely to harbour distinct assemblages of littoral Cladocera, were sampled: (1) bottom substrates: sand, stones, mud, leaf litter, wood branches; (2) plant substrates: filamentous algae, floating leaves, stem macrophytes (e.g. *Phragmites*), large-leaved and fine-leaved submerged macrophytes, *Sphagnum*, moss other than *Sphagnum*, submerged terrestrial vegetation; (3) water column: open littoral (accessible to waves) and protected littoral (no wave action due to a barrier of dense macrophyte stands). Sampling was conducted at 3–6 sampling points within each lake, where possible, multiple habitats were sampled at one point, e.g. a bottom substrate and the water column. No attempt was made to determine habitat specificity of cladoceran species, but rather to sample as many different habitats as possible. Habitat diversity per lake was scored according to the number of the habitats sampled in a lake on the two dates (Table 3.1).

Qualitative samples of littoral Cladocera were taken with a 100 μm mesh dip net by sweeping several times over the bottom substrate or within macrophyte stands and immediately preserved in ca. 4% formalin. Species identifications followed Flößner (2000), Korovchinsky (1992), Lieder (1996), Orlova-Bienkowskaja (2001), and Smirnov (1992, 1996). Besides, we have taken into account some recent taxonomic revisions (Sinev, 2009; Sinev & Atroschenko, 2011). *Daphnia hyalina* Leydig, 1860, *Daphnia galeata* Sars, 1863 and their putative hybrids were pooled together, as these taxa were rare and often represented solely by juveniles, which could not be reliably identified. For calculation of relative abundances a sample was mixed, loaded into a Bogorov chamber, and 100 specimens were counted. The rest of the sample was examined as well and all Cladocera species were identified. In 13 samples fewer than 100 individuals were found, only three of them containing fewer than 50 individuals.

Conductivity and pH were measured simultaneously with sampling for Cladocera. Data on other environmental parameters (Table 3.1) were obtained from the databases of the Department of Freshwater Conservation at the Brandenburg University of Technology at Cottbus (BTU Cottbus), Institute of Freshwater Ecology and Inland Fisheries (IGB, Berlin) and Landesamt für Umwelt, Gesundheit und Verbraucherschutz (LUGV) Brandenburg (State Office of Environment, Health and Consumer Protection of the Federal State of Brandenburg). For most parameters a yearly mean was calculated from several measurements in 2003. When data on year 2003 was not available,

measurements for the closest available year were taken.

3.2.3 Statistical analyses

Hierarchical agglomerative cluster analysis was applied to the data set of the measured environmental parameters (Table 3.1) in order to group the lakes according to their physico-chemical conditions. Clustering was performed in SPSS 15.0 software using the average linkage algorithm on a matrix of squared Euclidian distances. Prior to calculations all variables except for pH were ln-transformed and standardized by calculating Z-scores (obtained by subtracting the mean and dividing by the standard deviation).

In order to compare cladoceran species richness in acidic vs. non-acidic lakes and in acidic hard-water lakes vs. acidic soft-water lakes, we used sample-based rarefaction curves, computed in EstimateS v. 8.0.0. software (Colwell, 2006). Whereas among-habitat comparisons of observed species richness values are likely to be biased due to varying sampling effort, rarefaction provides unbiased species richness estimates, when species distributions are patchy and uneven both within and among habitats and species census is incomplete (Gotelli & Colwell, 2001). We calculated rarefaction curves based (1) on the counted data (100 individuals counted per sample) and (2) on the number of species recorded in the total samples. We applied one of the widely used and most reliable nonparametric estimators of asymptotic species richness Chao2 (Hortal et al., 2006), calculated in EstimateS, in order to estimate the completeness of the species inventory for each lake sampled.

The effects of pH and conductivity on species richness and diversity, as represented by PIE index (Hurlbert, 1971), were examined by regression methods implemented in OriginPro v. 8 software (OriginLab Corporation). Linear least-square regressions were calculated for pH effects and logistic regressions were fitted to the data on conductivity. We performed analysis of covariance (ANCOVA) in order to test the null hypothesis that no differences in species richness occurred between the two lake types (soft-water bog lakes and hard-water mining lakes) after accounting for the effect of pH. ANCOVA was implemented in SPSS 15.0 software, using GLM procedure for unbalanced designs, with species richness as dependent variable, lake type as fixed factor with two levels (soft-water lakes, mining lakes) and pH as covariate. ANCOVA analyses were run with two different data sets. Data set 1 was used to verify whether the sampled soft-water lakes in Germany were representative of a broader geographical range. It comprised observed total

species richness values for the lakes surveyed in this study, supplemented by the literature data for Canadian soft-water lakes from Walseng et al. (2008). All ANCOVA requirements, such as normality, homogeneity of variance and homogeneity of regression slopes, were fulfilled for the data set 1. Data set 2 comprised species richness estimates standardized by rarefaction to the smallest sampling size of 4 samples per lake, aiming to provide a rigorous test, which would not be biased due to differences in the sampling effort per lake. Unfortunately, it was not possible to obtain standardized species richness estimates for the literature data used in data set 1, due to the lack of counting data, hence, data set 2 included only those lakes, which were sampled in this study. Data set 2 deviated from normality due to the fact that the majority of the mining lakes were highly acidic with only one species occurring there. The Levene's test was significant ($p < 0.002$), indicating that the assumption of homogeneity of variance was violated. In order to comply with the requirements of ANCOVA the data set was ranked prior to analysis. Another important pre-condition for ANCOVA—the homogeneity of the regression slopes between the covariate (pH) and the dependent variable (species richness) of the respective lake types was fulfilled ($p > 0.05$).

In order to detect the major environmental gradients determining the cladoceran community composition in the studied lakes we applied multivariate ordination using CANOCO 4.5 software (ter Braak & Šmilauer, 2002). Mean relative abundances of species per lake, measured environmental parameters, and estimated micro-habitat diversity (Table 3.1) were used as input data. The length of the environmental gradient calculated by detrended correspondence analysis (DCA) was 2.6 SD, indicating that both linear and unimodal models would be appropriate (ter Braak & Šmilauer, 2002). Thus, we applied correspondence analysis (CA) and canonical correspondence analysis (CCA) to our data, which assume unimodal responses of species to environmental factors, but also have 'a second face', incorporating the linear model as well (ter Braak & Šmilauer, 2002). CA extracts intrinsic variation present in the species data, irrespective of any environmental data. The latter were projected onto the ordination plot after the CA analysis to help interpretation, but they did not influence the results. In contrast to that, in CCA ordination axes are constrained to be linear combinations of the measured environmental variables. Thus, only that variation is extracted from the species data, which is related to the variation in the environmental data. We compared the results obtained by CA and CCA, to see to what extent the measured variables accounted for the main patterns in the species composition. The degree to which each individual environmental variable

explained the observed variation in the species abundances was summarized as marginal and conditional effects in CCA. Marginal values represent the effects of environmental variables taken individually, whereas conditional values are calculated as additional variation explained by each variable in the order of inclusion into the multiple regression model constructed by CCA.

3.3 Results

Cluster analysis based on the measured abiotic parameters revealed three distinct lake groups: soft-water lakes, circumneutral hard-water, and acidic hard-water mining lakes (Fig. 3.2). AMLs formed the most divergent cluster, what reflects their peculiar water chemistry. On the other hand, abiotic conditions in the neutralized mining lakes (SFB56, SFB7 and 3W) were similar to those in the reference hard-water lakes, particularly, in the mesotrophic Lake Tiefer See (TIE).

3.3.1 Species richness of littoral Cladocera in relation to pH and conductivity

A total of 62 species were recorded in the studied lakes, 34 of them belonging to the family Chydoridae (Table 3.5 in Appendix). *Chydorus sphaericus* (O.F. Müller, 1785) was found in all lakes, and *Scapholeberis mucronata* (O.F. Müller, 1776) was found in all except for the most acidic mining lakes. On the other hand, 17 species were only found in a single lake. Total observed species richness ranged from one species per lake in the most acidic mining lakes (pH around 3) to 35 species in the circumneutral soft-water Lake Großer Milasee.

Sample-based rarefaction curves for the total observed species richness per sample are shown on Figure 3.3. We also calculated rarefaction curves based on the species richness values observed, when 100 individuals per sample were counted, these results being similar to those obtained with total species richness, with respect to ranking of lakes and saturation of the rarefaction curves, therefore, not shown here. Rarefaction curves for acidic mining lakes with $\text{pH} \leq 4$ as well as for acidic bog lakes have reached the asymptotes. Asymptotic rarefaction curves can be directly compared (Gotelli & Colwell, 2001), and thus it is apparent from Figure 3.3 that species richness was much lower in acidic mining lakes with $\text{pH} \leq 4$ (one or two

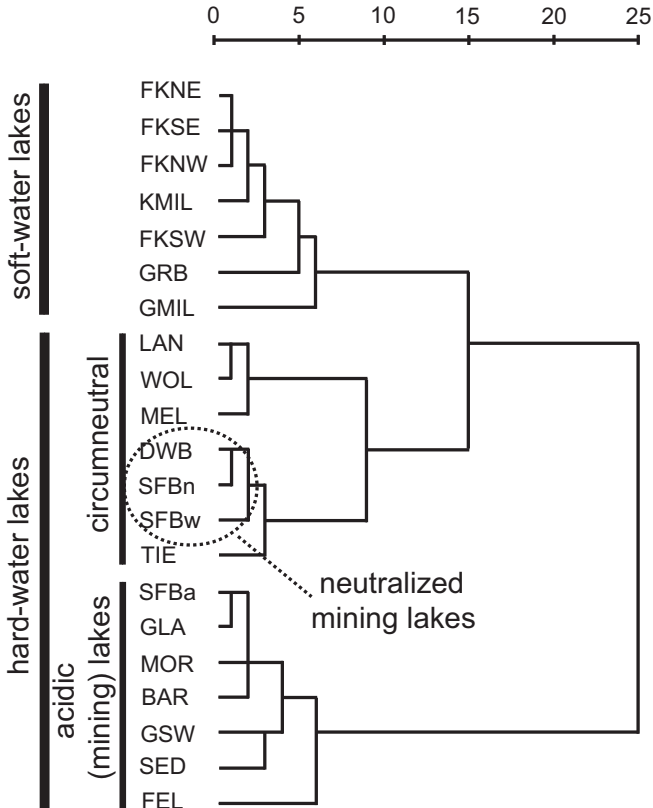


Figure 3.2 Lake groups based on the results of the cluster analysis of the environmental data (see Table 3.1 for the used parameters). Average linkage cluster analysis was conducted with ln-transformed (except for pH) and standardized data in SPSS 15.0

species), as compared to acidic bog lakes (ca. 15 species). For the less acidic mining lakes and non-acidic bog and reference lakes rarefaction curves were still rising, suggesting that our sampling effort was not sufficient to detect all species present in each lake. Hence, the ‘true’ total species richness could not be estimated for the studied non-acidic lakes, nevertheless, curves for non-acidic lakes lay clearly above the asymptotic estimates for the both types of acidic lakes (Fig. 3.3).

Species richness in the soft-water lakes sampled in this study ranged similarly to that in temperate soft-waters in Canada (Fig. 3.4 A), thus, our

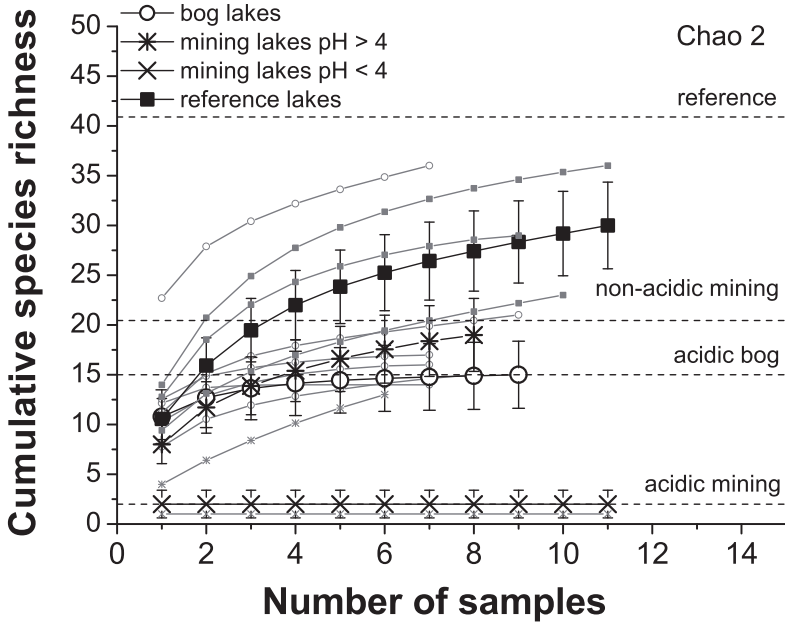


Figure 3.3 Sample-based rarefaction curves (plotted S_{obs} (Mao Tau) with standard deviation), computed in EstimateS v. 8.0.0. software based on the total observed species richness of littoral Cladocera. Large black data points represent examples of rarefaction curves for each lake type; the data for the other lakes are shown in gray. Dashed lines are Chao2 estimates of minimum species richness per lake for each lake type. The rarefaction curves for acidic mining lakes with pH < 4 and acidic bog lakes are asymptotic, the maximum S_{obs} values reaching the Chao2 estimates, whereas the curves for mining lakes with pH > 4 and for non-acidic bog and reference lakes are still rising, therefore, the ‘true’ species richness cannot be reliably estimated for the latter lakes

soft-water lakes’ data set appears to be representative of this lake type. A positive correlation between total observed species richness and pH was highly significant for both mining lakes ($adjusted\ r^2 = 0.97, p < 0.001$) and soft-water lakes ($adjusted\ r^2 = 0.42, p < 0.001$), mining lakes having lower species richness along the entire pH gradient as compared to soft-water lakes (Fig. 3.4 A). ANCOVA testing for the effects of pH and lake type on the total observed species richness with data set 1, which included both our and the literature data, was highly significant (Table 3.2, Test 1). A more rigorous test, applying standardization of species richness by rarefaction (data set 2, lakes from this study only), confirmed the significant effects of both pH and lake type on species richness (Table 3.2, Test 2). Thus, our null hypothesis

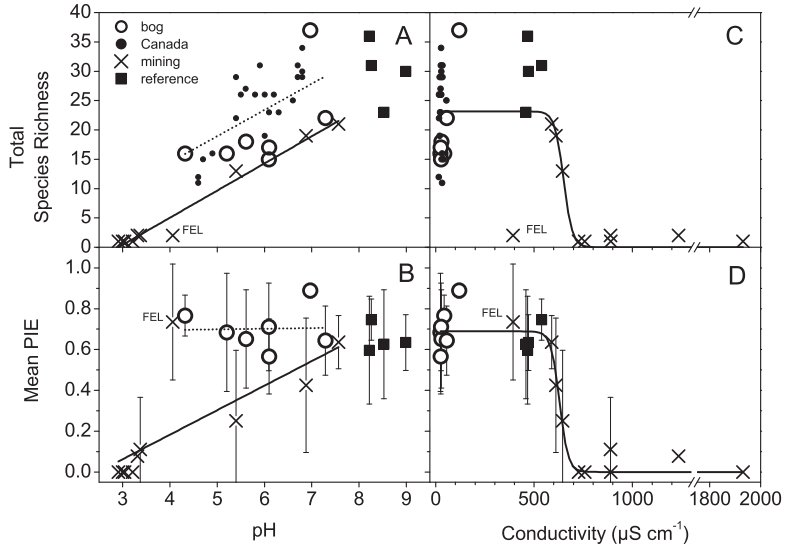


Figure 3.4 Results of the regression analyses: A and B—total observed species richness and PIE diversity index, respectively, versus pH. Solid lines—regressions for hard-water mining lakes, dotted lines—regressions for soft-water bog lakes; C and D—total observed species richness and PIE, respectively, versus conductivity. See Table 3.3 for the parameters of the logistic regression models

that pH was the only parameter that accounted for the decline of species richness in acidic lakes must be rejected. Additional factors associated with the lake type and modifying low pH toxicity have to be considered.

Acidic mining lakes tended to support lower species diversity, as represented by PIE index, than did acidic soft-water lakes (Fig. 3.4 B). The low

Table 3.2 Results of ANCOVA testing the effect of lake type on the species richness of littoral Cladocera. Test 1: dependent variable—total observed species richness; the data set for soft-water lakes included German lakes (this study) as well as published data on Canadian lakes from Walseng et al. (2008). Test 2: dependent variable—species richness rarefied to a sample size of four and ranked in order to achieve normality; only lakes sampled in this study were included

Source of variation	Test 1			Test 2		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
Lake type (factor)	1	19.74	< 0.0001	1	14.35	0.003
pH (covariate)	1	65.69	< 0.0001	1	23.53	< 0.001
Error	36			12		

Table 3.3 Parameters of the logistic regression model $y = a \times (1 + e^{(-k(x-x_c)})^{-1})$ fitted to the data plotted on Figures 3.4 C and 3.4 D. Abbreviations: *N*: no. of observations, *df*: degrees of freedom, *adj. r²*: adjusted correlation coefficient, *F*: F-value, *p*: probability of error, *a*: amplitude, *x_c*: center value, *k*: coefficient

Fitted logistic regression model	<i>N</i>	<i>df</i>	<i>adj. r²</i>	<i>F</i>	<i>p</i>	<i>a</i>	<i>x_c</i>	<i>k</i>
Species richness vs. conductivity	20	17	0.93	323	<0.0001	0.69	629	-0.05
PIE versus conductivity	43	40	0.52	123	<0.0001	23	650	-0.04

PIE values in mining lakes with pH from 3 to 6 were due to the high dominance by *C. sphaericus*. Mining Lake Felixsee (FEL) had an exceptionally high PIE due to very even distributions of the two species occurring there. Interestingly, no significant differences in species diversity were observed among soft-water lakes with varying pH and also between bog lakes and reference lakes (Fig. 3.4).

The relationships between conductivity and the species diversity parameters were non-linear, resembling dose-response curves, and they could be described by fitting logistic regression models (Table 3.3, Figs 3.4 C, 3.4 D). No relationship of diversity parameters to conductivity was apparent for soft-water and reference lakes, whereas in mining lakes a conspicuous decrease in both species richness and PIE occurred at the conductivity of around 650 $\mu\text{S cm}^{-1}$. Lake FEL was an outlier, having very low species richness for its conductivity, but a high PIE value (Figs 3.4 C, 3.4 D).

3.3.2 Patterns in species composition as related to the measured environmental variables revealed by ordination methods

CA analysis, based solely on the species abundance data (Fig. 3.5 A), revealed a similar grouping pattern as did cluster analysis of the environmental data (Fig. 3.2). For example, AMLs were distinct from other lakes both with respect to their environmental conditions and species composition, and neutralized mining lakes were similar to reference lakes. The first two CA axes with eigenvalues of 0.645 and 0.396, respectively, together accounted for 36.7% of the variance in the species data. The major direction, in which the species composition varied, represented by the first ordination axis, was most strongly correlated with conductivity ($r = 0.74$, $p < 0.001$) and related parameters—Ca, Mg, sulphate, Al, and Fe. Accordingly, hard-water mining lakes tended to have small scores on the first axis, and soft-water lakes tended to have large scores, with hard-water reference lakes being interme-

diate (Fig. 3.5 A). The second CA axis was strongly correlated with pH ($r = 0.81$, $p < 0.0001$) and it separated acidic from non-acidic lakes. It was also correlated with habitat diversity ($r = 0.46$, $p < 0.05$) and total Fe ($r = 0.44$, $p < 0.05$). The third CA axis was not correlated with any environmental parameters.

Ordination of lakes in CCA was very similar to that in CA and thus is not shown here. The first two axes had only slightly lower eigenvalues than in the CA analysis, 0.595 and 0.374, respectively, and they together explained 34.2% of the variance in the data. The high similarity of the CA and CCA results indicates that all important environmental variables were included in our data set (ter Braak & Šmilauer, 2002). Like in CA, the first CCA axis was correlated with conductivity ($r = 0.77$, $p < 0.0001$) and with related ions, while the second axis was strongly correlated with pH ($r = 0.88$, $p < 0.0001$) and weakly with chlorophyll a ($r = 0.45$, $p < 0.05$) and microhabitat diversity ($r = 0.49$, $p < 0.05$). The third CCA axis was correlated with total phosphorus concentration ($r = 0.71$, $p < 0.001$) and chlorophyll a ($r = 0.54$, $p < 0.05$). The stepwise regression model calculated in CCA revealed that only three environmental variables had significant effects on the species composition: conductivity, pH and total phosphorus (in the order of the explained variance) (Table 3.4). We then rerun CCA, including only the significant environmental variables (Fig. 3.5 B). The first two axes still explained a high proportion of the species variance (27.7%), and the ordination of lakes was very similar to that in the CCA analysis with all environmental variables included.

3.3.3 Occurrence of littoral cladoceran species in hard- and soft-water acidic lakes

Figure 3.5 C shows the CA ordination of species in relation to the measured environmental parameters, which is based on the same analysis as the CA plot for lakes (Fig. 3.5 A). Hence, the species, which were abundant in a certain type of lakes, are located within the respective part of the plot. Also, the projection of a species point onto the respective environmental vectors reflects its ecological preferences, and the species position on the ordination graph can be interpreted as its ecological optimum (ter Braak & Prentice, 1988). The observed species in situ occurrence ranges along the pH and conductivity gradients in the studied lakes are summarized on Figure 3.6. Two species with the broadest pH and conductivity ranges—*C. sphaericus* and *S. mucronata* (Fig. 3.6) occurred in all types of lakes, but their high

Table 3.4 Results of the forward selection in CCA analysis, showing the contribution of each measured environmental variable to explaining the variation in the species abundances. Marginal effects were calculated for each environmental variable independently from other variables, whereas conditional values were calculated as additional variation explained by each variable in the order of inclusion into the multiple regression model constructed by CCA. Significant *p*-values are indicated by an asterisk

Variable	Marginal effects lambda-1	Conditional effects lambda-A	<i>p</i> -value	<i>F</i> -value
Conductivity	0.40	0.40	0.002*	3.17
pH	0.39	0.34	0.002*	2.92
Total P	0.24	0.25	0.004*	2.25
Mg	0.34	0.13	0.202	1.25
Ca	0.27	0.12	0.302	1.13
DOC	0.19	0.14	0.292	1.29
Habitat diversity	0.27	0.11	0.348	1.13
Chlorophyll a	0.23	0.12	0.356	1.14
Sulfate	0.34	0.10	0.486	0.98
Total Fe	0.26	0.08	0.550	0.83
Total Al	0.21	0.04	0.856	0.35

relative abundances were characteristic for AMLs, what is reflected in their position on the CA ordination plot (Fig. 3.5 C). Indeed, these were the only two species found in the most acidic hard-water lakes with $\text{pH} \leq 4$, and *C. sphaericus* also dominated the communities in weakly acidic and neutral hard-water lakes (77 % and 46 % relative abundances, respectively).

A rather high proportion of species were associated with soft-water lakes (Table 3.5 in Appendix). *Acantholeberis curvirostris* (O.F. Müller, 1776), *Chydorus ovalis* Kurz, 1874, *Daphnia obtusa* Kurz, 1875, *Ilyocryptus agilis* Kurz, 1878 and *Scapholeberis microcephala* Sars, 1890 were restricted to acidic soft-water lakes (Figs 3.5C and 3.6). *Simocephalus serrulatus* (Koch, 1841) and *Streblocerus serricaudatus* (Fischer, 1849) were found in both acidic and non-acidic soft-water lakes, but they were absent from hard-water lakes. *Alonopsis elongata* (Sars, 1861), *Alona rustica* Scott, 1895, *Ilyocryptus cuneatus* Štifter, 1988, *Latona setifera* (O.F. Müller, 1785), *Paralona pigra* (Sars, 1862), *Rhynchotalona falcata* (Sars, 1861), and *Simocephalus expinosus* (Koch, 1841) were restricted to non-acidic soft-water lakes. It should be noted that our sampling was inadequate for sediment-dwelling *Ilyocryptus* species, hence, their occurrence in the samples was accidental and should not be interpreted as environmental preferences. The following species were positioned on the right side of the CA plot (Fig. 3.5 C), because they tended to have higher relative abundances in soft-water lakes, although they were also present at hard-water conditions: *Alona affinis* (Leydig, 1860), *Alona guttata* Sars, 1862, *Alonella excisa* (Fischer, 1854), *Alonella nana*

acids) for biological colonization (Uéno, 1934, 1958 and references therein). In particular, Uéno (1934, 1958) found that zooplankton communities differed considerably among the acidic hard-water lakes and acidic soft-water bog lakes in Japan. A few studies are available on zooplankton in man-made acidic hard-water mining lakes in Europe and North America, which also indicate that communities in AMLs are rather distinct from those, found in acidic soft-water lakes, although available studies mostly lack taxonomic resolution (see Chapter 2 for a review). Overall, zooplankton communities in acidic hard-water lakes remain poorly studied, the taxonomic knowledge and assessment of factors, determining colonization patterns, have hardly advanced since early work (see Chapter 2). This situation is no longer satisfying, inasmuch as acidic hard-water lakes have become numerous worldwide due to mining activities; there are hundreds of AMLs alone in Germany, and these provide important ecosystem services (Nixdorf et al., 2005). More studies are required on zooplankton in natural and artificial acidic hard-water lakes for at least two reasons. Firstly, the prevalence of studies on acidic soft-water lakes due to the vulnerability of the latter to acid rain presumably has influenced general concepts of biological effects of low pH. A more comprehensive view of colonization patterns, physiological responses and adaptations in acidic waters may emerge, if the entire range of acidic environments on Earth, both natural and anthropogenically acidified, is studied. In particular, the roles of multiple stressors can be better disentangled, when comparing communities in chemically diverse lakes. Secondly, many AMLs are large water bodies that have to be monitored and managed in order to achieve or maintain a good water quality state according to the EC Water Framework Directive (WFD). Zooplankton was proposed as a sensitive indicator group to characterise diverse ecological states in AMLs (Nixdorf et al., 2005), but littoral Cladocera, although being a promising indicator group, have not yet been studied in detail. In this study, we provide the first detailed account of the littoral cladoceran communities in chemically diverse mining lakes and draw a comparison to cladoceran communities in acidic soft-water lakes and non-acidic reference lakes.

3.4.1 Colonization patterns by littoral Cladocera in acidic hard- and soft-water lakes

Acidic hard-water mining lakes are characterized by rather peculiar water chemistry conditions (Fig. 3.2), and the unique combination of abiotic stressors occurring there (see below) should lead to communities distinct

from those colonizing acidic soft-water lakes. Indeed, we found that littoral cladoceran communities differed drastically between the two types of acidic lakes studied, in terms of both species richness and composition (Table 3.2, Figs 3.3, 3.4 and 3.5). Species richness was very low in AMLs with $\text{pH} \leq 4$, comprising just two species—*C. sphaericus* and *S. mucronata*. Weakly acidic mining lakes had much higher species richness (Fig. 3.3), yet, rather low diversity, represented by PIE (Fig. 3.4 B), what possibly indicates a high level of ecosystem disturbance in these lakes. In contrast to AMLs, acidic soft-water lakes in the same region harboured much more diverse communities, both in terms of their species richness (Fig. 3.3: ca. 15 species) and PIE index (Fig. 3.4). Interestingly, although species richness in soft-water lakes showed a significant decrease at acidic pH, PIE index did not differ significantly among the studied acidic soft-water lakes and non-acidic lakes. Presumably, this characterises acidic soft-water bog lakes as natural habitats, capable of supporting diverse communities of littoral Cladocera, and these communities appear to be rather distinct from those found in other types of lakes (Fig. 3.5). Therefore, although our study was limited with respect to the number of lakes studied, the observed differences in the species richness and composition of littoral Cladocera between the two types of acidic lakes were striking and unequivocal.

We detected considerable changes in species diversity and composition along the pH gradient in mining lakes (Figs 3.4 and 3.5), and thus littoral Cladocera can be used as biological quality components for monitoring of environmental conditions in mining lakes in accordance with EC WFD. The observed diversity patterns corresponded well with the classification scheme for mining lakes developed by Nixdorf et al. (2005), based on chemical conditions as well as on colonization by phyto- and zooplankton. All very acidic mining lakes (pH 2.8–3.5, iron buffer), except for Lake Geierswalder See, contained only *C. sphaericus*. Lake Geierswalder See also contained *S. mucronata*, what is possibly explained by within-lake pH variation, as some measurements did record pH values of 3.5, whereas the mean pH was only 3.24. Moderately acidic mining lakes (pH 3.5–4.5, aluminium buffer), represented by Lake Felixsee and possibly Lake Geierswalder See in our data set, contained two cladoceran species, and weakly acidic lakes (pH 4.5–7, lacking buffer), represented by the pH gradient in Lake Senftenberger See had diverse cladoceran communities. Therefore, our data supported the proposal of Nixdorf et al. (2005) for classification of mining lakes.

It is also in agreement with Nixdorf et al. (2005) that studied neutralized mining lakes were very similar to reference hard-water lakes with respect

to their diversity indicated by PIE index (Fig. 3.4) and species composition (Fig. 3.5), although they tended to have somewhat lower species richness (Fig. 3.3). These findings indicate that once mining lakes are neutralized, the living conditions for littoral Cladocera may become as favourable as in natural hard-water lakes. Unfortunately the 'true' species richness could not be reliably estimated for any of non-acidic lakes in our data set, as the rarefaction curves were still rising. Thus, it remains unclear, whether species richness of littoral Cladocera in neutralized mining lakes is reduced, compared to natural hard-water lakes, in response to some abiotic stressors, such as elevated sulphate concentration, which was shown to affect communities in neutralized mining streams (Kennedy et al., 2005).

Whereas comparative studies on communities in acidic hard-water vs. soft-water lakes are scarce (but see Uéno, 1934, 1958), diversity patterns similar to our findings have been documented for macroinvertebrates (Collier et al., 1990; Dangles et al., 2004; Petrin et al., 2007) and fish (Greig et al., 2010) in acidic streams with mineral vs. organic acidity sources. Furthermore, a large-scale meta-analysis of macroinvertebrate communities in the two types of acidic streams revealed the generality of the divergent effects of mineral and organic acidity on species diversity and composition (Petrin et al., 2008). Two non-exclusive explanations have been invoked to explain this pattern in acidic streams, and these are likely to play a role in acidic lakes as well. An ecological explanation invokes additional abiotic stressors, such as toxic metal ions, present in acidic hard-waters as compared to acidic soft-waters and/or the presence of ameliorative compounds, such as, for example, humic substances, in acidic soft-waters (Petrin et al., 2008). The second explanation of the distinctiveness among the faunas of hard- and soft-waters is provided by the evolutionary species pool hypothesis (Pither & Aarssen, 2005), which predicts that more species would occur in long-existing wide-spread habitats compared to young and/or rare types of habitats, such as acidic hard-waters, not because the conditions there are stressful per se, but simply because there has not been enough time for adaptation to their specific water chemistry (Petrin et al., 2008). Indeed, AMLs are young habitats with peculiar water chemistry, which are isolated from natural acidic hard-water lakes by large distances, what makes exchange of colonisers improbable. It is likely that both the ecological and the evolutionary explanation are relevant for the diversity patterns in man-made acidic mining lakes. Accordingly, natural acidic hard-water lakes in Japan, which have existed for evolutionary relevant time scales, appear to support more diverse zooplankton communities than do man-made AMLs

(see Chapter 2), supporting the evolutionary hypothesis. In the meantime, acidic soft-water lakes in Japan harbour yet greater diversity compared to acidic hard-water lakes in the same region (Uéno, 1934, 1958), thus, corroborating the ecological stress hypothesis.

3.4.2 Environmental factors determining colonization of acidic lakes by littoral Cladocera

We detected strong effects of the water chemistry at both community and species levels in the studied acidic lakes. Our null hypothesis that pH was the only abiotic factor shaping cladoceran communities had to be rejected, as the two types of acidic lakes under study differed greatly in their communities, irrespective of pH. Although being, apparently, not the only important abiotic factor, pH, nevertheless, significantly affected both species diversity and composition of littoral Cladocera in the studied lakes. These results agree with other studies on littoral Cladocera in acidic lakes, which established the value of this group as biological indicators of pH changes (Walseng et al., 2001, 2003).

We found that species richness of littoral Cladocera decreased with lowering pH in both hard- and soft-water lakes. So far, the empirical evidence for adverse effects of low pH on species richness for this group has been equivocal. Species richness of littoral Cladocera was found to be positively correlated with pH in some studies (Fryer, 1980; Krause-Dellin & Steinberg, 1986; Walseng et al., 2003), whereas other ones reported no relationship within a pH range 4.3–7 (Arvola et al., 1990; Hann & Turner, 2000) or even a slightly negative correlation (Uimonen-Simola & Tolonen, 1987; Nilssen & Sandøy, 1990). These inconsistencies may have several reasons, and before discussing the possibility of essential differences in lake water chemistry affecting cladocerans, we have to consider some methodological problems related to differences in the sampling effort among lakes. If sampling effort is insufficient to reveal all species present at a given site, species richness estimates may be biased by differences in the number of individuals examined per site, even if the sampling effort was comparable (Gotelli & Colwell, 2001). Rarefaction curves can circumvent this problem either by indicating that the species census was nearly complete (asymptotic curves) or for incomplete census (rising curves) by providing standardized species richness estimates, which facilitate rigorous among-site comparison. In our analysis the rarefaction curves for both acidic mining lakes with $\text{pH} \leq 4$ and acidic soft-water lakes proved asymptotic, and the curves for

the non-acidic lakes though still rising, lay higher than the curves for the acidic lakes. Furthermore, ANCOVA using rarefied species richness estimates revealed significant effect of pH in both types of acidic lakes. Thus, our results provide a strong evidence for the following ranking of the species richness in the studied lakes: acidic hard-water lakes < acidic soft-water lakes < non-acidic hard- and soft-water lakes.

Another reason for the disagreement among studies concerning the effect of pH on species richness might be systematic differences in water chemistry and/or differences in biogeographical colonization history among the surveys. The first point has been rarely addressed in studies, because pH was often implicitly regarded as the only abiotic factor limiting colonization in acidic lakes. Our results illustrate that the effects of other than pH abiotic factors can indeed be important determinants of colonization patterns (discussed below). The second point has become obvious, as many previously conspecific taxa in Europe and North America were shown to be closely related, but separate species (Frey, 1995), and, therefore, they may exhibit different environmental preferences, what indeed was shown for some species (Walseng & Schartau, 2001). We still know little about how regional factors determine colonization by Cladocera, particularly, this information is lacking for the poorly studied acidic hard-water lakes.

Total dissolved solids (TDS) content, measured as conductivity, likely underlined the significant effect of the lake type on the species richness of littoral Cladocera detected by ANCOVA (Table 3.2), and it also accounted for the most explained variation in the species composition, having even a larger effect than pH (Table 3.4). This environmental gradient has been largely neglected in the acidic lakes' research up to now, yet, some field studies indicated the importance of TDS for colonization by Cladocera (e.g. DeSellas et al., 2008). Both ends of the gradient may act as stressors, i.e. some species may be limited by low ionic content in soft-waters (Aladin & Potts, 1995; Kappes & Sinch, 2005), while other organisms may become stressed by very high TDS typical for AMD-affected waters (Kennedy et al., 2005). Although the toxicity of high TDS content is in many cases attributed to Al and Fe ions (Soucek et al., 2001), also high salt content (in AMLs mostly made up by sulphate) in itself has been shown to have a significant toxic effect on organisms (Goodfellow et al., 2000; Kennedy et al., 2005). The picture is complicated by possible interactive effects between pH, Al and Fe, which are not always additive, but rather some toxicants may also ameliorate the toxicity of the other ones (Soucek et al., 2001). Another important major component of TDS, water hardness, comprising Ca and Mg ions, is known

to mitigate toxicity related to low pH, high ionic content and metals (Yim et al., 2006). Therefore, the resultant effect of high TDS in AMLs on organisms is difficult to predict. Our results suggest that adverse effects of high TDS prevailed over positive effects, as the cladoceran communities were greatly impoverished in the studied AMLs. We were not able to separate the effects of individual ions, constituting TDS, partly because some of them, namely Ca, Mg, sulphate, Al and Fe, were strongly correlated with the total TDS represented by conductivity and thus, these parameters were excluded from the forward regression model, despite their rather high marginal effects (Table 3.4). It might be still possible to separate the effects of individual TDS constituents, if more AMLs with broader ranges of abiotic conditions are sampled. On the other hand, even more data might not help to solve the problem of multi-collinearity in regression analysis, as changes in TDS, Fe and Al concentration are non-linearly correlated with one another and with pH in mining lakes. Indeed, the non-linear relationships were indicated by the steep declines of species richness and diversity at the conductivity values around 600 $\mu\text{S}/\text{cm}$ (Fig. 3.4 C). A further complication for the statistical analysis is the bimodal rather than normal distribution of the TDS values among the hard- and soft-water lakes, which represent two discrete types of lakes with no intermediate states. Therefore, laboratory testing should ideally complement field data, when studying the individual and interactive effects of the TDS components in AMLs.

Although our study was not designed to study the effects of nutrients on littoral Cladocera, phosphorus concentration, which is a proxy for the trophic state, proved to be significant in the CCA forward selection (Table 3.4). Acidic hard-water lakes are mostly oligotrophic, as phosphorus is bound to Fe ions in the presence of oxygen and it precipitates from the water column, whereas soft-water lakes can cover a much broader trophic range. The low diversity of littoral Cladocera in AMLs may be partly attributed to the low nutrients concentration in these lakes. Many other studies have shown nutrients to be important determinants of littoral cladoceran communities in lakes (e.g. De Eyto et al., 2003; Nevalainen, 2010). Our results emphasize that trophy-related parameters should be taken into account, also when studying acidic lakes, and sampling should ideally cover a broad trophic range.

DOC was not a significant factor in our analyses, possibly because it was highly correlated with TDS in our data set, i.e. all studied acidic soft-water lakes had high DOC concentration, while all acidic hard-water lakes were low in DOC. Inclusion of acidic clear soft-water lakes into analysis would

possibly help to single out the effect of DOC, as other studies did find that it affected communities of littoral Cladocera (DeSellas et al., 2008). Both extremes of the DOC gradient, especially its coloured fraction, can limit species occurrences in acidic waters (e.g. Kullberg, 1992). It is likely that due to the lack of cDOC in AMLs its beneficial effects are negligible in these lakes. In the studied bog lakes cDOC can likely protect organisms against Fe and Al toxicity, but it can also reach concentrations, which are known to be toxic (Steinberg et al., 2003).

The diversity of littoral micro-habitats is known to limit species occurrence of littoral Cladocera (Di Fonzo & Campbell, 1988; Tremel et al., 2000). Micro-habitat structure is unlikely to be a limiting factor in highly acidic mining lakes, which already have very low species diversity due to abiotic stress, but it may play a role at weakly acidic and neutral pH. As mining lakes are created completely from scratch, macrophytes are absent or rare in the early years. Poorly structured littoral zone might have contributed to the observed lower species diversity in the neutralized mining Lake Dreiweibern, which reached its end water volume around year 2000 (Nixdorf et al., 2001), as compared to the higher diversity observed in the much older Lake Senftenberger See, filled with water in 1970s. The latter lake resembles natural hard-water lakes in the complexity of littoral micro-habitats (Table 3.1; van de Weyer et al., 2009), and somewhat lower species richness, compared to reference lakes, can be possibly attributed to the factors related to water chemistry rather than to micro-habitat structure.

Biotic factors appear to play a lesser role than do abiotic conditions in structuring communities of littoral Cladocera (Kurek et al., 2011), particularly in acidic lakes, where the role of biotic factors weakens with decreasing pH with the exception of invertebrate predation (Arnott & Vanni, 1993). While fish is completely extirpated from AMLs, some invertebrate predators, such as corixids, can tolerate both soft- and hard-water acidic conditions, although their role in AML communities is so far poorly understood (but see Wollmann et al., 2000). Competition in the AMLs with $\text{pH} \leq 4$ is restricted to a metazooplankton community, consisting of two cladoceran, one copepod and about four rotifer species (see Chapter 2). Thus, competition as a cause for species exclusion in highly acidic mining lakes is quite improbable (see also Weithoff, 2004). In contrast, in naturally acidic soft-water lakes, characterised by lower level of abiotic stress and more diverse communities, indirect effects of pH via food web interactions may be more pronounced.

3.4.3 Littoral cladoceran species associated with acidic conditions in hard- and soft-water lakes

No truly acidophilic species, restricted solely to acidic conditions, probably exist among Cladocera (Bērziņš & Bertilsson, 1990; Flößner, 2000). Nevertheless, this group has been widely used as indicators of acidification (Jeppesen et al., 2001; Walseng et al., 2001, 2003) due to the fact that some species are more common and/or more abundant in acidic lakes (Krause-Dellin & Steinberg, 1986; Sandøy & Nilssen, 1986), which is possibly related to competition with more efficient pelagic species in less acidic lakes. We found that a number of species, previously proposed as indicators of acidic conditions (Fryer, 1980; Krause-Dellin & Steinberg, 1986; Flößner, 2000; Walseng & Schartau, 2001), were associated with acidic and non-acidic soft-water lakes in our data set (Table 3.5 in Appendix). Although these species commonly occur at low pH in soft-water lakes, they were apparently not able to colonise acidic hard-water lakes. We suggest that the majority of so-called acidity indicator species, possibly also in other taxonomic groups apart from Cladocera, are in fact specialists adapted to the complex of abiotic conditions occurring in acidic soft-water lakes, rather than to low pH per se. Some of them may be more sensitive to changes in other abiotic parameters rather than pH. For example, low TDS content, low calcium concentrations, and high cDOC in bog lakes may as well limit colonization of acidic soft-water lakes by some species (discussed above). Although some field data are available on the occurrence of cladoceran species along these environmental gradients (e.g. DeSellas et al., 2008; Korosi & Smol, 2011), the physiological thresholds of most littoral cladoceran species in relation to TDS, Ca and DOC are unknown. Future studies should verify the indicator value of littoral cladoceran species in relation to pH and other abiotic parameters by analysing chemically diverse lake data sets as well as conducting laboratory experiments on physiological thresholds of single species.

In accordance with the prediction of the evolutionary species pool hypothesis (Pither & Aarssen, 2005) we found that specialists occurred in more wide-spread and historically common acidic soft-water lakes and they were lacking in young man-made AMLs, which were colonized by generalists only. Based on our results, high abundances of the two generalist species, *C. sphaericus* and *S. mucronata*, can serve as indicators of mining lakes' conditions. Interestingly, *C. sphaericus* is regarded as an indicator of eutrophic conditions, yet, we recorded it in high abundances in oligotrophic mining

lakes (see Chapter 4; this study) and also in oligotrophic high mountain lakes of the Altai region (Belyaeva, 2003). In our opinion, high abundance of this generalist species should indicate stressful environments, but no specific stressor can be identified based on *C. sphaericus* abundance data alone. Overall, the absence of specialists in AMLs and high dominance by few generalist species, even in weakly acidic and neutralized mining lakes, characterises these lakes as extreme environments with high level of ecosystem disturbance.

3.4.4 Concluding remarks

Acidic hard-water mining lakes are unique ecosystems, characterized by high levels of abiotic stress, and their communities are distinct from other acidic lakes. Ecological research on the so far poorly studied acidic hard-water lakes has a potential to broaden our view on the colonization patterns and adaptations to acidic environments in general. Similar to much better studied acidic streams, our results suggest that different combinations of stressors likely act in hard- and soft-water acidic lakes, resulting in distinct colonization patterns. Although rarely accounted for in previous studies, TDS and its constituents proved to be the major abiotic factors, determining species diversity and composition of littoral Cladocera in the studied lakes. Overall, our results illustrate that multiple factors (not only stressors) might be crucial for understanding and correctly interpreting the observed diversity patterns in acidic lakes. The multiple-stressor paradigm is currently being established in the acidic lakes' research, what is reflected in the increased number of recent publications dealing with effects of multiple stressors at low pH conditions (Christensen et al., 2006; Yan et al., 2008; Keller; 2009; Ormerod et al., 2010; Valois et al., 2011). However, a detailed understanding of the ecological roles of abiotic factors and their interactive effects in acidic lakes is still lacking. We suggest that not only multiple anthropogenic stressors' effects should be taken into account in future research on acidic lakes, but also sampling programs should include lakes, broadly ranging in naturally occurring water chemistry conditions. The advantages of such a comparative approach, illustrated by our study, would be integration of other important environmental axes apart from pH into analysis as well as at least partial circumvention of multi-collinearity among abiotic parameters, what would eventually result in a less biased view on the community regulation in acidic lakes.

Appendix

Table 3.5 List of the recorded littoral cladoceran species and their relative abundances in the studied lakes (Abbr.—Abbreviations)

Species	Abb.	Lake type				
		acidic mining	neutral mining	acidic bog	neutral bog	neutral reference
<i>Acantholeberis curvirostris</i> (O.F. Müller, 1776)	acan			1.4		
<i>Acroperus angustatus</i> Sars, 1863	acra		0.5		4.5	4
<i>Acroperus harpae</i> (Baird, 1843)	acha	1.9	18.3		1.8	10.0
<i>Alona affinis</i> (Leydig, 1860)	alaf	0.2	0.4	2.5	0.7	0.4
<i>Alona costata</i> Sars, 1862	alco	0.1	1.6		1.6	0.9
<i>Alona guttata</i> Sars, 1862	algu	0.01	0.03	3.2	1.7	0.3
<i>Alona protzi</i> Hartwig, 1900	alpr					0.2
<i>Alona quadrangularis</i> (O.F. Müller)	alqu					0.3
<i>Alona rectangula</i> Sars, 1861	alre		0.8		0.1	1.3
<i>Alona rustica</i> Scott, 1895	alru				0.1	
<i>Alonella excisa</i> (Fischer, 1854)	alec			5.9	29.6	5.6
<i>Alonella exigua</i> (Lilljeborg, 1853)	aleg				0.2	1.3
<i>Alonella nana</i> (Baird, 1850)	alna		2.3	8.2	5.9	1.8
<i>Alonopsis elongata</i> (Sars, 1861)	alel				7.6	
<i>Anchistropus emarginatus</i> Sars, 1862	anch				0.5	0.4
<i>Bosmina coregoni</i> Baird, 1867	boco		3.8		0.2	1.6
<i>Bosmina longicornis</i> Schoedler, 1866	bolb					0.3
<i>Bosmina longirostris</i> (O.F. Müller, 1785)	bolo	0.4	0.4		0.7	11.5
<i>Bythotrephes longimanus</i> Leydig, 1860	byth		0.2			
<i>Camptocercus biserratus</i> Schoedler, 1862	camb				0.2	0.6
<i>Camptocercus lilljeborgi</i> Schoedler, 1862	caml					0.3
<i>Camptocercus rectirostris</i> Schoedler, 1862	camr	0.7	1	0.6	0.1	0.8
<i>Ceriodaphnia megops</i> Sars, 1862	ceme		0.8			
<i>Ceriodaphnia pulchella</i> Sars, 1862	cepu	1.9	3.2		2.6	10.0
<i>Ceriodaphnia quadrangula</i> (O.F. Müller, 1785)	cequ		0.9	3.6	7.2	1.1
<i>Chydorus gibbus</i> Lilljeborg, 1880	chgi					0.9
<i>Chydorus ovalis</i> Kurz, 1874	chov			0.2		
<i>Chydorus sphaericus</i> (O.F. Müller, 1785)	chyd	77.6	46.4	21.6	3.5	33.1
<i>Daphnia cucullata</i> Sars, 1862	dcuc					2.7
<i>Daphnia galeata</i> Leydig, 1860,						
<i>Daphnia hyalina</i> Sars, 1863 and hybrids	dga		0.9		0.1	1.0
<i>Daphnia obtusa</i> Kurz, 1875	dobt			0.2		
<i>Diaphanosoma brachyurum</i> (Lievin, 1848)	diab	0.7	0.4	1.2	0.8	0.3
<i>Diaphanosoma mongolianum</i> Ueno, 1938	diam					0.2
<i>Disparalona rostrata</i> (Koch, 1841)	disp				0.1	0.2
<i>Eurycerus lamellatus</i> (O.F. Müller, 1776)	eury	0.2	0.8	1.4	1.7	0.3
<i>Graptoleberis testudinaria</i> (Fischer, 1848)	grap			0.7	1.8	1.9
<i>Ilyocryptus agilis</i> Kurz, 1878	ilag			0.3		
<i>Ilyocryptus cuneatus</i> Stifter, 1988	ilcu				0.1	
<i>Ilyocryptus sordidus</i> (Lievin, 1898)	ilso					0.3
<i>Kurzia latissima</i> (Kurz, 1874)	kurz			0.3		0.3
<i>Latona setifera</i> (O.F. Müller, 1785)	lato				0.1	
<i>Lathonura rectirostris</i> O.F. Müller, 1785	latr				0.6	0.2
<i>Leptodora kindtii</i> (Focke, 1844)	lept					0.7
<i>Leydigia leydigii</i> (Schoedler, 1863)	leyd					0.3
<i>Macrothrix laticornis</i> (Jurine, 1820)	macr					0.3
<i>Monosoplitus dispar</i> Sars, 1861	mono		0.5		0.7	0.4
<i>Paralona pigra</i> (Sars, 1862)	para				0.8	
<i>Picripleuroxus laevis</i> (Sars, 1862)	picr				0.5	0.2
<i>Pleuroxus aduncus</i> (Jurine, 1820)	plad					0.4
<i>Pleuroxus trigonellus</i> (O.F. Müller, 1785)	ptri				0.1	0.4
<i>Pleuroxus truncatus</i> (O.F. Müller, 1785)	ptru			4.0	4.4	4.7
<i>Pleuroxus uncinatus</i> Baird; 1850	punc					0.6
<i>Polyphemus pediculus</i> (Linne, 1761)	poly	0.7	0.4	5.8	1.6	0.2
<i>Pseudochydorus globosus</i> (Baird, 1843)	psch				0.2	0.1
<i>Rhynchotalona falcata</i> (Sars, 1861)	rhyh				1.7	0.3
<i>Scapholeberis microcephala</i> Sars, 1890	scmi			0.1		
<i>Scapholeberis mucronata</i> (O.F. Müller, 1776)	scmu	16.8	1.4	4	8.2	4.0
<i>Simocephalus vetulus</i> (O.F. Müller, 1776)	seve		0.2			1.2
<i>Sida crystallina</i> (O.F. Müller, 1776)	sida	1.1	19.3		7.0	1.1
<i>Simocephalus exspinosus</i> (Koch, 1841)	siox				0.2	
<i>Simocephalus serrulatus</i> (Koch, 1841)	sise				5.5	2.1
<i>Streblocerus serricaudatus</i> (Fischer, 1849)	stre			3.8	2.0	

Chapter 4

Colonization of acidic mining lakes: *Chydorus sphaericus* and other Cladocera within a dynamic horizontal pH gradient (pH 3–7) in Lake Senftenberger See (Germany)

Abstract

Acidic mining lakes are man-made habitats, which differ greatly from natural acidic lakes in their water chemistry. In order to study the potential for colonization of mining lakes by Cladocera we investigated their in situ distributions within a pH gradient from 3 to 7.5 occurring in Lake Senftenberger See (Germany). We found that species in situ pH minima were higher and the overall diversity at the respective pH values was lower in the investigated mining lake in comparison to natural acidic lakes. Possible explanations involve the specific water chemistry in mining lakes. *Chydorus sphaericus* was the most acid-tolerant species and occurred along the entire pH gradient. We experimentally tested the effect of lake water of different pH values on *C. sphaericus*. Surprisingly, its survival was the highest at low pH (3–4), while moderately acidic and neutral pH (5–7) had a well-expressed toxic effect on the animals. About 20% of *C. sphaericus* survived when transferred from pH 3 to pH 7 and vice versa, which suggests that this species is a generalist in relation to pH. As Cladocera display species-specific pH tolerances, we suggest that they could be a useful group for ecological quality assessment of acidified mining lakes.

4.1 Introduction

Acidic mining lakes provide a harsh environment for zooplankton organisms (Nixdorf et al., 1998). Only a few species of metazoan zooplankton are able to colonize those lakes, in spite of the abiotic stress imposed by the combination of low pH and high concentrations of toxic metal ions (Deneke, 2000; Wollmann et al., 2000; Nixdorf et al., 2005). Cladocera are the most acid-tolerant crustaceans and are important colonizers at pH above 3 (Nixdorf et al., 1998; Steinberg et al., 1998; Deneke, 2000; Wollmann et al., 2000; Nixdorf et al., 2005). However, little is known about the community composition and physiological thresholds of colonizing species, especially in moderately acidic and neutral mining lakes (Nixdorf et al., 1998). On the other hand, there have been many reports, considering zooplankton communities in acidified soft-waters and naturally acidic bog lakes, which discussed the influence of species-specific pH tolerance ranges and additional stressors on the species colonization success (e.g. Brett, 1989; Fryer, 1993; Havas & Rosseland, 1995). Although low pH is considered to be the most important factor limiting colonization of soft-water acidic lakes, it has been also shown that additional stressors, such as metal pollution, lack of calcium and high content of humic substances, can further decrease in situ tolerances of colonizing species (Fryer, 1993; Kappes & Sinsch, 2005). Some of the potential stressors are highly correlated with pH, what made it difficult to separate the effects of individual factors. Acidic mining lakes, which represent hard-water acidic habitats, can help to assess the limiting role of pH in a different combination of abiotic conditions. Namely, these water bodies are characterized by elevated concentrations of iron, aluminium and sulphuric ions, resulting in a very high conductivity, as well as by high calcium concentrations and a lack of humic substances (Geller et al., 1998; Nixdorf et al., 1998). Littoral Cladocera display species-specific tolerances in relation to a number of factors, and are therefore useful indicators of present and past environmental conditions (Krause-Dellin & Steinberg, 1986; Schartau et al., 2000; Walseng et al., 2003; De Eyto et al., 2003). This group is also both species-rich and includes many tolerant species (Smirnov, 1971), and thus they can be especially useful for water quality assessment in habitats subjected to multiple abiotic stress, such as mining lakes, where the number of potential biological indicators is generally low. Lake Senftenberger See, with its water chemistry typical of mining lakes (Table 4.1), is well suited to study the response of the littoral cladoceran community to these multiple factors. A peculiar pH gradient from highly

Table 4.1 Characteristic data of Lake Senftenberger See (data of water chemistry are given separately for the North Basin (Elsterfeld) and the South Basin (Südfeld)). Data indicated by an asterisk are original measurements; all other data are taken from Nixdorf & Hemm (2001)

Catchment area	km ²	779	
Area of lake	km ²	11	
Area of island	km ²	3.5	
Maximum depth	m	23	
Mean depth	m	7.0	
Volume	10 ⁶ m ³	80	
Water level	m NN	97.8–99.25	
		North	South
pH		7.4 *	3.2 *
Conductivity	μS cm ⁻¹	577 *	941 *
Acidity (KB 8.2)	mmol l ⁻¹	0.06 *	1.72 *
Chlorophyll a	μg l ⁻¹	2–6	≈ 1
Total nitrogen	mg l ⁻¹	4	3
Total phosphorus	μg l ⁻¹	6	2
Sulfate	mg l ⁻¹	266	300
Ca	mg l ⁻¹	85	79
Total Fe	mg l ⁻¹	0.27	3.1
Total Al	mg l ⁻¹	< 0.01	0.31

acidic (pH 3) to neutral (pH 7.5) has been formed between its two basins, which enables the study of almost the entire pH range occurring in mining lakes. Given the age of the lake (40 years) and the lack of physical barriers within the pH gradient, all potential colonizers should have reached the lake and distributed according to their pH tolerances. In our study, a single cladoceran species—*Chydorus sphaericus* (O.F. Müller, 1776)—was found along the entire pH gradient in the lake. Therefore, we decided to test its pH tolerance in mining lake water experimentally. This species was previously reported as an important colonizer of acidic mining lakes (Nixdorf et al., 1998; Wollmann et al., 2000), although its optimum pH range is unknown. Generally, the ability of a single cladoceran species to live under a wide range of conditions was doubted by Frey (1980, 1986), who discovered a number of cryptic chydorid species complexes consisting of ecologically distinct but morphologically similar species. Hence, he proposed that a wide distribution and eurytopy indicates plurality of species (Frey, 1980, 1986). The present study of littoral Cladocera in Lake Senftenberger See aimed to assess their in situ pH tolerances in a hard-water acidic mining lake in order to predict the potential for colonization of these waters. In order to investigate the pH range at which *C. sphaericus* can survive we experimentally assessed the

acute toxicity of mining lake water for *C. sphaericus*, as well as its within-lake variation in pH tolerance.

4.2 Description of the study site

Lake Senftenberger See (51°29'03" N, 14°01'14" E) is located near the town of Senftenberg in the Lusatian mining area (Germany) (Fig. 4.1). The lake was formed by flooding of the abandoned open-cast mining pit Niemtsch with groundwater and water from the River Schwarze Elster from 1967 to 1972. Due to the mining technique applied, several small and larger islands divide the lake into two main parts, connected by a shallow channel: the large North Basin with a maximum depth of 23 m, and the small South Basin. As the ground water in the region is extremely acidic (ca. pH 2) due to pyrite oxidation, neutral water from the River Schwarze Elster has been used for continuous flushing of the lake, in order to prevent acidification. However, the South Basin, which is not influenced by the river, acidified. Mixing of water from the highly acidic South Basin and the neutral North Basin occurs within the connecting channel forming a horizontal pH gradient. The mixing zone is a shallow littoral habitat with dense stands of macrophytes dominated by *Juncus bulbosus*.

Water chemistry is essentially different at both sides of the gradient (Table 4.1). The hydrogen ion concentration is about 10 000 times higher in the acidic part (ca. pH 3.2) that is strongly buffered by high concentrations of iron (3.1 mg l^{-1}), resulting in an acidity of 1.72 mmol l^{-1} . The neutral part is buffered by the carbonate system, and metal ion concentrations are much lower here, which corresponds to the higher pH. Furthermore, high concentrations of sulphate, carbonate, calcium and magnesium were detected on both sides of the gradient and are characteristic of mining lakes in general (Nixdorf et al., 1998). The measured concentrations of heavy metal ions, such as cadmium and copper, are negligible. Within the mixing zone the chemical conditions are unstable due to continuous chemical processes, such as the oxidation and precipitation of Fe (II) and Al, which leads to the loss of buffer capacity. These processes presumably account for visible changes in water colour and clarity as well as for the formation of a dense layer of precipitate covering macrophytes within the mixing zone.

The acidic part of the lake is oligotrophic, while the neutral part seasonally becomes more productive reaching a mesotrophic level. Apart from

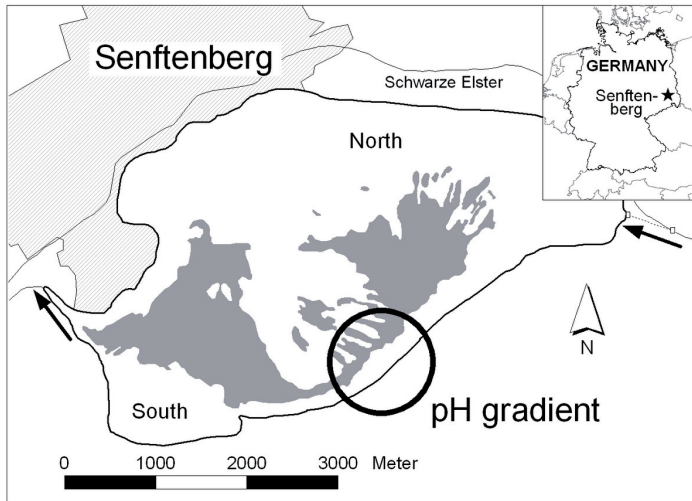


Figure 4.1 Map of Lake Senftenberger See in Lusatia (Germany). Inset: location of the lake in Germany, circle: location of the pH gradient in the channel connecting the neutral North and the acidic South Basin, grey area: islands. Arrows indicate the in- and out-flow of water from the River Schwarzer Elster

tourism and fishery the lake plays an important role in the water management of the mining region, in particular, for flood control and has been used as a reservoir.

4.3 Materials and methods

Sampling of Cladocera and recording of physicochemical parameters within the pH gradient in Lake Senftenberger See were carried out in 2003 (08.05., 03.09.) and 2005 (19.04., 28.04.). Horizontal profiles of temperature, conductivity and pH were taken at ca. 0.5 m depth by a multi-parameter probe (Hydrolab H20) connected to a field computer (Husky Hunter). Simultaneously locations of point measurements were recorded on three dates (03.09.2003, 19.04.2005, 28.04.2005) using a hand-held GPS receiver (Garmin GPS 72). On each date about 200 points were measured over a transect of ca. 1.5 km. GPS coordinates were transformed from WGS 84 to DHDN GK5 format using WGeo 3.0 software. Contour maps were created using the GIS software package ArcView 3.2a with 3D extension.

Samples of Cladocera were taken from up to 1 m depth using a 100 μm

mesh dip net. A total of 37 samples were collected, 6–14 samples on each respective date, and preserved with 37% formalin to a final concentration of approximately 4%. Cladocera species were identified at 400× magnification under a light microscope according to Flößner (2000), Lieder (1996) and Smirnov (1996). For calculation of relative abundances each sample was examined under a stereomicroscope using a Bogorov chamber. 100 specimens were counted starting from the second row of the chamber.

In a first set of experiments, we assessed the acute toxicity of lake water from the pH gradient in Lake Senftenberger See for *C. sphaericus* in relation to in situ environmental conditions at the sampling localities. Both *C. sphaericus* and lake water were sampled on 28 April 2005 at pH values of 7.4, 6.1, 5.3, 4.3 and 3.4, taken to the laboratory and used for the experiments within the next 10 h. We used two different experimental setups to test for the influence of acclimation on survival rates. First, in a sequential setup the treatments consisted of stepwise transfers of test animals from their respective in situ pH to the final pH in steps of ca. 1 pH unit every 18 h to account for acclimation. This procedure was applied to all possible combinations of in situ pH and final pH using water from the gradient and amounted to overall 60 vials (20 pH combinations with 3 replicates each). Given that the most extreme transfers consisted of four 18h-steps, the duration of the whole experiment was 72 h. Survival rates were calculated separately for each single transfer step. Only those transfer steps with at least 10 individuals were included into the statistical analysis, which resulted in overall 81 data points. In the second setup animals were transferred from their respective in situ pH in one step to the final pH without prior acclimation. This procedure was restricted to the transfers from pH 3.4 and 5.3 to 7.4 and from 7.4, 6.1 and 5.3 to 3.4, respectively, and summed up to overall 15 vials (5 pH combinations with 3 replicates each). The same controls were used for both setups, and they consisted of *C. sphaericus* kept at their original in situ pH, media being renewed and dead animals removed at 18 h intervals, so the handling was the same as in the treatments. Overall 15 vials (5 pH values with 3 replicates each) were used as controls. All replicates in both treatments and controls consisted of 20 individuals per vial, which were randomly selected from field samples. All the tests were performed in glass vials filled with 25 ml 30 µm filtered lake water and kept under a 8:16 h light/dark regime at ca. 11 °C (the recorded in situ temperature varied from 10 to 14 °C among the sampling localities). No additional food was added, apart from phytoplankton that naturally occurred in the lake water. Survival was calculated as the ratio of the final

and the initial numbers of live animals. The controls were included into calculations as 'zero treatments', rather than used for treatments' correction, because of an unexpected mortality of the controls at pH 6.1 and 7.4.

In order to address this phenomenon, we conducted a second set of experiments to investigate factors, diminishing the survival of *C. sphaericus* at circumneutral pH. The setup of the first set of experiments was then simplified in the following way: *C. sphaericus* was sampled on the 10.05.2005 at two locations, at pH 6.5 within the pH gradient and at pH 7.4, 800 m off the gradient. The treatments consisted of the transfers of animals in one step from their respective in situ pH to 3.2, 6.5, 7.4 (Lake Senftenberger See) and to pH 8.1 (a natural eutrophic Lake Scharmützelsee, 52°17'27" N, 14°03'04" E, for the description of the water chemistry parameters see Hämmerling et al., 2006). Five replicates each for the treatments and controls were incubated for 48 h without prior acclimation at ca. 17 °C, the standard temperature for laboratory cultures, overall resulting in 30 vials for treatments and 10 for controls. A shorter incubation time of 48 h was applied because no additional mortality occurred after this period in the first experiment.

4.3.1 Statistical analysis

For statistical tests (χ^2 , t-test for independent samples) we used the software package SPSS 12.0 G according to Backhaus et al. (2003). The nonlinear regression analysis was based on the Levenberg-Marquardt algorithm and used the nonlinear least-squares curve-fitting procedure implemented in the OriginPro 6.1 (OriginLab Corporation) software. Survival data in the experiments, as well as presence/absence field data of species occurrences were fitted to the logistic (logit) regression model $y = a \times (1 + e^{-z})^{-1}$ with $z = k \times (x - x_c)$, where k is the regression coefficient and $(k \times x_c)$ is the intercept of the logit-linearized function, x_c is the centre value of the regression line, and a is the amplitude which was set to 1. In order to test for significant differences between the regression coefficients a Monte Carlo resampling simulation (bootstrapping) procedure was applied using Microsoft Excel (Simon, 1997). Randomized regressions of the experimental data with treatment pH as the independent variable were calculated by drawing 1500 replicate samples from the original survival data (reshuffling with replacement), each time calculating regression coefficients, as well as the differences between them. The probability to erroneously reject the null hypothesis, i.e. the differences between coefficients are random, was

calculated as the ratio of cases with a higher difference in comparison to the original data and the sum of all simulated cases.

4.4 Results

4.4.1 Cladocera species richness and distribution within the pH gradient

We found a total of 25 species of Cladocera, the majority of them (14) from the family of Chydoridae (Table 4.2). The most common genera were *Alona* Baird, 1843 (5 species) and *Ceriodaphnia* (Dana, 1853) (3 species). Only 2 species—*C. sphaericus* and *Scapholeberis mucronata* (O.F. Müller, 1776)—were found at pH 3, whereas other species occurred starting at pH 4.9. *C. sphaericus* was the most abundant and the only species present in all the samples ($N = 37$). In 32 samples (91%) its share of the total abundance of Cladocera was higher than 10% (Table 4.2) and in many cases

Table 4.2 Comparison of the minimum in situ pH recorded for 25 Cladocera species within the pH gradient in Lake Senftenberger See on four sampling dates and the minimum pH values given in the literature for the respective species. N: frequency of occurrence in 37 samples, Dominance: frequency of records with a share > 10% of the total abundance

pH range	Cladocera	N	Dominance	Minimum in situ pH	Other locations	Citations
3	<i>Chydorus sphaericus</i>	37	32	3.1	3.2	Flößner, 2000
	<i>Scapholeberis mucronata</i>	13	2	3.0	3.8	Bērziņš & Bertilsson, 1990
5	<i>Eurycercus lamellatus</i>	15	-	4.9	3.9	Flößner, 2000
	<i>Pleuroxus truncatus</i>	2	-	4.9	4.2	Flößner, 2000
	<i>Bosmina longirostris</i>	15	1	5.0	4.3	Bērziņš & Bertilsson, 1990
	<i>Sida cristallina</i>	14	2	5.0	4.6	Fryer, 1993
	<i>Alona affinis</i>	7	-	5.0	4.0	Flößner, 2000
6	<i>Acroperus harpae</i>	12	8	5.7	4.6	Bērziņš & Bertilsson, 1990
	<i>Alona costata</i>	10	-	5.7	4.7	Bērziņš & Bertilsson, 1990
	<i>Ceriodaphnia pulchella</i>	9	2	5.7	5.0	Bērziņš & Bertilsson, 1990
	<i>Polyphemus pediculus</i>	7	-	5.7	3.9	Flößner, 2000
	<i>Alona guttata</i>	3	-	5.7	3.8	Bērziņš & Bertilsson, 1990
	<i>Diaphanosoma brachyurum</i>	2	-	5.7	3.9	Flößner, 2000
	<i>Simocephalus vetulus</i>	8	-	6.1	4.4	Flößner, 2000
	<i>Camptocercus rectirostris</i>	2	-	6.8	4.7	Uimonen-Simola & Tolonen, 1987
	<i>Daphnia galeata</i>	2	-	6.8	5.0	Flößner, 2000
	<i>Bosmina coregoni</i>	1	-	6.8	3.3	Almer et al., 1974
7	<i>Monospilus dispar</i>	1	-	6.9	5.5	Flößner, 2000
	<i>Alona rustica</i>	3	-	7.0	3.3	Fryer, 1993
	<i>Pleuroxus trignonellus</i>	1	-	7.0	5.0	Flößner, 2000
	<i>Alona quadrangularis</i>	1	-	7.0	5.0	Flößner, 2000
	<i>Alonella excisa</i>	2	-	7.2	3.8	Flößner, 2000
	<i>Ceriodaphnia quadrangula</i>	2	-	7.2	3.8	Bērziņš & Bertilsson, 1990
	<i>Acroperus angustatus</i>	1	-	7.4	-	
	<i>Ceriodaphnia megops</i>	1	-	7.4	5.0	Flößner, 2000

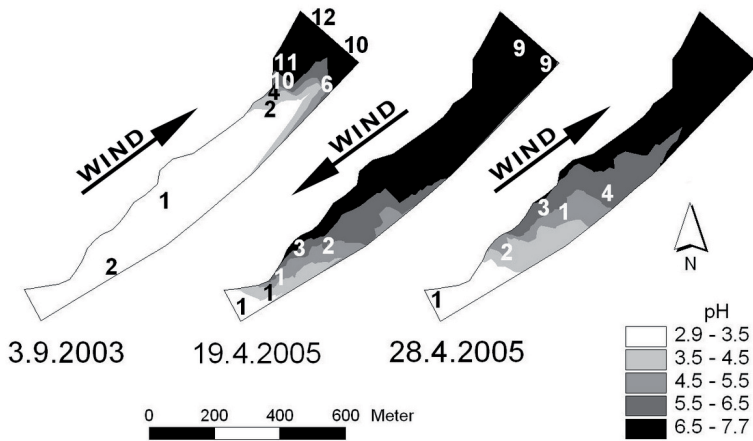


Figure 4.2 Contour plots of pH within the transitional zone between the neutral and the acidic basins of Lake Senftenberger See (Lusatia, Germany) on the 3 dates (03.09.2003, 19.04.2005, 28.04.2005). Numbers: species richness of Cladocera species, arrows: main wind direction at the respective date

close to 100%. Other occasionally dominant taxa were *Acroperus harpae* (Baird, 1835) (8 times), *Ceriodaphnia pulchella* Sars, 1862 (2 times), *Sida crystallina* (O.F. Müller, 1776) (2 times) and *S. mucronata* (2 times). None of the species found was restricted to the acidic conditions, but all acidtolerant species were found at neutral pH as well. Therefore, they tended to occur more often, than the species that were found only at neutral pH. Most of the species found are generally common in the littoral zone. *B. longirostris* was the most tolerant pelagic species, having been found at pH 5.0, whereas the other three pelagic taxa occurred at pH 5.7 or higher in the following order: *Diaphanosoma brachyurum* (Liévin, 1848), *Bosmina coregoni* Baird, 1857 and *Daphnia galeata* Sars, 1863 (Table 4.2).

Figure 4.2 illustrates the highly dynamic nature of the pH gradient and the spatial distribution of the species richness. Depending on the wind direction, the pH gradient changed its location within the connecting channel by approximately 800 m, and its length varied from 100 to 600 m. The decrease in species numbers was towards the acidic part, concordant with the gradient. The mean species richness per sample declined from 8.2 ± 3.2 under neutral conditions to 1.5 ± 0.7 below pH 4.6 (Fig. 4.3 A). The concomitant reduction of variation within the groups indicates the growing

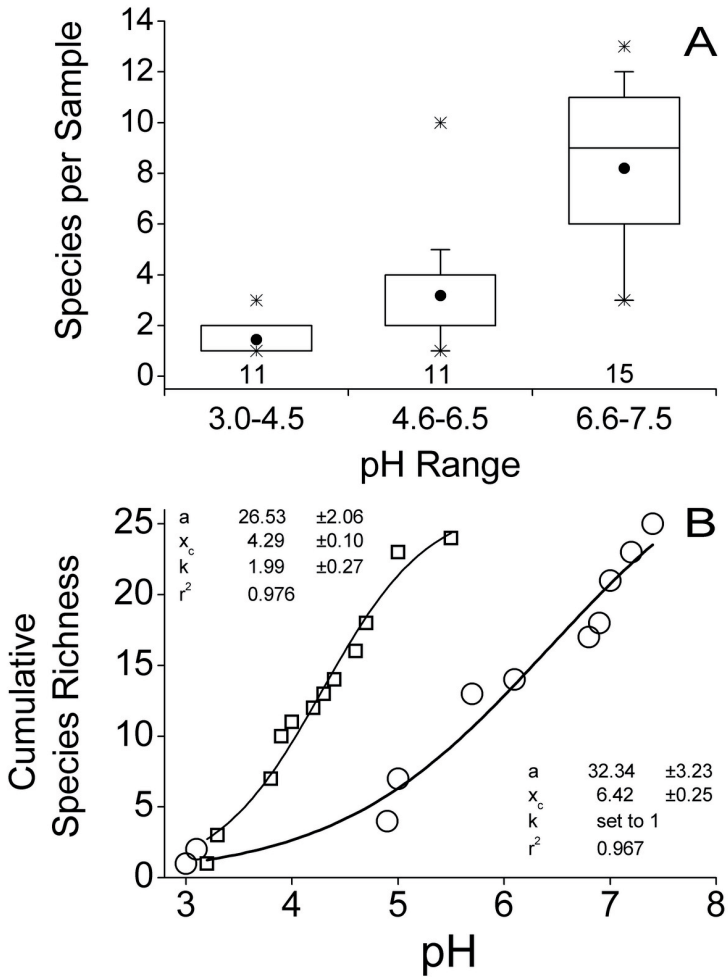


Figure 4.3 Species richness of Cladocera within the pH gradient in Lake Senftenberger See (Germany). (A) Box-plots of species richness on the basis of 37 samples aggregated to three pH ranges between 3 and 7.5 with similar sample sizes. Cross: maximum and minimum values, whisker: 10th and 90th percentile, box: interquartile range (50%), solid circle: mean value, horizontal line: median, numbers above the X-axis give the numbers of samples examined. (B) Nonlinear regression curves for the potential cumulative species richness of Cladocera. Data represent the in situ pH minima at which species were found at the study site (open circles, bold solid line) as well as the lowest in situ pH values reported for these species in the literature (open squares, thin solid line, for data see Table 4.2). For explanation of the model parameters see Methods

importance of pH as a limiting factor towards more acidic conditions.

In order to estimate if the potential for cladoceran colonization differs between mining and soft-water lakes we used cumulative species richness curves based on the minimum in situ tolerances of species. We fitted a nonlinear (logistic) regression to our field data, as well as to the in situ pH minima given in the literature for the same species. A substantial discrepancy exists between the two curves (Fig. 4.3 B), the centre values x_c of the regression line differing by more than 2 pH units, and the slope k of the regression being steeper for the literature values. Thus, the cumulative species richness of Cladocera found in our study was considerably lower than predicted from the species in situ pH minima in softwater acidic lakes.

4.4.2 Is the pH gradient a toxic environment for *Chydorus sphaericus*?

In the first experimental setup using sequential transfers we aimed to test the null hypotheses that the probability of survival of *C. sphaericus* within the pH gradient is (1) equal at all tested pH values and (2) independent of the location, where the animals were collected. A χ^2 -test, assuming an even distribution of survival data over the observed pH range, revealed highly significant differences ($p < 0.01$) compared to the distribution of experimental data. Hence, the probability of survival is significantly different among the tested pH values, and the first null hypothesis can be rejected. The evidence to reject the second null hypothesis comes from plotting the survival rates of *C. sphaericus*, grouped according to the in situ pH, against the treatment pH as the independent variable (Fig. 4.4). The overall response pattern can be described by fitting a logistic (logit) regression model to the experimental data. The two most divergent regression coefficients (in situ pH 5.4 and 3.4) were tested for significant differences by a Monte Carlo re-sampling method (see Methods for the test procedure). The second null hypothesis (differential response among the sampled localities) could not be rejected because no significant differences were found ($p > 0.05$).

In order to explore the general response pattern the data from all experiments were pooled and a combined regression model was calculated describing the observed asymmetrical response over the entire pH gradient (dashed line in Fig. 4.4). The centre value x_c of the pooled regression line is at pH 5.5 and it assigns the place in the gradient with a 50 % probability of survival ($k = -2.5$, $r^2 = 0.69$). No regression could be fitted to the data of in situ pH 6.2 and 7.4, because no animals could pass further

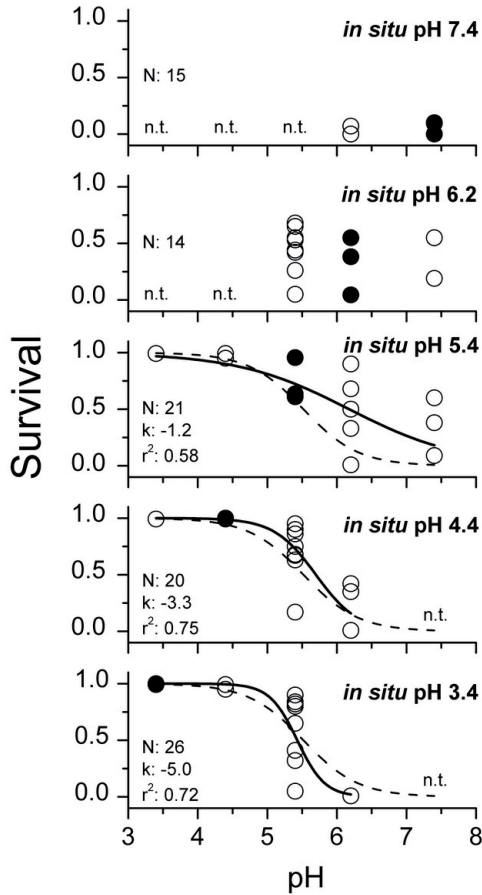


Figure 4.4 Survival of *C. sphaericus* in acute toxicity experiments with mining lake water (Senftenberger See, Germany) of 3.4, 4.4, 5.4, 6.2 and 7.4 pH. Test animals have been transferred from the respective *in situ* pH by a stepwise method (1 pH unit every 18 h) to all other pH to account for acclimation. Results are grouped according to the *in situ* pH, i.e. the original pH at the sampling station. Solid circles: controls, hollow circles: survival in treatments after ca. 18 h of exposure, 'n.t.': no transfer possible because less than ten individuals survived in the previous transfer step, solid lines: logistic regressions fitted to the data of the respective *in situ* pH, dashed lines: logistic regression line fitted to the pooled data, N : number of observations, k : regression coefficient of the linearized function, r^2 : correlation coefficient

than a pH of 5.4. Furthermore, an increased mortality was observed in all treatments and controls at weakly acidic and neutral conditions (Fig. 4.4). The survival rates were extremely low at pH 6.2 and 7.4 (median values of 0 and 0.15 respectively), indicating nearly no chance for survival, while 100% survival occurred at the allegedly stressful pH 3.4 and 4.4. Lake water of intermediate pH 5.4 also caused an intermediate effect with a high variability. Transfers starting from here were successful in both directions with the highest survival rates towards acidic pH (Fig. 4.4).

We applied the second setup using one-step transfers in order to study the effect of abrupt pH changes, which may occur within the gradient, for example, via intensive mixing. In the most extreme transfers bridging 4 pH units we observed survival rates of about 0.2 (Fig. 4.5). When both transfer methods were compared, survival was significantly higher in one-step transfers from pH 7 to 3 ($p < 0.05$, t-test), but no significant differences were observed in pH 3 to 7 transfers. At a smaller pH increment of 2 units, there were no significant differences related to the method used. In both stepwise and one-step transfers survival was higher under acidic compared to neutral conditions ($p < 0.05$, t-test, Fig. 4.5). At an intermediate pH increment of 3 units (pH 6.1 to 3.4) the survival rate was intermediate as well (mean 0.88 ± 0.03 , data not shown).

In the second set of experiments, we tested the null hypotheses that (1) neutral pH rather than other chemical characteristics of the tested lake water caused the mortality of *C. sphaericus* in the previous experiments and (2) that there is no difference in the sensitivity of *C. sphaericus* collected from different circumneutral locations in the lake. The results confirmed the strong lethal effect at pH 6.4 and 7.4 with mean survival rates of 0.02 and 0.04 (Fig. 4.6), respectively. When the test animals were transferred to water from a natural lake with a pH of 8.1, the survival rate increased significantly to 0.57 ± 0.24 (pooled mean for both test groups, t-test, $p < 0.05$), suggesting that other chemical characteristics of the Lake Senftenberger See water, rather than pH, caused the increased mortality of *C. sphaericus*.

No significant difference in survival was found between the test groups (t-test: $p > 0.05$), suggesting that there is no difference in response to pH 6.4 and 7.4 between the two sampling localities. However, a significant difference in survival between the two groups was found at a highly acidic pH of 3.2 (t-test: $p < 0.01$). Animals collected at a pH of 6.5 within the gradient survived much better under the harsh acidic conditions, which were lethal to those collected 800 m further off the gradient at a pH of 7.4 (Fig. 4.6). The mean survival rates were not significantly different in the

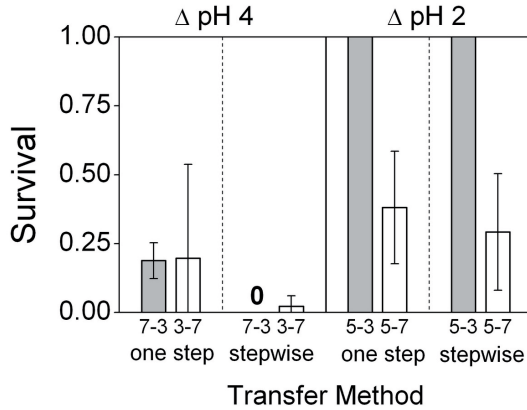


Figure 4.5 Survival of *C. sphaericus* in acute toxicity experiments using two different transfer methods: Comparison of transfers over 2 and 4 pH units, respectively. (method 'one step': transfers in one step without prior acclimation, method 'stepwise': transfer in steps of ca. 1 pH unit per 18 h, grey bars: transfers to acidic pH 3.4, white bars: transfers to neutral pH 7.4, error bars: standard deviation of 3 replicates). Numbers below the X-axis indicate the pH increment and direction of change, e.g. '7-3' means transfer from pH 7.4 to 3.4

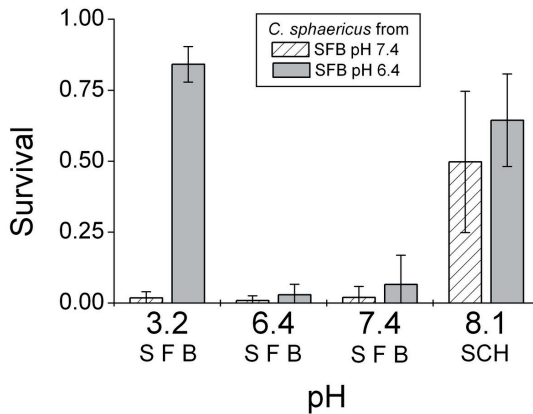


Figure 4.6 Survival of *C. sphaericus* collected at two circumneutral locations in Lake Senftenberger See: at pH 6.4 within the gradient and at pH 7.4 800 m off the gradient. The test animals were exposed for 48 h to lake water of pH 3.2, 6.4 and 7.4 from Lake Senftenberger See (SFB) and water of pH 8.1 from a natural, eutrophic lake (SCH: Lake Scharmützelsee). Error bars: standard deviations

transfers from pH 6.4 to 3.2 in this set of experiments as compared with the corresponding one-step transfers in the previous experimental setup (0.84 ± 0.07 , and 0.88 ± 0.03 , respectively). Meanwhile, *C. sphaericus* collected 800 m off the gradient survived significantly worse when transferred to acidic pH than those animals which were collected at pH 7.4 close to the gradient (mean survival rates 0.02 ± 0.02 and 0.19 ± 0.07 , respectively; t-test: $p < 0.05$). Our findings suggest that both the effect of mining lake water chemistry as well as variation in tolerance among circumneutral locations within the lake should be incorporated to account for the response of *C. sphaericus*.

4.5 Discussion

4.5.1 Unfavourable conditions in mining lake water at pH 5–6

Acidic mining lakes represent hard-water acidic habitats that are poorly investigated in respect of their cladoceran community composition and species-specific physiological thresholds. In Lake Senftenberger See, the unique situation of a within-lake pH gradient from 3 to 7 enabled us to study the community response of Cladocera to acidic water with a chemical composition typical of mining lakes. Our results show that the acidic pH alone is neither sufficient to explain the decreased cumulative species richness; nor does it explain the observed mortality of an acid-tolerant species *C. sphaericus* at weakly acidic and neutral pH values. In mining lakes at a pH below 4, water is strongly buffered by the iron buffer system, whereas mixing with neutral water results in a loss of buffer capacity. This causes chemical instability, and intense precipitation of metal compounds occurs, as the buffer system changes towards the aluminium and afterwards to the carbonate system (Nixdorf et al., 2003). In the mean time, precipitation of Al and Fe was shown to be harmful for fish in acidic streams which were limed to achieve a neutral state (Weatherley et al., 1991). In our study the centre value of the logistic regression at pH 5.5 and the high variation in survival of *C. sphaericus* indicate unfavourable conditions at this pH range. It seems likely that it is mainly precipitation of Al compounds that prevents expansion of cladoceran species towards more acidic conditions and that this caused the mortality of *C. sphaericus* at this pH in the experiments. While high toxicity at moderately acidic conditions seems to be related to

the specific water chemistry typical of mining lakes, this effect would be only weakly expressed in soft-water acidic lakes, where relatively diverse cladoceran communities have been reported at this pH (e.g. Fryer, 1980; Walseng et al., 2003). A possible explanation for this might be that much higher concentrations of inorganic dissolved Al are achieved in mining lakes accompanied with a lack of metal-binding humic substances, which have been shown to decrease toxicity and prevent precipitation of inorganic metal compounds in soft-water lakes (Steinberg, 2003).

Due to geogenic acidification, most of the mining lakes in the Lusatian Area (Germany) are highly acidic, with a pH of 2.5–3.5, unless they have been flushed with neutral water and achieved circumneutral conditions (Geller et al., 1998). However, the latter tend to be unstable, due to continuous influx of acidic groundwater and once the flushing is stopped the lake may revert to an acidic state. Our findings predict that during such a re-acidification, as well as in mixing zones, the conditions can become highly unfavourable even for acid-tolerant species.

4.5.2 Community response of Cladocera in a changing environment

In Lake Senftenberger See the investigated cladoceran community consisted of wide spread species (Flößner, 2000), which are also common in nearby neutral lakes (unpubl. authors' data). Communities comprised of generalist species and forming simple food webs indicate environmental stress (Simon, 2002), and acidic mining lakes can be regarded as extreme environments, where animals are subjected to multiple abiotic stressors.

The cumulative species richness of Cladocera was essentially lower in Lake Senftenberger See at the respective pH values than it is predicted from the in situ pH minima taken from literature. Assuming that our sampling was sufficient to reveal species in situ distributions and no physical barrier prevents dispersal within the gradient, we can hypothesize that some lake water characteristics hamper colonization by cladoceran species. It has been previously shown that additional stressors, such as metal pollution, can decrease species pH tolerances (Fryer, 1993; Kappes & Sinch, 2005). At pH 3–5 relatively high concentrations of Al and Fe in lake water are likely to contribute to its toxic effect on cladocerans. At a pH range of 5–6 precipitation of metal compounds discussed above probably plays the most important role in the extinction of even acid- and metal-tolerant species. Furthermore, also at neutral pH we have found much lower diversity, than

is commonly found in temperate lakes. The reasons are unclear and are probably related to the specific water chemistry in mining lakes.

Dynamic conditions within the gradient is another factor, which may bias the correspondence between the observed in situ species distributions and the true species preferences, as water shifts could be too fast for small cladocerans to avoid unfavourable conditions and to occupy space that becomes less acidic. Thus, the question arises: Can small species actively adjust their horizontal position according to their preferences despite of the dynamic nature of the gradient? Considering the average speed of the slowest cladocerans—chydorids of $2.5\text{--}7\text{ mm s}^{-1}$ (Smirnov, 1971), we can roughly estimate the distance potentially covered by a chydorid during one day. Using the lowest value of 2.5 mm s^{-1} and assuming 8 h of movement in one direction the distance amounts to ca. 72 m, what exceeds by far the observed shift of ca. 21 m d^{-1} of the pH gradient between the two sampling dates in April 2005, when the wind direction changed. The spatial pattern of species richness corresponded well to the gradient extension, and no irregularity was detected suggestive of possible impact of other environmental factors such as water mixing or micro-habitat structure. Thus, we can hypothesize that despite the dynamic nature of the gradient Cladocera are capable to adjust their position within the gradient according to their preferences. It signifies that in situ distributions of cladocerans are likely to reflect their true tolerances.

As cladocerans in the investigated mining lake displayed differential species-specific tolerances, they can be useful indicators of environmental conditions in mining lakes. Supplementary to the suggestions by Nixdorf et al. (2005) to use zooplankton as bio-indicators in ecological quality assessments, we recommend using the species richness and composition of littoral Cladocera as a valuable parameter, particularly, under neutral and weakly acidic conditions.

4.5.3 *Chydorus sphaericus*—a specialist in tolerance?

In Lake Senftenberger See *C. sphaericus* has been found across the entire pH gradient, however, in the experiments its survival varied greatly among the treatment pH values. Acidic conditions seem to be most favourable for the studied population, as survival in the controls was absolute, and of all treatments those at pH 3.4 and 4.4 caused the lowest mortality irrespective of the origin of the specimens. While the decrease in survival at a pH range of 5–6 is likely to be an effect which also occurs in situ (see above), high

mortality at pH around 7 was apparently an artefact. This is suggested by the high survival of the test animals in natural lake water of pH 8.1. Besides, abundant populations were collected in the neutral part of the Lake Senftenberger See far from the gradient zone. Presumably, this effect can be attributed to the chemical instability of the lake water at pH 6.4 and 7.4 and some undetected changes that had occurred during the transport or laboratory incubations in small volumes. Although pH in the incubation vials, measured every 18 h, remained stable, self-toxication of lake water apparently occurred in a few hours after the experiments had started.

The response pattern in the acute toxicity experiments was not significantly different among *C. sphaericus* collected at pH 3.4, 4.4 and 5.4. However, animals collected at pH 6.2–6.4, 7.4 near the gradient and pH 7.4 800 m off the gradient displayed significant differences in survival, when transferred to the acidic pH in one step. The proportion of individuals, which survived the transfers to acidic mining lake water, was lowest for the animals collected 800 m off the gradient. This may be due to either environmentally induced or genetic variation in tolerance of *C. sphaericus* and further investigations are required to distinguish between these two cases.

The results of the acute toxicity experiments suggest that the *C. sphaericus* population from Lake Senftenberger See is capable of enduring both extremes of the pH range of the gradient. We found that ca. 20% of the individuals survived the most extreme transfers. A possible explanation could be that those individuals survived, which were in the insensitive stage of their moulting cycle. Some investigations suggest that resistance to low pH is related to the effectiveness of ionic regulation (Havas & Likens, 1985; Havas & Advokaat, 1995). Moulting of crustaceans was shown to be accompanied by changes in ionic fluxes (Ahearn et al., 2004), and thus life cycle stages are likely to be differentially sensitive to a high-ambient protons' concentration. Essentially, the results of the experiments have shown that *C. sphaericus* can cope both with fast environmental changes and with a wide pH range, though a chemical barrier, represented by a highly toxic zone at pH 5–6, should to a great extent limit the exchange between the sub-populations at the two ends of the gradient in Lake Senftenberger See. However, this barrier for dispersal is not likely to be absolute. According to the calculations above a specimen of *C. sphaericus* should theoretically be able to pass the chemical barrier within the gradient in 5 to 20 h depending on the length of the zone on the respective day. An exposure time of that duration allows a high probability of survival, as it has been shown for

C. sphaericus in the acute toxicity experiments. Thus, exchange of animals between both sides of the gradient should be possible, however, limited, and thus it might allow an existence of two sub-populations at each side of the gradient. The selection pressure should be very different between these two lake parts, as they exhibit essential differences in water chemistry, trophic state, food web structure and interactions (Table 4.1; Wollmann et al., 2000). It seems probable, that this might have led to colonization of each part by distinct genotypes with adaptations to the local conditions, which existed prior to the colonization or/and have been developed during the 40 years of the site's existence. Alternatively, we even have to consider the presence of sibling species, each with a restricted tolerance range, which would be suggested by the non-cosmopolitanism hypothesis that regards existence of eurybiotic cladoceran species as unlikely (Frey, 1980, 1986). Yet, contrary to this hypothesis, our study experimentally confirms the generalism of *C. sphaericus* in relation to pH, therefore, showing the principal possibility of the existence of generalists among cladoceran species. Overall, *C. sphaericus* displays to our knowledge the broadest range of pH tolerance among crustaceans, though some rotifer species are known to be even more eurybiotic (Deneke, 2000). Some studies also report on the wide tolerance ranges of this species to other environmental factors such as temperature, trophic state and salinity (Belyaeva, 2003; Boronat et al., 2001; De Eyto et al., 2003), so *C. sphaericus* seems to be a 'specialist' in tolerance to a wide range of abiotic 'conditions'.

Chapter 5

Cryptic species within the *Chydorus sphaericus* species complex (Crustacea: Cladocera) revealed by molecular markers and sexual stage morphology

Abstract

The cosmopolitanism paradigm in the biogeography of freshwater invertebrates is currently being replaced by non-cosmopolitanism or continental endemism. Benthic water fleas (Cladocera) from the family Chydoridae were the first group of freshwater invertebrates for which non-cosmopolitanism and cryptic diversity was substantiated by morphological studies. Yet, little is known about genetic differentiation and evolutionary history of chydorid species complexes. Here we present the first analysis of the genetic versus morphological differentiation in a benthic cladoceran species complex—*Chydorus sphaericus* s. str. using sequence variation in a nuclear (ribosomal internal transcribed spacer 2, ITS-2) and a mitochondrial (cytochrome c oxidase subunit I, COI) genes in 50 Holarctic localities. We tested for continental endemism and cryptic diversity predicted by previous morphological studies. We found evidence for the presence of at least seven putative regional species in the Holarctic, at least three of them being distributed beyond a single continent. While the molecular and sexual stage characters showed general concordance on species lineages, parthenogenetic female characters lacked resolution or were unas-

sociated with molecular lineages. We conclude that cryptic regional lineages of benthic cladocerans are apparent and that the sexual stages represent the most informative morphological source of species characters for this environmental indicator group.

5.1 Introduction

Darwin famously concluded that priority effects and local selection led to the frequent evolution of independent lineages among terrestrial islands. His Galapagos bird example illustrates that lineage formation can occur despite the possession of strong dispersal abilities and short distances among islands. Yet, Darwin reasoned a different evolutionary scenario for the inhabitants of freshwater islands or lakes. Here, he proposed that migration would serve to unify aquatic species separated by vast geographic distances because the rates of modification are inherently much slower among freshwater species (mostly invertebrates) compared to terrestrial species. But, lineage formation in the 'lower forms' could merely be difficult to detect by humans. Indeed, genetic studies have revealed considerable cryptic diversity in freshwater invertebrates (e.g. Gómez et al., 2002; Jackson & Resh, 1998; Lefébure et al., 2006; Taylor et al., 1998). The failure to distinguish sibling species has partly accounted for the long-standing concept of cosmopolitanism in freshwater invertebrates (Bohonak & Jenkins, 2003). Later, as evidence of strong geographic structuring accumulated, the opposite concept of non-cosmopolitanism or continental endemism became generally accepted (Bohonak & Jenkins, 2003). Likewise, models of lineage formation in freshwater invertebrates now more closely resemble Darwin's model for strongly dispersing vertebrates on oceanic islands (e.g. De Meester et al., 2002; Taylor et al., 1996).

Still, our understanding of the mechanisms and evolutionary consequences of dispersal is limited. Some phylogeographic studies indicated that there might be a high variation in rates, magnitude and success of dispersal among aquatic insects employing similar dispersal strategies (Hughes et al., 1999; Wishart & Hughes, 2003). Pelagic zooplankton groups, such as rotifers and cladocerans, contain well-documented cases of both endemism and cosmopolitanism (Colbourne et al., 1998; Gómez et al., 2002; Schwenk et al., 2000; Taylor et al., 1996). Benthic passively dispersing groups remain poorly studied. Benthic Cladocera mostly show lower growth rates and rely on different food resources than pelagic zooplankton (Smirnov, 1971). According to the monopolization hypothesis cyclic parthenogens that require

more time to exhaust the available resources should exhibit less pronounced geographic structure (De Meester et al., 2002). On the other hand, intimate associations of benthic Cladocera with substrata may restrict the choice of habitats suitable for colonization and thus enhance structuring. Therefore, generalizations about freshwater invertebrate biogeography remain elusive.

5.1.1 State of the art in the taxonomy and evolution of chydorid Cladocera

Benthic Cladocera of the family Chydoridae are species-rich and abundant in freshwater habitats (Smirnov, 1971, 1996). They have been widely used as indicator and test species in ecological, ecotoxicological and paleolimnological studies (De Eyto et al., 2003; Dekker et al., 2006; Frey, 1960; Hofmann, 1987; Walseng et al., 2003). However, uncertainties in chydorid taxonomy have often hampered the applied research on this group, for instance, cryptic diversity may bias results of ecological studies if sibling species possess different ecological characteristics. The establishment of the non-cosmopolitanism concept (Frey, 1986), substantiated by morphological studies, encouraged further detailed investigations on conspecifics with inter-continental distributions (Frey, 1995). As a result, substantial cryptic diversity was revealed, greatly advancing the taxonomic knowledge of this group. Yet, the state of the chydorid taxonomy is immature (Korovchinsky, 1996, 2000). The morphological approach appears to underestimate the diversity in the most species-rich chydorid taxa, as the latter may often display a combination of morphological stasis and high intra-specific polymorphism (Frey, 1980). Ecological and genetic investigations are likely to provide more taxonomic resolution within closely related species complexes (e.g. Gómez et al., 2002; Taylor et al., 1996).

Frey (1995) noticed, that “demonstrating that cosmopolitanism . . . is less common than formerly believed . . . is only the first (and possibly the easiest) part of the problem to tackle. The major question now is: How did these species groups come into being, over what time periods, and why do we have so many of them?”. The existing hypotheses on the evolution in benthic cladocerans are based on the assumption of the ancient age of all present taxa. Frey (1995) proposed that most diversifications were promoted by continental drift of both Pangea and Gondwanaland. He noticed that dispersal into yet-uninhabited areas might be “the initiator of many of the troubling species groups”, but such events must have been rare and occurred many millions of years ago. Sacherová & Hebert (2003) regarded continental

drift as an important factor promoting diversification at the subfamily level. Chydorid genera and species are thought to evolve sympatrically via niche differentiation within the same water bodies. The most recent hypothesis explaining biogeography and evolution of chydorids is the ‘theory of ejected relicts’ (Korovchinsky, 2006). It considers continental drift an important factor, but emphasizes the role of mass extinctions and range contractions in shaping chydorid biogeography. Korovchinsky’s hypothesis predicts that species diversity was lost during the climatic changes in the Tertiary. None of these hypotheses explains how closely related complexes with several co-existing sibling taxa on a continent might have evolved. However, such species complexes are common within species-rich chydorid genera such as *Alona*, *Chydorus*, *Pleuroxus* and others (Smirnov, 1996).

5.1.2 Diversity of the *C. sphaericus* species complex

Chydorus sphaericus s. l. is a widely distributed species complex in the Holarctic. A few additional records exist from Africa, Australia and South America (Smirnov, 1996). Regional studies have suggested that these small chydorids (200–500 μm) occur under a widest range of environmental conditions and inhabit almost every investigated lake or pond in the Holarctic (e.g. Alonso, 1996; Chengalath, 1987; Duigan, 1992; Flößner, 2000; Walseng et al., 2003). Like many other zooplankton taxa, *C. sphaericus* was previously regarded as a single cosmopolitan species, but detailed morphological studies revealed cryptic diversity and continental endemism. Even a new genus (*Ephemeroporus*) and a few *Chydorus* species were described from what was previously regarded as *C. sphaericus* (Frey, 1995). At least three potentially valid species are presently recognized within the complex: *C. sphaericus* s. str. (Palearctic distribution), *Chydorus biovatus* (northern Nearctic distribution), and *Chydorus brevilabris* (southern Nearctic distribution) (Frey, 1980, 1985). Frey also distinguished *sphaericus* and *brevilabris* species groups within the complex. He argued that *C. sphaericus* s. str. and *C. biovatus* belong to the former and *C. brevilabris*—to the latter, and each group consists of many undescribed cryptic species (Frey, 1980, 1985). The taxonomic status of three recently described or redescribed taxa *Chydorus latus*, *Chydorus arcticus* and *Chydorus patagonicus* is unclear. They display the same general morphology of females and males that is characteristic for the complex, but their present descriptions are insufficient for the reliable discrimination of these taxa from *C. sphaericus* s. str. (Korovchinsky, 1996; Smirnov, 1996). Some morphotypes, which were previously regarded as separate species,—

Chydorus caelatus, *Chydorus herrmanni*, *Chydorus mutilus* and *Chydorus rylovi*—are now synonymized with the *C. sphaericus* s. str. (Brancelj, 1996; Frey, 1980; Smirnov, 1996).

The *C. sphaericus* complex is a challenging group for taxonomists. Differences in morphology of parthenogenetic females between the species are subtle, while considerable within-species variation has been described (Belyaeva, 2003; Duigan & Murray, 1987; Flößner, 2000; Frey, 1980; Hann, 1975; Smirnov, 1971, 1996). Consequently, the majority of morphological characters are of unknown taxonomic utility, as there is a possibility that they are characteristic just for the described populations, but not necessarily for the entire species. Alternatively, convergence may have occurred between closely related species (Frey, 1980). The distinct morphology of the ephippial females and males suggests independent taxonomic status of the three species named above. However, it is not unlikely that there might be unrevealed sibling taxa displaying similar morphology of gamogenetic individuals. Overall, a large amount of morphological variation remains unexplained and could be either due to the presence of sibling species, phenotypic plasticity or simple morphotypes. Based on the analysis of morphological variation in Nearctic populations, Frey predicted that many more species still masquerade under the present species names. Yet, recent investigations have failed to find morphological evidence for further separation of taxa within the *C. sphaericus* complex (e.g. Belyaeva, 2003; Duigan & Murray, 1987). It seems that applicability of the morphological approach is limited in this particular case. On the contrary, the genetic approach seems a promising tool for taxonomy, as many chydorid species are believed to be ancient (Frey, 1995; Sacherová & Hebert, 2003) and, consequently, they should be well-differentiated genetically.

In the present study we analyzed sequence variation in one mitochondrial-cytochrome c oxidase I (COI) and one nuclear-rDNA internal transcribed spacer 2 (ITS-2) gene in the *C. sphaericus* complex in the Holarctic. We also examined the morphological characters, which were previously proposed to be useful for species identifications. The objectives of this study were to (1) provide a phylogenetic framework for the diversity of the *C. sphaericus* complex; (2) test the predictions based on morphological investigations of continental endemism and cryptic diversity; and (3) obtain insights into the morphological and molecular evolution in a benthic cladoceran species complex.

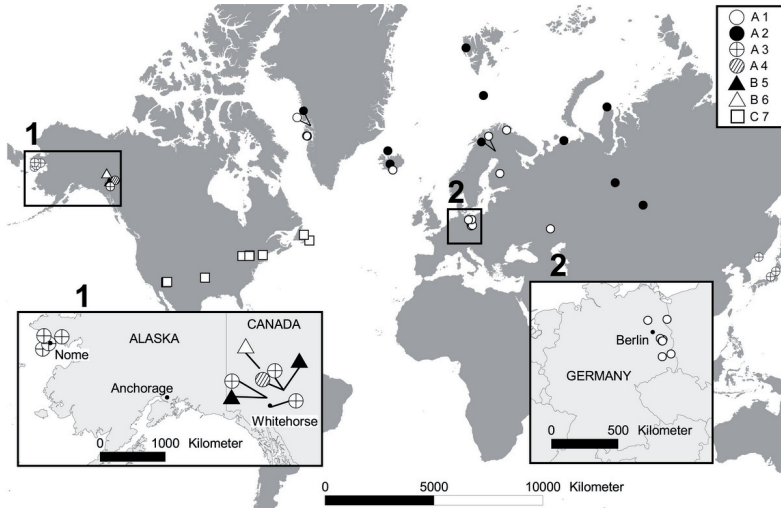


Figure 5.1 Collection sites for the studied Holarctic populations of the *C. sphaericus* species complex. (A1–C7)—putative species lineages identified from mtDNA and ncDNA phylogenies and the morphology of sexual stages

5.2 Materials and methods

5.2.1 Sampling and scoring of morphological characters

Specimens were collected from 50 Holarctic localities (Table 5.1; Fig. 5.1) and either preserved in 95% ethanol, acetone or liquid nitrogen. Prior to DNA extractions, all specimens were placed into a drop of water on a slide, examined under a light microscope to assign to either the *brevilabris* or the *sphaericus* morphotype *sensu* Frey (1980) (Fig. 5.2) and photographed (Leica equipment and software). The available gamogenetic populations were scored for the following morphological characters: number of ehippial eggs, shape of male postabdomen, presence of postabdominal claw in males and shape of male rostrum. These are the major characters on which species diagnoses are based in the currently known species within the *C. sphaericus* complex (Frey, 1980, 1985). We also examined some morphological traits of parthenogenetic females, suggested by Frey (1980, 1985) as being of potential taxonomic utility, such as shell sculpture (polygons along the ventral margin, dimples, lines, connecting major head pores) and shape of the tip of the rostrum (sharply emarginated versus weakly emarginated).

Table 5.1 List of the sampling localities and morphotype of collected *Chydorus* populations

Code	Locality, state/region	Latitude	Longitude	Morphotype	COI clade
Germany					
Ger1	Senftenberger See, Brandenburg	51°29'03" N	14°01'14" E	'sphaericus'	A1
Ger2	Großer Barsch See, Brandenburg	53°07'01" N	13°00'04" E	'sphaericus'	A1
Ger3	Tiefer See, Brandenburg	52°09'12" N	13°59'42" E	'sphaericus'	A1
Ger4	Oder flood plain, Brandenburg	53°02'46" N	14°16'45" E	'sphaericus'	A1
Ger5	Große Fuchskuhle, Brandenburg	53°06'23" N	12°59'07" E	'sphaericus'	A1
Ger6	Scharmützelsee, Brandenburg	52°17'28" N	14°03'05" E	'sphaericus'	A1
Ger7	Felixsee, Brandenburg	51°36'48" N	14°32'48" E	'sphaericus'	A1
Ger8	Wolziger See, Brandenburg	52°15'07" N	13°48'07" E	'sphaericus'	A1
Ger9	Langer See, Brandenburg	52°14'28" N	13°47'10" E	'sphaericus'	A1
Ger10	Melangsee, Brandenburg	52°09'36" N	13°59'18" E	'sphaericus'	A1
Ger11	Kleiner Milasee, Brandenburg	52°09'14" N	13°57'26" E	'sphaericus'	A1
Norway					
Nor12	Nameless pond, Finnmark	70°06' N	28°37' E	'sphaericus'	A2
Nor13	Nameless pond, western Swabard	78°55' N	11°56' E	'sphaericus'	A2
Nor14	Lake, Bear Island	74°25' N	19°02' E	'sphaericus'	A2
Iceland					
Ice15	Nameless pond, central Iceland	64°44' N	19°26' W	'sphaericus'	A2
Ice16	Nameless pond, northern Iceland	66°58' N	20°22' W	'sphaericus'	A1, A2
Ice17	Lake Fljótsbótin, southern Iceland	63°39'59" N	18°17'00" W	'sphaericus'	A1
Finland					
Fin18	Lake Kejtele	63°02'41" N	25°49'16" E	'sphaericus'	A1
Fin19	Lake Orajärvi	66°54'19" N	24°05'48" E	'sphaericus'	A1, A2
Greenland					
Gree20	Nameless pond, Disko Island	69°16' N	53°50' W	'sphaericus'	A1
Gree21	Nameless pond, Disko Island	69°15' N	53°38' W	'sphaericus'	A1
Gree22	Nameless pond, Uummannaq	70°41'24" N	52°10'12" W	'sphaericus'	A1, A2
Russia					
Rus23	Nameless pond, Tomsk	56°29' N	84°58' E	'sphaericus'	A2
Rus24	Nameless pond, Belyj Island	73°06' N	70°06' E	'sphaericus'	A2
Rus25	River Eruslan, Saratov Area	50°43' N	46°46' E	'sphaericus'	A1
Rus26	Lake Krasnogo Plastika, Arkhangelsk Area	68°35' N	52°18' E	'sphaericus'	A2
Rus27	Nameless lake, Khanty-Mansi Area	61°14' N	73°26' E	'sphaericus'	A2
Rus28	Lake Primorskoe, Region of Primorsky	42°49' N	132°53' E	'sphaericus'	A3
Japan					
Jap29	Lake Midori-ga-ike, Toyama	36°33' N	137°39' E	'sphaericus'	A3
Jap30	Lake Misuma-lke, Yamagata	38°22'05" N	139°49'16" E	'sphaericus'	A3
Canada					
Yuk31	Lake Kookatsoon, Yukon Territory	60°33'28" N	134°52'32" W	'sphaericus'	A3
Yuk32	Nameless pond, Canol Road, Yukon	61°44'53" N	133°04'21" W	'sphaericus'	B6
Yuk33	Nameless pond, Yukon Territory	63°03'29" N	136°25'42" W	'sphaericus'	B5
Yuk34	Fox Lake, Yukon Territory	61°10'38" N	135°23'29" W	'sphaericus'	A3, B6
Yuk35	Lapie Lake, Yukon Territory	61°39'34" N	133°03'37" W	'sphaericus'	A3, A4
Newf36	Octagon Pond, St. John's, Newfoundland	47°31'00" N	52°52'60" W	'brevilabris'	C7
Newf37	Nameless pond, Newfoundland	49°07'14" N	55°05'19" W	'brevilabris'	C7
ON38	Silver Lake, Ontario	43°05'23" N	80°31'48" W	'brevilabris'	C7
USA					
Ala39	Nameless pond, Teller, Alaska	65°14'30" N	166°19'46" W	'sphaericus'	A3
Ala40	Council pond, Alaska	64°57'27" N	163°41'41" W	'sphaericus'	A3
Ala41	Nameless pond, Teller, Alaska	65°01'13" N	166°08'31" W	'sphaericus'	A3
Ala42	Nameless pond, Nome, Alaska	64°33'37" N	165°29'14" W	'sphaericus'	A3
Ari43	Nameless pond, Coconino, Arizona	34°47'30" N	111°29'19" W	'brevilabris'	C7
Ari44	Nameless pond, Yavapai, Arizona	34°34'22" N	111°51'22" W	'brevilabris'	C7
NY45	Nameless pond, Buffalo, NY	43°01'40" N	78°42'39" W	'brevilabris'	C7
NY46	Nameless pond, Buffalo, NY	43°01'42" N	78°45'16" W	'brevilabris'	C7
NY47	Irondequoit Bay, NY	43°11'01" N	77°31'58" W	'brevilabris'	C7
NY48	Deep pond, Mendon, NY	43°01'29" N	77°34'18" W	'brevilabris'	C7
OK49	Swan Lake, Oklahoma	36°08'06" N	95°58'06" W	'brevilabris'	C7
NH50	Sunapee Lake, New Hampshire	43°22'32" N	72°04'12" W	'brevilabris'	C7

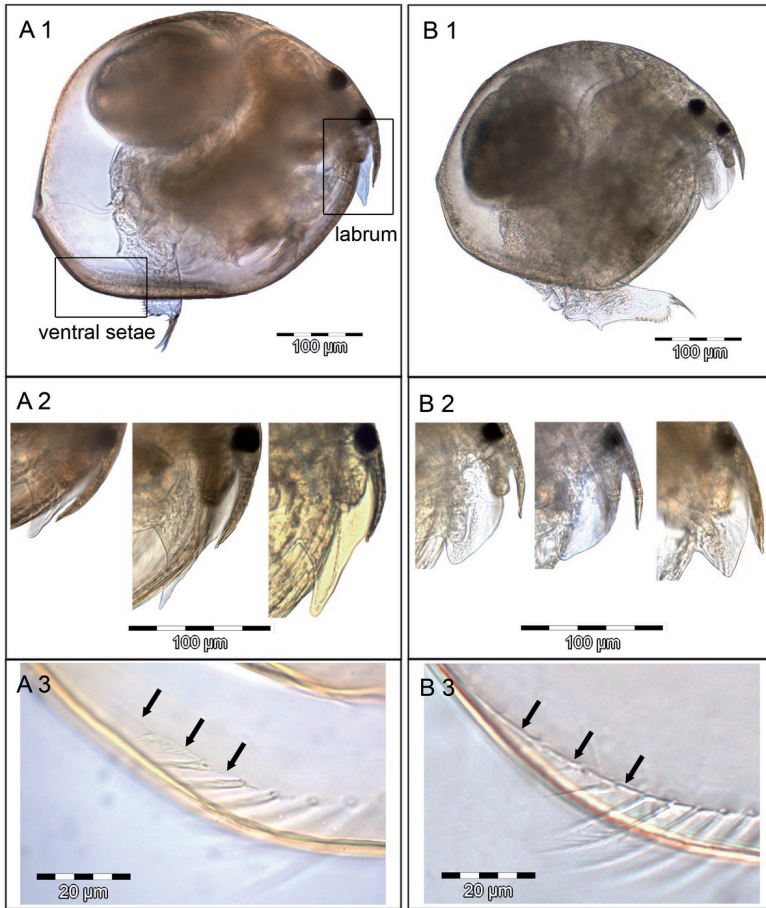


Figure 5.2 Diagnostic morphological characters of the two *Chydorus* morphotypes indicating the major groups of species according to Frey (1980). (A1-A3)—'*sphaericus*', (B1-B3)—'*brevilabris*'. (A1 and B1)—adult parthenogenetic female, lateral view; (A2 and B2)—labrum (note the difference between the two species groups despite the within-group variability); (A3 and B3)—ventral setation on the inner shell surface: (A3)—ventral setae not connected by a line (inter-setal ridges), (B3) 'connected' ventral setae. The position of the line is indicated by arrows

5.2.2 DNA sequencing

For DNA extraction specimens were transferred into reaction tubes and dried. For each extraction 25 μl of Quicckextract (Epicentre Technologies) was added, the specimens were homogenized, and the tubes were placed for 2 h at 65 °C, then for 10 min at 95 °C. DNA extracts from the same individuals were used to obtain PCR products for both genes. A total of 158 individuals were sequenced for the mitochondrial cytochrome oxidase subunit I (COI) gene. A subsample of 60 individuals were sequenced for the nuclear fragment, which included the complete sequence of internal transcribed spacer 2 (ITS-2) as well as small partial sequences of 5.8S and 28S ribosomal genes. In the text below we refer to the nuclear fragment as ITS-2 for convenience. The following taxa were sequenced for the outgroups: *Chydorus pubescens*, *Paralona pigra* and *Pleuroxus procurvus*—COI (GenBank Accession Nos. EU719119, EU719117 and EU719118, respectively), and *C. pubescens* and *P. pigra*—ITS-2. Other available chydorid COI sequences, obtained from the GenBank or kindly provided by Veronika Sacherová from her published (Sacherová & Hebert, 2003) and unpublished data, were also tested as outgroups. As COI amplification had a low success with universal arthropod primers (Folmer et al., 1994), internal primers—Chy-f 5'-TTG GGG ATG ATC AAA TTT ATA ATG T-3' and Chy-r 5'-AGA GGT ATT CAG ATT TCG ATC TGT CA-3'—were designed from the conserved regions in the sequences of *Eurycercus lamellatus* (Seiji Ishida, unpublished data) and *C. sphaericus* from Germany. These primers were used for amplification of all ingroups and outgroups, except for some specimens from the Yukon belonging to clade B6. Those failed to amplify with our specific primers due to mutations in the primer regions. Yet, we obtained PCR products with the universal COI primers from Folmer et al. (1994). The primers 5.8SF (Taylor et al., 2002) and D2r (Omilian and Taylor, 2001) were used to amplify the ITS-2 fragment. Each 50 μl PCR reaction consisted of 35 μl dd H₂O, 5 μl PCR buffer, 1.5 μl each primer, 1 μl dNTPs, 1 μl Taq DNA polymerase and 5 μl DNA extract. The PCR conditions for the COI amplification were 40 cycles of 30 s at 94 °C (denaturation), 30 s at 50 °C (annealing) and 90 s at 72 °C (extension) followed by 1 cycle of 7 min at 72 °C. The PCR conditions for the amplification of ITS-2 were the same, but the annealing temperature was 60 °C. PCR products were sequenced on an ABI 3700 sequencer. DNA sequences were submitted to the GenBank database (Accession Nos. EU719117-EU719163, EU822324- EU822330 for COI and EU719164-EU719188 for ITS-2 sequences).

5.2.3 Phylogenetic analyses

The authenticity of the sequences was verified by BLAST comparisons. Sequences were edited and assembled in Sequencher 4.1 (Gene Codes Corporation), Bioedit (Hall, 1999) and MEGA 4.0. (Tamura et al., 2007). COI sequences were aligned manually in MEGA 4.0. ITS-2 sequences were aligned in CLUSTAL X (Thompson et al., 1997) using default options and after that manually adjusted in MEGA 4.0. The best-fit models of nucleotide substitution were selected in jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) based on likelihood scores for 88 different models and the AIC criterion. Within- and among-clade distances were calculated and neighbor-joining (NJ) phylogenetic analyses were carried out in MEGA 4.0. using Kimura 2-parameter (K2P) model and gamma rates distribution with the shape parameter estimated by jModelTest and with pairwise deletion of gaps. All phylogenetic analyses were performed separately for each gene. Maximum parsimony (MP) analyses were performed in PAUP* 4.0b10 (Swofford, 2003). Heuristic MP searches were done using equal weighting, 10 random sequence addition replicates and TBR branch swapping. Non-parametric bootstrapping was performed to assess the nodal support using 1000 pseudoreplicates for NJ and 100 for MP. Bayesian analyses (BI) were performed in MrBayes v.3.0b3 (Huelsenbeck & Ronquist, 2001). The number of substitution types (nst) was set to 6 and rates were set to gamma with a proportion of invariable sites, all priors were left default to allow estimation of the parameters from the data. Four independent Markov chain Monte Carlo (MCMC) analyses were run simultaneously for 5 million generations and sampled every 100 generations. The first 20 % of the generations were discarded as the burn-in and a 50 % majority rule consensus tree was calculated from the remaining trees. Additionally BI analysis of a mixed ITS-2 data set was performed, where indels were coded as binary characters and added to the nucleotide data set. In the binary partition the model was corrected for the ascertainment bias (lset coding = variable), the settings for the nucleotide partition were as before.

Both data sets were tested for recombination events using GENECONV (Sawyer, 1989) and GARD (Kosakovsky Pond et al., 2006 a, b) software. In GENECONV default settings were used (10 000 permutations, no mismatches allowed) and with mismatches allowed and penalties set to 1–4. Prior to the analysis the first 295 bases were deleted from the ITS-2 alignment as they contained only very few polymorphisms and caused apparently false detection of gene conversion. GENECONV has a reasonable rate of

false positives (ca. 5%) at a sequence divergence range of 5–20% (Posada & Crandall, 2001 a). As sequence divergences ranged from 5% to 11% between the main clades in our data, the program should reliably detect recombination between the sequences belonging to different clades. However, within-group recombination might remain undetected, for the method has a low power at divergences < 5%. Besides, rare recombination events may also remain undetected (Posada & Crandall, 2001 a). The GARD algorithm detects putative recombination break points based on phylogenetic incongruence among partitions within the data set (Kosakovsky Pond et al., 2006 a). The method was shown to be more sensitive than most other recombination tests, however, it sometimes may detect fragments that are underlined by significantly different phylogenies due to substitution rate variation rather than recombination (Kosakovsky Pond et al., 2006 b). To check for this we applied a conservative SH test implemented in HyPhy software (Kosakovsky Pond et al., 2005). The model selection tool available on the GARD server was used to obtain the input nucleotide substitution model. The other settings were: general discrete model for rate variation with four rate classes.

5.3 Results

5.3.1 Sequence variation and alignments

Of 158 ingroup specimens from 50 locations sequenced for COI, 51 unique haplotypes were detected. A summary of the diversity is presented in Table 5.2, but only unique sequences were included in the phylogenetic analyses. The COI alignment for all ingroups and 3 outgroups was 461 bp long, unambiguous and contained no indels. There were 178 variable and 141 parsimony informative sites. The average base composition for the 51 ingroup sequences was as follows: T = 37.4%; C = 21.0%; A = 22.8%; G = 18.7%. The observed A-T content around 60% is common for COI of chydorids (Sacherová & Hebert, 2003). The translated amino-acid alignment had 153 characters, of which 12 were variable and 5 parsimony informative.

Of 60 individuals, for which ITS-2 was amplified, 27 ingroups and 3 outgroups were homozygous in ITS-2. These gave good-quality sequences with no or few ambiguities. The rest of the ITS-2 sequences were unreadable due to within-individual variation in the sequence and its length, resulting in ca. 60% of double electropherogram peaks. Such ITS-2 heterozygotes were not associated with a particular clade, but rather they were present in

Table 5.2 Number of the sampled *Chydorus* populations, specimens, and detected COI haplotypes, and sequence divergence within the putative species lineages in the *C. sphaericus* complex. For the definition of the clades A1–C7 see Fig. 5.3 and the text

COI clade	Putative species lineages	No. populations	No. specimens	No. haplotypes	Max within-group COI sequence divergence (%)
A1	<i>C. sphaericus</i> s. str.	18	75	19	4.1
A2	undescribed species	12	22	13	2.5
A3	undescribed species	10	27	6	1.8
A4	undescribed species	1	1	1	-
B5	undescribed species	1	12	1	-
B6	undescribed species	2	5	1	-
C7	<i>C. brevilabris</i>	11	16	10	2.9
Total		55	158	51	

each of the COI-defined clades. The final ITS-2 alignment included only non-identical sequences from homozygous individuals, and all putative species lineages revealed by the COI phylogeny were represented. Outgroups were excluded as well, because their inclusion substantially decreased the quality of the alignment. The length of the sequences varied from 900 to 1016 bp. The ITS-2 alignment was 1057 characters long, with 197 variable and 126 parsimony informative sites and contained numerous indels 1–46 bp long. There were both very variable regions, which were difficult to align, and very conserved ones which displayed no variation. The average ITS-2 base composition was: T = 23.2%; C = 29.4%; A = 22.6%; G = 24.7%.

The K2P distances among COI ingroup taxa varied between 0% and 22.8%. Between ingroups and outgroups the following sequence divergences were observed: *C. pubescens* 21.1–25.4%, *P. procurvus* 21.9–29.2%, *P. pigra* 25.8–29.9%. The ITS-2 sequence divergences varied between 0% and 26.2% among ingroup taxa.

5.3.2 Mitochondrial gene tree

The best-fit model selected by jModeltest for the COI data set was TIM3 + I + G with a relative AIC weight of 0.3343, the next best model was GTR + I + G with a weight of 0.2338. Based on the cumulative relative weight, five more models were within the 95% confidence interval, all of them assuming the presence of invariable sites and gamma substitution rates.

All phylogenetic methods resulted in trees that did not differ in their main topology, i.e. all specimens were assigned to the same main clades and the relationships between these clades held. In the MP analysis 7791 best trees each of 473 steps were found, with a consistency index (CI)

of 0.567 and retention index (RI) of 0.858. Three major clades within the *C. sphaericus* complex, named A, B and C were well-supported in all phylogenetic analyses of the COI data set (Fig. 5.3). These clades were very distinct from each other, the mean between-group K2P distances being 18.4–20.0%. The phylogenetic relationships between the major clades remained unresolved. In the phylogeny based on the COI amino-acid composition (not shown) clades A and C were unresolved in a single clade and clade B was divergent.

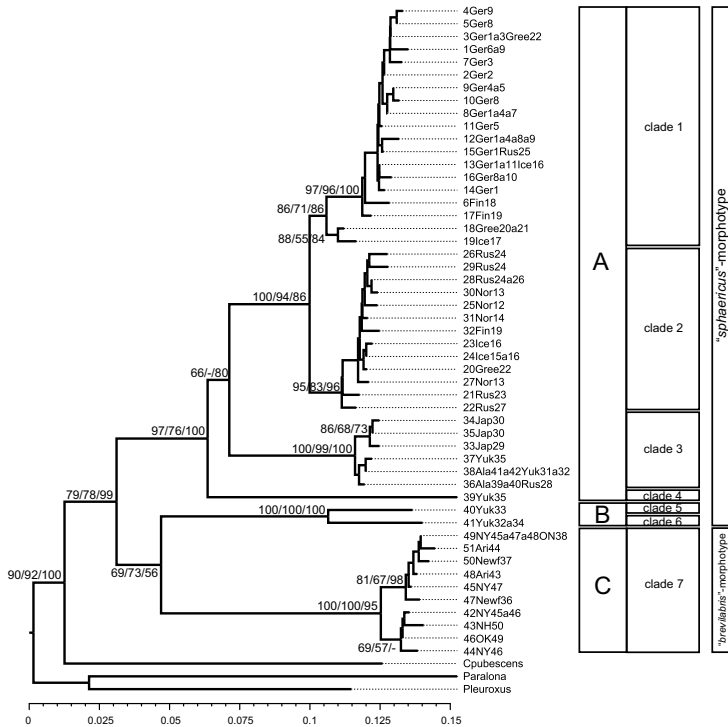


Figure 5.3 COI phylogeny: NJ tree based on Kimura 2-parameter distances as indicated by the scale axis. Gamma rates were assumed with a gamma parameter of 1.838 (estimated in jModelTest). The tree is rooted using *Pleuroxus* and *Paralona* sequences as outgroups. The node support: bootstrap NJ/MP/Bayesian posterior probabilities. (A–C): main branches in the *C. sphaericus* complex; 1–7: putative species lineages (see Table 5.1 for the location codes and the text for further explanations)

Table 5.3 Between-group Kimura 2-parameter distances (in percent) among the putative species lineages within the *C. sphaericus* complex for COI/ITS-2 genes. Distances were calculated with assuming gamma distributed rates and the gamma parameter estimated in jModelTest. ITS-2 distances were calculated with pairwise deletion of gaps. For the definition of the clades A1-C7 see Fig. 5.3 and the text

Clade	A1	A2	A3	A4	B5	B6
A1						
A2	4.9/0.7					
A3	10.5/3.4	10.3/3.4				
A4	15.6/3.3	14.3/3.2	15.2/2.3			
B5	20.0/24.8	19.1/24.6	19.8/22.0	25.2/26.0		
B6	20.3/15.1	19.9/15.4	19.2/13.9	24.8/15.9	6.4/14.9	
C7	20.8/15.6	20.2/16.1	17.1/16.1	20.9/16.0	18.8/19.2	18.0/6.7

Clade A contained four well-supported lineages with the mean K2P-distances of 4.9–15.6 % between and 0–4.1 % within them (Table 5.3; Fig. 5.3): (A1) all specimens from Germany and some from Finland and Greenland; (A2) Finland, Norway, Iceland, Greenland, Russian Arctic and Siberia; (A3) Alaska, Yukon, Japan and eastern Siberia; (A4) one specimen from the Yukon. Clades A1 and A2 were sister groups with high support for this node. The relationships between clades A1 + A2, A3 and A4 were unresolved (Fig. 5.3). Clade B contained two lineages from Yukon: (B5) was represented by twelve identical sequences sampled in one location and (B6) by five identical sequences from two locations. Despite the high nucleotide distance of 6.4 % between clades B5 and B6, they had an identical amino-acid composition, which was, however, distinct from all other clades. A highly supported Clade C included only Nearctic specimens and constituted just one putative species lineage, showing moderate sequence variation comparable with that observed in other putative species lineages (Table 5.2). Also, no phylogeographic subdivision was apparent within the *brevilabris* clade C7.

The age of the *C. sphaericus* species complex and the time scale for the diversification can be only approximately estimated, as no fossil calibration exists for Cladocera. For Arthropoda the rates of 1.4–2.6 % per MY have been reported (Knowlton & Weigt, 1998; Schubart et al., 1998). Then a crude estimation of diversification time of the major clades A, B and C, given that the Kimura-2-parameter model is appropriate for the data, is ca. 5–15 MYA and subsequent speciation within clades A and B—ca. 2–10 MYA. Thus, the youngest putative species detected by our study are *C. sphaericus* s. str. (A1) and the undescribed species from the European Arctic, Greenland and Siberia (A2), which possibly diverged from a common ancestor in late

Pliocene—early Pleistocene.

GENECONV recombination test applied to the COI data set detected no significant global fragments under either of the settings used. Some significant pairwise fragments were found, involving the outgroup *Pleuroxus* and C7; A1 and A4; A2 and A4; sequences within A1; sequences within A3. Nevertheless, as the more conservative global test detected no recombination, and also because pairwise fragments did not involve species found to co-exist, we consider the test results as providing no reliable evidence for recombination. GARD detected no significant recombination breakpoints by either criteria used.

5.3.3 ITS-2 gene tree and concordance among the mitochondrial and the nuclear phylogenies

The best-fit model selected by jModeltest for the ITS-2 data set was TPM2uf + G with a relative AIC weight of 0.3178, the next best model was TIM2 + G with a weight of 0.2181. Six more models were within the 95% confidence interval, all of them assuming gamma substitution rates and some of them also assuming a proportion of invariable sites.

MP analysis of the ITS-2 data set resulted in 40 best trees with a length of 270 steps, CI of 0.848, and RI of 0.924. All phylogenetic analyses of the ITS-2 data set revealed four major clades that corresponded to A, B5, B6 and C7 mitochondrial clades (Fig. 5.4). Thus, A and C clades were recovered and well-supported in both gene trees, whereas clades B5 and B6 were much more divergent from each other in the nuclear than in the mitochondrial tree (14.9% versus 6.4%, respectively). The monophyly of clade B received no support in the ITS-2 phylogeny. Similar to the mitochondrial phylogeny, the relationships among the major ITS-2 clades remained unresolved, i.e. neither grouping yielded any significant support. BI analysis of a mixed ITS-2 data set that included binary coded gaps resulted in higher posterior probabilities for the groupings A1 + A2 (100%) and (A1 + A2) A3 (99%) and (B5, B6) (95%). Overall, the nuclear phylogeny provided even less resolution than the mitochondrial one. For example, most recent, but well-separated in the COI tree A1 and A2 clades merged in one well-supported clade. A3 and A4 clades were also, respectively, divergent in ITS-2, but no relationships among putative species lineages were resolved except the 99% support for the (A1 + A2) A3 in BI analysis of the mixed data set that received no support at all in other analyses.

Age estimations based on ITS-2 would probably be even more unreliable

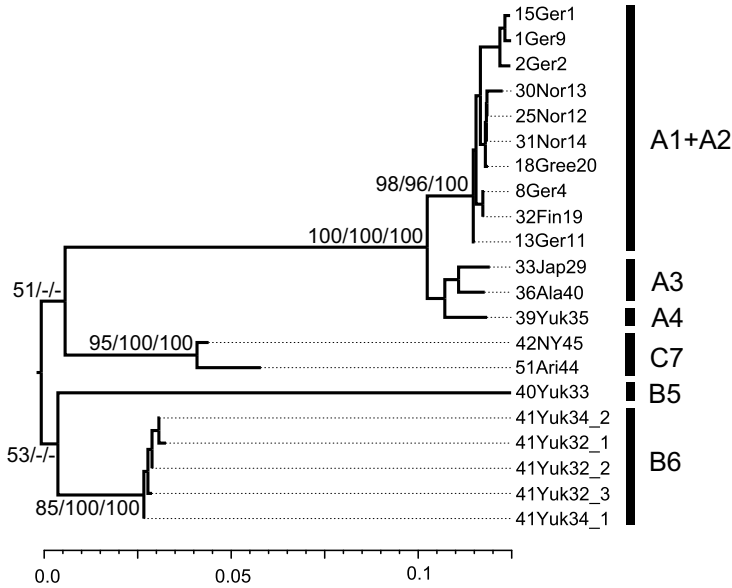


Figure 5.4 ITS-2 phylogeny: NJ tree based on Kimura 2-parameter distances as indicated by the scale axis, calculated with pairwise deletion of gaps and assuming gamma rates with a gamma parameter of 0.129 (estimated in jModelTest). The tree is rooted on the midpoint. The node support: bootstrap NJ/MP/Bayesian posterior probabilities for the nucleotide data set

than those based on mitochondrial DNA divergences. A great variation in substitutional rates for this gene has been observed in insects (Schlötterer et al., 1994). Although a rate of 2.4% per MY is reported for *Drosophila* and it has been used for age estimations in other arthropods (e.g. Schwenk et al., 2000), it was based on a sophisticated algorithm aiming to exclude invariable sites assumed to be ‘conserved’ by chance (Schlötterer et al., 1994). Thus, a straightforward application of the reported rate would give (negatively) biased results. Also our ITS-2 data suggest the presence of a considerable rate heterogeneity, as indicated by the highly divergent sequences representing B5 clade (see above). Even though we give only very rude age estimations for the putative species clades, nevertheless, we decided to limit them to the mitochondrial gene phylogeny, as it seems much more reliable.

GENECONV recombination test detected no significant global inner fragments (p -values < 0.05) and one significant outer fragment involving

a sequence from clade B6. Pairwise significant fragments were detected between sequences belonging to clade A and between clades B5, C and B6. Thus, no reliable evidence for recombination between the clades have been found. GARD test detected two significant breakpoints. Of these only one fragment was found to be significant by SH test. Furthermore, close examination of the significant breakpoint revealed that it corresponded to the boundary between the ITS-2 and 28S genes, the latter being very conservative with almost no variation within this alignment fragment. Thus, the significance of the test most likely results from the rate variation rather than recombination events. We conclude that no unequivocal evidence for recombination was found by either of the tests applied.

5.3.4 Biogeographical patterns

We found a greater diversity of the *C. sphaericus* s.l. in the Nearctic than in the Palearctic, both regarding the presence of the major evolutionary clades (three in the Nearctic versus one in the Palearctic) and the number of the putative species lineages (7 in the Nearctic versus 3 in the Palearctic). The *C. sphaericus* complex comprises both rather widely distributed species (A1, A2, A3 and C7) and those that are likely to be more restricted geographically (A4, B5 and B6). Lineages A4, B5 and B6 were found only in Yukon and might represent Beringian glacial relict species. We detected clade A3 from both the western Nearctic and the eastern Palearctic, yet, it was restricted to the Beringian region. Among the widely distributed lineages *C. brevilabris* (C7) was restricted to the North American continent, whereas *C. sphaericus* s. str. (A1) and clade A2 were encountered both in Nearctic and Palearctic locations. *C. sphaericus* s. str. was restricted to sites in western Eurasia and Greenland.

Each of the widely distributed species showed considerable intraspecific variation in mitochondrial DNA (Table 5.2; Fig. 5.3). The lack of observed variation within the geographically restricted lineages is probably the result of the low number of the sampled locations and analysed specimens (Table 5.2), the real variation present in nature remaining unknown. Some phylogeographic structuring was observed in the *C. sphaericus* s. str., as northern populations possessed COI haplotypes that were distinct from those in the Central- and East-European populations. Yet, no phylogroups were apparent within other species lineages, perhaps due to the low number of sampled populations (Table 5.2).

5.3.5 Morphological variation within the *C. sphaericus* species complex and differentiation of the newly discovered lineages

Examination of the specimens prior to DNA extractions assigned clades A and B to the *sphaericus*-morphotype *sensu* Frey (1980) and clade C to the *brevilabris*-morphotype (Table 5.1; Figs 5.2 and 5.3). The *sphaericus*-morphotype was generally larger (230–480 μm versus 220–420 μm in the *brevilabris*-morphotype according to Frey (1980)), possessed a longer labrum plate of variable shape and the ventral setae were not connected by a line (inter-setal ridges), whereas the *brevilabris*-morphotype could be readily distinguished by its somewhat smaller size, short, broadly rounded labrum and ‘connected’ ventral setae (Fig. 5.2).

Where possible, the morphology of the newly found genetically divergent lineages was studied. Only those characters that are helpful for the delimitation of these lineages are described here, all of them being traits of gamogenetic individuals—ephippial females and males. The latter occurred in the sampled populations of the two new species found in this study—lineages A2 and A3, thus enabling their reliable delimitation from the *C. sphaericus* s. str. (clade A1). The formal taxonomic descriptions of these two new species as well as a detailed assessment of the intra-species morphological variation within *C. sphaericus* s. str. (A1) will be published elsewhere. The other newly discovered putative species—clades A4, B5 and B6—were represented each by just one or a few parthenogenetic individuals, therefore, no morphological investigations were conducted.

Gamogenetic individuals belonging to clade A2 could be readily distinguished from the occasionally co-existing sister species *C. sphaericus* s. str. based on the characters described below. The ehippial females carried two eggs and the males lacked the preanal angle of postabdomen (Fig. 5.5). Although these particular characters are diagnostic of *C. biovatus*, the males from A2 populations also showed some substantial morphological differences from this species. The shape of rostrum was similar to that of *C. sphaericus* s. str. and not to *C. biovatus* (Fig. 5.5). The postabdomen of males had a well-developed basal spine on the claw, which was approximately of the same size as that of females. All five adult males studied, all had a basal spine, suggesting normal development. Male postabdomens with basal spines are unknown in the well-described species of the *C. sphaericus* complex. However, this feature was mentioned in Lilljeborg’s description of *C. latus* (Lilljeborg, 1900, cited in Flößner, 2000), which is a poorly

investigated species and is not regarded as valid according to the modern taxonomic standards (Smirnov, 1996). However, the specimen drawn by Lilljeborg had a well-developed preanal angle and none of our specimens had it. Therefore, we conclude, that clade A2 is a new yet-undescribed species belonging to the *sphaericus*-branch of the *C. sphaericus* species complex.

Beringian clade A3 was very similar to *C. sphaericus* s. str., yet, differing in the shape of the rostrum tip of adult males that was narrowly rounded as in *C. biovatus* and *C. brevilabris*, rather than broadly truncate as in A1 and A2 clades. Five adult males were examined, all possessing narrowly rounded rostrum. Juvenile males from the studied A3 clade population also displayed rostrum morphology similar to that described for *C. biovatus* (Frey, 1985; Figs 16 and 17) and not to *C. sphaericus* s. str. (Frey, 1985, Figs 19 and 20). However, other characters, such as the shape of male postabdomen and one egg in the ephippium clearly delineate the new species from *C. biovatus* (Fig. 5.5).

5.4 Discussion

5.4.1 Phylogenetic framework for the taxonomy of the *C. sphaericus* species complex: is there an agreement between the morphological and genetic data?

The *C. sphaericus* species complex, as it is defined by morphological criteria, also proved to be monophyletic in our phylogenetic analyses. However, it should be noted that rigorous testing for the monophyly of the species complex would require inclusion of all species from the genus *Chydorus*. The nuclear gene tree contained only ingroups and thus monophyly could not be established. Nevertheless, a rather high similarity among the ingroup sequences might be an indication of their monophyletic origin.

Both nuclear and mitochondrial phylogenies were concordant in revealing three major lineages within the *C. sphaericus* complex (Figs 5.3 and 5.4): (A) *sphaericus*-lineage with a Holarctic distribution, (B) a previously undescribed Yukonian lineage and (C) a *brevilabris*-lineage—widely distributed in North America, but possibly absent from the northernmost part of the continent, where it is replaced by the two other major lineages. The major clades A, B and C probably reflect an ancient radiation within the *C. sphaericus* complex and correspond to a higher than specific taxonomic level, as at least the *sphaericus*-branch (A) shows clear morphological, geographical,

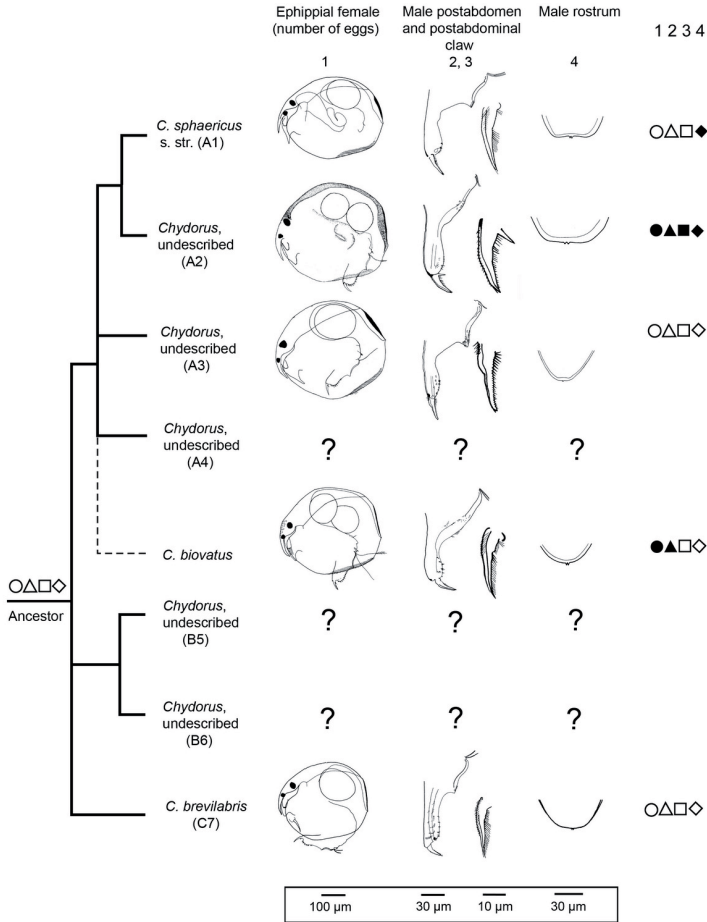


Figure 5.5 Morphological versus molecular evolution within the *C. sphaericus* complex. The summary tree shows the phylogenetic relationships between the putative species lineages (A1–C7) estimated from the mitochondrial (COI) and nuclear (ITS-2) sequence variation. Branches with support < 70% in the COI phylogeny are collapsed. Genetic data on *C. biovatus* were lacking, hence, its position on the tree is unknown (shown by a dotted line), possibly it belongs to the major clade A. Morphological characters: 1—number of ephippial eggs; 2—shape of preanal angle of male postabdomen (position indicated by an arrow); 3—presence of a spine on postabdominal claw (position indicated by an arrow); 4—shape of male rostrum. On the right: empty symbols are ancestral character states, filled symbols—derived characters. All drawings of *C. sphaericus* s. str., *C. biovatus* and *C. brevilabris* except for male rostrums of *C. sphaericus* s. str. and *C. biovatus* are modified from Frey (1980), with kind permission from Springer Science and Business Media. Male rostrums are redrawn from SEM photos in Frey (1985), with kind permission from Wiley-VCH Verlag GmbH & Co. KGaA. All drawings for Clades A2 and A3 are original

and phylogenetic evidence for further speciation. Two of the three major lineages revealed by our study—A and C—correspond to the traditional separation of the complex into two species groups (Frey, 1980)—*sphaericus* and *brevilabris*, respectively. Thus, there is some agreement between morphological and genetic approaches in recognition of supra-specific lineages. However, our study showed that some deeper divergences in both nuclear and mitochondrial genomes might have arisen without any recognizable morphological changes in parthenogenetic individuals. Specimens belonging to the Yukonian clade B, which was genetically the most divergent one, did not display any substantial morphological differences from those belonging to the *sphaericus*-branch (A). However, taking into account the evolutionary patterns within the complex (see below) it can be expected that gamogenetic individuals would be morphologically distinct also in clade B. Overall, an important taxonomic implication is that the morphology of parthenogenetic females, even if one studies fine morphological structures, may sometimes be misleading or of no taxonomic use, even in case of relatively ancient diversifications. It seems possible that more major lineages belonging to the complex can be still found in the regions, which were not sampled in this study. In particular, there are some records of *C. sphaericus* from Africa, Asia, South America and Australia, and those might prove to comprise yet undetected highly divergent lineages.

Several independent lines of evidence support the species status of seven lineages belonging to the three major clades within the *C. sphaericus* complex. These are concordance between the phylogenies of two independently evolving genes, high between-group divergences versus lower within-group divergences and high support for the proposed specific lineages in the COI phylogeny. Further evidence of the independent status of the Beringian lineages (A3, A4, B5 and B6) as well as two Palearctic lineages (A1 and A2) comes from their occurrence in the same sampling sites. All these arguments are commonly used as a prove of an independent evolutionary history and specific status of lineages (Avice & Ball, 1990). Furthermore, the youngest phylogenetic lineages—A1 and A2—proved to display substantial differences in the morphology of gamogenetic individuals. These can possibly lead to reproductive isolation between closely related species (Van Damme & Dumont, 2006). Unfortunately, one of the three of the currently described valid species in the *C. sphaericus* species complex—*C. biovatus*—was lacking from our samples. According to the morphological description (Frey, 1980, 1985) it is likely to belong to clade A in our phylogenetic framework (Fig. 5.5). Further studies are required to clarify the taxonomic status (a single

species or a group of cryptic species) and the position of this taxon within the complex.

Recombination tests failed to detect any reliable evidence for ongoing recombination between the putative species lineages, thus, inter-specific hybridization should be absent or rare despite the frequent sympatry. However, it should be noted that recombination rates are likely to be underestimated in our ITS-2 data set, which included only homozygous individuals (see Section 5.2). In spite of the observed morphological differences, which should hamper hybridization between the most closely related lineages A1 and A2, an intermediate position of some haplotypes from Greenland and Iceland (Fig. 5.3: 18Gree20a21 and 19Ice17) might indicate a mitochondrial introgression or, more likely, a recent speciation event. It is also unknown, whether allopatric lineages belonging to the *sphaericus*-branch (A) would be capable of hybridization once they are brought into a secondary contact, for example, via human-mediated introduction. Although inter-specific hybridization is poorly studied in chydorids, there is experimental evidence for it in a chydorid genus *Pleuroxus* (Shan & Frey, 1983) and in the well-studied *Daphnia* it is a wide-spread phenomenon (Schwenk & Spaak, 1995). Thus, we cannot completely exclude the possibility of hybridization in the studied species complex. Nevertheless, our study clearly shows that even if inter-specific hybridization does occur within the *C. sphaericus* complex, hybrids are rare in nature, and putative species lineages are capable of maintaining genetic differentiation in sympatry.

5.4.2 Cryptic diversity and non-cosmopolitanism within the *C. sphaericus* complex

Prior morphological investigations on populations of the *C. sphaericus* s. l. from a wide range of locations in North America indicated that each of the two major lineages—*sphaericus* and *brevilabris*—may consist of numerous sibling species (Frey, 1980, 1985). We have tested this prediction with molecular data and found that the *sphaericus*-clade (A) is comprised of several independent lineages, though not so numerous as anticipated. However, the *brevilabris*-clade (C), despite its ancient origin, included just one species, widely distributed in North America. This was an unexpected result, given the high phenotypical polymorphism and wide distribution of this taxon (Fig. 5.1; Frey, 1980, 1985). This finding is also in agreement with other studies, which detected only one *brevilabris*-like species with a low level of COI diversity in different climatic zones in Mexico (Elías-Gutiérrez et al.,

2006; Elías-Gutiérrez, pers. comm.). Although our analysis did reveal two subclades within *C. brevilabris*, they were shallow, not well-supported and did not correspond to any geographic structuring (Fig. 5.3).

Frey (1986, 1995) argued that cladoceran species, particularly chydorids, display continental endemism, restricted distributions within continents and narrow ranges of environmental preferences. Indeed, continental endemism and regionalism were found to be a common pattern for cladocerans with sexual propagules (Adamowicz et al., 2004; Cox & Hebert, 2001; Haney & Taylor, 2003; Taylor et al., 1996, 2002). In our first assessment of this pattern in a benthic cladoceran we find that three species from the *C. sphaericus* clade A are present in both the Nearctic and the Palearctic, and thus they are not continental endemics in the strict sense. Yet, these species lack cosmopolitan status as they appear to be restricted to Greenland and Eurasia (clades A1 and A2) or eastern and western parts of the former Beringia (clade A3). The remaining species (A4, B5, B6 and C7) appear to be restricted to the Nearctic. Also, contrary to Frey's predictions, at least two species in the complex—*C. sphaericus* s. str. and *C. brevilabris*— are widely distributed within each continent and capable of living under a broad range of conditions. Hence, our results provide evidence for isolation among the European and North American faunas and for some regionalism, on the other hand, they also show that chydorid Cladocera are capable of dispersing over large distances within continents and to isolated islands.

Whereas studies on pelagic cladocerans detected several cases of recent inter-continental introductions (Haney & Taylor, 2003; Taylor et al., 1996), we did not find any evidence for these in the *C. sphaericus* complex, nor local faunistic studies ever reported of any indications of such introductions. A number of thorough recent revisions of the local faunas in the northern, western and southern Europe (e.g. Alonso, 1996; Duigan, 1992; Flößner, 2000) failed to detect the presence of *C. brevilabris* or *C. biovatus* in Europe. On the other hand, there are numerous records of *C. sphaericus* from North America, but it seems probable that all of them are in fact misidentifications of *C. biovatus* or even *C. brevilabris*. There is still a possibility that some recent human-mediated introductions are undetected, but the presence of *C. sphaericus* s. str. in North America so far lacks any reliable evidence, so we suggest that the name *Chydorus sphaericus* s. str. should not be applied to North American populations.

5.4.3 Taxonomic and phylogenetic utility of morphological characters

The phylogenetic framework developed in this study allows us to assess the taxonomic and phylogenetic utility of the previously proposed morphological characters (Frey, 1980, 1985). A number of characters in the morphology of parthenogenetic females, which have been traditionally used in chydorid taxonomy (such as armament of the first and second antennae, trunk limbs and postabdomen), appear to have remained unchanged in all investigated species from the *C. sphaericus* complex (Frey, 1980). Conversely, other characters (shell sculpture, shape of rostrum, postabdomen armament) show high intra-specific variability, at the same time, similar morphotypes occurred across several distantly related lineages (Belyaeva, unpublished data), indicating convergence. Hence, only very few of presumably selectively neutral characters in the morphology of parthenogenetic females seem invariable within the species and are diagnostic of the major monophyletic lineages (e.g. the short labrum and 'connected' ventral setae in *C. brevilabris*). On the contrary, the morphology of gamogenetic individuals proved very useful for distinguishing species, although, due to frequent convergences, it probably has less if any value for phylogenetic reconstructions. Convergences might have occurred in such characters as the number of eggs in the ephippium and presence/absence of the preanal angle in the postabdomen of males. Furthermore, both gains and losses of characters have occurred in the evolution of chydorids (Adamovicz & Sacherova, 2006), that may complicate phylogenetic reconstructions. The second egg in the ephippium and the spine at the base of male postabdomen are examples of such gains in the *C. sphaericus* complex. Taking into account the above considerations, a joint genetic and morphological approach would be advisable for determining the affinities of newly discovered species in the *C. sphaericus* complex.

5.4.4 Evolution in the Holarctic *C. sphaericus* complex

Our results suggest that the major branches within the *C. sphaericus* complex evolved from a common ancestor in the Tertiary. Furthermore, the resistance to cold temperatures displayed by all studied taxa including the most southern species *C. brevilabris* possibly developed, when in Tertiary sharp climatic gradients established between high and low latitudes and promoted adaptation to cold climate in a number of freshwater invertebrate taxa (Ko-

rovchinsky, 2006). There are also some indications for mass extinctions and range contractions during Tertiary suggested by Korovchinsky (2006) for Cladocera. Namely, the major branch B has a very restricted distribution in Beringia and was possibly more widely distributed in the past. Besides, the observed lack of genetic variation in the ancient lineage of *C. brevilabris* (C) as compared to the other two major lineages can be explained by elimination of the previous diversity by severe climate changes in Tertiary and survival of just one species. It is possible that more species from the two other major lineages survived, because they were better adapted to cold climate, as suggested by their more northern distributions at present.

We detected not only ancient, but also some putatively recent speciation events in the *C. sphaericus* species complex. The youngest species A1 and A2 probably diverged ca. 2 MYA. These results show that not all existing chydorid species might deserve a relict status, as it was proposed by Korovchinsky (2006). It is not unlikely that a substantial proportion of chydorid taxa will prove to be recently evolved, and there is still ongoing speciation in some benthic Cladocera. With the moderately evolving DNA markers used in this study we could not reliably determine whether the variation that had accumulated within the complex during the Pleistocene lead to further speciation.

Analysis of the genetic versus phenotypical differentiation within the *C. sphaericus* complex reveals some evolutionary patterns and trends characteristic of this group of chydorids. The striking morphological similarity of parthenogenetic females from genetically divergent lineages is most likely plesiomorphic, as a near-identical morphology is shared by some *Chydorus* species, which probably do not belong to the complex, such as *C. ovalis*. It seems, that there is a strong selective constraint, which preserves the bauplan of parthenogenetic females, even given the great variety of abiotic and biotic conditions, under which these animals live in Holarctic water bodies. This constraint possibly includes two components. Conservatism in body shape and size is characteristic of the family Chydoridae, which do not display such environmentally induced phenotypical changes, common for pelagic bosminids and daphniids. The specific conservatism in the *C. sphaericus* complex probably includes the constraint on the antennae and trunk limb morphology and ventral valve setation—the features, which are invariable in the *C. sphaericus* complex, but commonly vary among other closely related chydorids (Frey, 1980; Smirnov, 1971, 1996). Thus, the conservatism in the morphology of parthenogenetic females common for chydorids (Frey, 1986; Smirnov, 1971) seems to have reached its maximum

in the presumably advanced *C. sphaericus* complex. More investigations on the functional morphology of this species complex are needed to answer, why this particular bauplan is so strongly preserved and how it has contributed to its apparent ecological success.

Whereas parthenogenetic females have remained almost unchanged for millions of years, the morphology of gamogenetic individuals has undergone considerable evolutionary changes. Ehippial females in at least two species within the *sphaericus*-branch have gained a second egg and males have undergone changes in the shape and armament of the postabdomen, the armament of the postabdominal claw, the shape and structures of the copulatory hook, the shape of the rostrum and possibly some other yet undetected changes. Unfortunately, the lack of sequence data for *C. biovatus* does not allow a more detailed inference of evolution of these morphological traits. Nevertheless, it seems likely that a two-egged ehippium has evolved in parallel in two species in the *C. sphaericus* species complex, as it is also the case in several other chydorid species (Fryer & Frey, 1981), whereas one-egged ehippium is clearly the ancestral state. A two-egged ehippium may have an advantage in the cold climate, where the growing season is short and the importance of sexual reproduction increases in chydorids (Sarmaja-Korjonen, 2004). An increased number of eggs in the ehippium in some chydorids may ensure a faster re-establishment of the population in spring and a better chance for colonizing new habitats. Interestingly, another *Chydorus* species with two ehippial eggs is *C. ovalis*, which also has a northern Holarctic distribution (Smirnov, 1996). However, a two-egged ehippium, even if it is advantageous in the cold climate, is apparently not a necessity, as one-egged *C. sphaericus* s. str. co-occurs with the two-egged species in northern Palearctic water bodies (this study). Considerable evolution of the male morphology was probably mainly associated with advances in the mating process and may ensure reproductive isolation between closely related species (Van Damme & Dumont, 2006). Indeed, the latter study showed that males of *C. ovalis* were even not able to attach to females of *C. sphaericus*, though they reacted to the chemical signal of heterospecific females. A narrow postabdomen of males lacking denticles is probably a major synapomorphy of the *C. sphaericus* complex. This type of postabdomen appears to be the most derived one in the genus *Chydorus*, whereas a postabdomen similar to that of females is the most primitive one (Van Damme & Dumont, 2006). Some other species of *Chydorus* also show the tendency for narrowing of the postabdomen and decreasing its armament, e.g. *C. pizzari*, *C. faviformis*, *C. baicalensis*, but this is most pronounced in the

C. sphaericus complex. Within the latter the armament of the postabdomen is reduced to few fine setules within the *sphaericus*-branch, while further reduction of the preanal angle of the postabdomen leads to the narrowing of its proximal part. The functional meaning of these morphological changes is still unknown, but it is likely related to a more specialized mating behaviour in this species (Van Damme & Dumont, 2006).

Chapter 6

Genetic erosion in a littoral cladoceran species, colonizing extreme acidic mining lakes, and the colonization sources for the acid tolerant populations

Abstract

Genetic diversity is of major concern for the long-term persistence of species and populations. In extreme environments genetic erosion, i.e. loss of genetic diversity due to abiotic stress, may occur, and this may have consequences for ecosystem stability. Yet, in evolutionary old extreme environments genetic diversity may increase over time due to adaptation, despite adverse abiotic conditions. The aims of this study were to test for genetic erosion in the populations of a tolerant cladoceran species, *Chydorus sphaericus*, colonizing young acidic hard-water mining lakes and evolutionary old softwater lakes, and to reveal the potential colonization sources for the acid tolerant populations. Genetic diversity was assessed as sequence variation in a mitochondrial COI gene. Changes in the genetic structure of the populations, colonizing acidic hard- and soft-water lakes, were contrasted to the patterns of natural genetic variation at both regional and local scales. We found evidence for genetic erosion in acidic mining lakes; in contrast, the genetic structure of acidic bog lakes' populations was indistinguishable from that of non-acidic reference sites. Analysis of the genealogical relationships among the sampled mitochondrial haplotypes suggested that

acidic mining lakes were most probably colonized from adjacent non-acidic water bodies, and the broad physiological tolerance in *C. sphaericus* is likely to be maintained via locally adapted genotypes. The observed genetic erosion in acidic mining lakes and the absence of divergent genetic lineages characterizes them as highly disturbed environments, which should be subjected to restoration measures.

6.1 Introduction

The effects of anthropogenic contaminants on the genetic diversity of resident populations have become a focus of an emerging field of research—evolutionary toxicology (Bickham, 2011). Still, we know rather little of how environmental conditions in general and anthropogenic stressors in particular shape genetic diversity in natural populations. For example, one of the central hypotheses in evolutionary toxicology—so-called genetic erosion, i.e. the loss of genetic diversity under toxic chemicals' exposure (van Straalen & Timmermans, 2002), was not always supported by empirical data, and the reasons for these discrepancies among studies are not well understood (e.g. Martins et al., 2009). Apparently, more information is required to understand the long-term effects of stressors at the population genetic level, as intraspecific genetic diversity is of crucial importance for the long-term persistence of populations, especially when they are subjected to multiple and successive stressors (Lopes et al., 2009; Jansen et al., 2011).

Extreme habitats of natural and anthropogenic origin, characterized by ecologically stressful values of one or, more often, several abiotic factors, are good model sites to study the impact of stressors on genetic diversity and the mechanisms of species' adaptations to abiotic conditions. This is because selection pressure tends to be higher under extreme abiotic conditions, and thus the environmental effects on the population genetic structure should become more easily observable. Acidic mining lakes, originating after closure of open-cast coal mines, are extreme freshwater habitats (Geller et al., 1998). They are impacted by so-called acid mine drainage (AMD)—severe acidification due to pyrite oxidation resulting in large inputs of sulphuric acid, aluminium and iron ions. At the same time, these lakes are embedded in a non-extreme landscape, and thus they may be colonized from adjacent natural water bodies or, alternatively, from similar extreme habitats via long-distance dispersal. In Germany there are hundreds of mining lakes, which are commonly acidic, unless they have been subjected to neutralization measures (Nixdorf et al., 2005). The status of these water

bodies remains controversial, as there exist proposals either to neutralize extremely acidic mining lakes or to preserve them as unique ecosystems (Nixdorf et al., 2005). Data on intraspecific genetic diversity can provide unique population-level information and, when complemented by ecological data, it can serve as a solid basis for bio-monitoring and ecological quality assessment (Bagley et al., 2002). We have chosen a tolerant cladoceran species, which is also a frequent colonizer of acidic mining lakes, *Chydorus sphaericus* (O.F. Müller, 1785), in order to test for genetic erosion in two types of acidic lakes in Germany: man-made acidic hard-water mining lakes and naturally acidic soft-water bog lakes. We then used these data to assess the ecological quality of acidic mining lakes and to find out whether our results support the appraisal of these lakes as unique and valuable ecosystems (Nixdorf et al., 2005) or, alternatively, they represent highly disturbed environments and should be subjected to restoration measures.

Studies on organismal communities in acidic mining lakes have shown that their biodiversity at both species and ecosystem levels is severely reduced, as compared to naturally acidic soft-water lakes at the respective pH values (Deneke, 2000; Wollmann et al., 2000; Nixdorf et al., 2005, see Chapters 2 and 4). Reduced diversity in acidic mining lakes likely results from higher abiotic stress in these habitats, compared to naturally acidic soft-water lakes (see Chapters 2 and 4). In addition, evolutionary species pool hypothesis predicts that species diversity should be higher in evolutionary older and more wide-spread habitats, also under stressful abiotic conditions, due to adaptation (Pither & Aarssen, 2005). Analogous to species diversity, we expect that for both ecological (abiotic stress) and evolutionary (time for adaptation) reasons, intraspecific genetic diversity should be lower in young acidic mining lakes, as compared to evolutionary old acidic soft-water lakes, although populations in both habitat types are subjected to multiple abiotic stressors. Some reduction in genetic diversity can be expected also in acidic bog lakes, compared to non-acidic sites, in the case, if abiotic stress still limits occurrence of non-tolerant genotypes, despite the possibility for long-time adaptation.

Previous studies, aiming to test for occurrence of genetic erosion in cladoceran populations, colonizing acidic mining lakes, yielded contradictory results (Lopes et al., 2009; Martins et al., 2009). Lopes et al. (2009) found evidence for genetic erosion in laboratory clonal mixtures; conversely, Martins et al. (2009) detected no genetic erosion in field populations exposed to a mild AMD-stress (pH 6–7, conductivity 272–437 $\mu\text{S cm}^{-1}$). Both studies used *Daphnia* as a test object, which is a model taxon in ecotoxicol-

ogy, yet, it is rather sensitive to low pH and other AMD-related stressors, and this taxon was never recorded from acidic mining lakes at $\text{pH} < 6$ (see Chapter 2). However, it remains unknown, whether genetic diversity of tolerant cladoceran species, which do colonize highly acidic mining lakes, is negatively affected by multiple abiotic stressors in these water bodies. That's why, in order to test for genetic erosion, we used a tolerant cladoceran species, *C. sphaericus*, which often dominates zooplankton communities in acidic mining lakes at $\text{pH} 3\text{--}6$ (see Chapter 2).

When assessing the impact of abiotic stress on the genetic structure of populations, it is advisable to contrast the observations at the impacted sites with the natural 'genetic landscape' at both local and regional scales (Belfiore & Anderson, 2001; Staton et al., 2001). We followed these recommendations and first described the regional-scale genetic structure in the northern and middle European populations of *C. sphaericus*. Secondly, we explored the population genetic structuring at the local scale by sampling several non-acidic sites and by interpreting these data in the light of the monopolization hypothesis (De Meester et al., 2002), which describes population genetic structuring in animals with cyclic parthenogenetic life cycle, such as Cladocera. Indeed, this life cycle may have profound consequences for the genetic structure of local populations. Specifically, cyclic parthenogens commonly exhibit high levels of among-population differentiation and low within-lake genetic diversity, despite their high dispersal potential. The monopolization hypothesis explains these patterns by persistent founder effects, which are further enhanced by rapid adaptation to local environmental conditions (De Meester et al., 2002). Therefore, we expect that in Cladocera genetic erosion due to abiotic stress should be superimposed on the naturally reduced genetic diversity due to 'monopolization' effects, thus, causing even more drastic alterations in the genetic structure. Hence, cyclic parthenogens may be particularly vulnerable to the negative effects of abiotic stress on the population level, on the other hand, according to the theoretical predictions, they should also possess greater capacities for rapid adaptation to stressful environmental conditions, as compared with obligate sexuals (De Meester et al., 2002).

Apart from testing the genetic erosion hypothesis, our assessment of the genetic diversity of *C. sphaericus* in acidic lakes may through light on the mechanisms enabling broad tolerance in Cladocera. According to the non-cosmopolitanism paradigm in cladoceran biogeography, broad tolerance of single species should be uncommon in this group, and when observed, it should indicate the failure of the current taxonomy to distinguish among

ecologically specialized but morphologically similar cryptic species (Frey, 1980, 1986, 1995). Yet, our previous studies provided genetic and physiological evidence for the broad pH tolerance in a single species—*C. sphaericus* s. str. (see Chapters 4 and 5), although the mechanisms of this, exceptional for Cladocera, ability remain unknown. Here we aimed to determine the colonization sources for the populations in acidic lakes in order to distinguish among the following hypotheses, explaining how broad pH tolerance is achieved in *C. sphaericus*: (1) Broad pH tolerance under hard-water conditions is inherent for the species and is shared by all genotypes. The prediction here is similar population genetic structuring in acidic and non-acidic lakes, with no genetic erosion in the former lakes; (2) Acid tolerant genotypes have evolved in naturally acidic bog lakes over time, and these are also able to colonize acidic mining lakes; (3) Colonization of acidic hard- and soft-water lakes each require specific adaptations, thus the two types of acidic lakes are colonized by distinct genotypes. In the latter case the potential colonization sources for acidic hard-water mining lakes are adjacent non-acidic lakes, implying pre-adaptation, or, alternatively, natural acidic hard-water environments, implying long-distance dispersal. Each of these alternative colonization patterns should leave distinct signatures in the genetic structure of populations.

6.2 Methods

6.2.1 Sampling strategy

In order to provide the information on the genetic structuring in *C. sphaericus* on a broader geographical scale (Staton et al., 2001), we sampled several localities in Europe: one lake in Russia, two in Finland, two in Iceland and two in Greenland.

Fourteen lakes in north-eastern Germany (State Brandenburg) were sampled, among them, two types of acidic lakes—natural soft-water bog lakes and artificial hard-water mining lakes—as well as adjacent non-acidic lakes, which were used as reference sites (Table 6.1). A mining lake Senftenberger See is divided into the South and North Basins, which are connected by a channel. The South Basin is highly acidic (pH 3) and the North Basin has been neutralized in 1970s and maintained a neutral pH to the point of our sampling. The horizontal pH gradient that is established within the connecting channel is described in detail in Chapter 4.

Table 6.1 Sampling localities and analyzed samples for the studied populations of *C. sphaericus*

Locality #	Country	Locality name	Abbreviation	pH	Water body type	Collection date	No. samples	Field / Culture
1a	Germany	Senftenberger See	SFB36	3–6	acidic mining lake	28.04.05	5	field
1b	Germany	Senftenberger See	SFB7	7	neutral mining lake	10.05.05	3	field
2	Germany	Großer Barsch See	GB	4–5	acidic bog lake	09.11.06, 2003	2	culture, field
3	Germany	Tiefer See	TIE	7–8	reference	20.05.05	2	field
4	Germany	Oder	Oder	7	reference	01.06.05	1	field
5	Germany	Große Fuchskuhle	FK	5–6	acidic bog lake	2003	1	culture
6	Germany	Scharnitzelsee	SCH	7	reference	2003	1	culture
7	Germany	Felixsee	FEL	4	acidic mining lake	2003	1	culture
8	Germany	Wolziger See	WOL	8–9	reference	01.06.05	4	field
9	Germany	Langer See	LAN	8–9	reference	01.06.05	3	field
10	Germany	Melangsee	MEL	8–9	reference	20.05.05	1	field
11	Germany	Kleiner Mflasee	KMIL	7	neutral bog lake	2000, 04.12.06	2	culture, field
12	Germany	Grünewalder Lauch	GLA	3	acidic mining lake	2003	1	culture
13	Germany	Sedlitzer See	SED	3.5	acidic mining lake	03.11.06	1	field
14	Germany	Mortitzteich	MOR	3	acidic mining lake	11.10.06, 12.04.07	2	field
15	Russia	River Eruslan	RUS	7	reference	04.08.06	1	field
16	Iceland	Nameless pond, northern Iceland	ICE16	7	reference	10.07.93	1	field
17	Iceland	Lake Fjótstöðin	ICE17	7	reference	27.08.07	1	field
18	Finland	Lake Keitele	FIN18	7	reference	26.08.04	1	field
19	Finland	Lake Orajärvi	FIN19	7	reference	27.08.04	1	field
20	Greenland	Nameless pond, Fortune Bay, Disko Island	GRE20	7	reference	15.07.93	1	field
21	Greenland	Nameless pond, Godhavn, Disko Island	GRE21	7	reference	18.07.93	1	field
22	Greenland	Nameless pond, Uummannaq	GRE22	7	reference	21.07.93	1	field

C. sphaericus specimens were collected at several sampling points in Lake Senftenberger See along the pH gradient. All other sampled acidic lakes were isolated water bodies without any waterway connections (Nixdorf et al., 2001). Some of the reference sites constituted a connected lake chain. The connection sequence was as follows (the numbers correspond to the localities as indicated in Table 6.1): 3—10—6—8—9.

The analyzed specimens were taken either from field populations or from long-term laboratory cultures (Table 6.1). When several individuals were sequenced from a monoclonal culture, all of them resulted in identical sequences. Therefore, in order to obtain a sequence data set that would be representative of the genetic variation in nature, only one sequence per monoclonal culture was included into the alignment. All the sequences obtained from field samples, including identical ones from a single location, were included into the data set.

6.2.2 DNA amplification and sequencing

In order to amplify a 461 bp fragment of the COI gene we used specific primers—Chy-f 5'-TTG GGG ATG ATC AAA TTT ATA ATG T-3' and Chy-r 5'-AGA GGT ATT CAG ATT TCG ATC TGT CA-3'—that were developed in our previous study (see Chapter 5). Each 50 μ l PCR reaction consisted of 35 μ l dd H₂O, 5 μ l PCR buffer, 1.5 μ l each primer, 1 μ l dNTPs, 1 μ l Taq DNA polymerase and 5 μ l DNA extract. The PCR conditions for the COI amplification were 40 cycles of 30 s at 94 °C (denaturation), 30 s at 50 °C (annealing) and 90 s at 72 °C (extension) followed by 1 cycle of 7 min at 72 °C. PCR products were sequenced on an ABI 3700 sequencer. Sequences were edited and assembled in Sequencher 4.1 (Gene Codes Corporation) and manually aligned in MEGA 4.0. (Tamura et al., 2007).

6.2.3 Statistical analyses

The alignment was first examined in MEGA 4.0., using its basic options for data exploration. For calculation of among-haplotype genetic distances the Kimura 2-parameter model was used with the gamma parameter set to 0.092, as estimated by jModelTest (Guindon & Gascuel, 2003; Posada, 2008).

Inter-population differentiation

Genetic structure of the sampled populations was explored using a statistical package for population genetics ARLEQUIN ver. 3.11 (Excoffier et al., 2005). The exact test of population differentiation (Raymond & Rousset, 1995) was performed on the data set with only German populations included. This method employs the Markov chain approach to compare the contingency table containing the observed data to randomly constructed contingency tables, while sample sizes are kept equal to those in the original data set. The resulting p -value is the probability that the observed haplotype distributions can be obtained by chance. The null hypothesis of no population differentiation is rejected at $p > 0.05$.

We also performed analysis of molecular variance (AMOVA), which estimates the covariance components for different hierarchical levels of genetic structure: within populations, within pre-defined groups of populations, and among groups (Excoffier et al., 1992). The statistical significance is tested using non-parametric permutation procedures, thus, in contrast to the classical analysis of variance (ANOVA), non-normal distribution of data and unequal sample sizes are allowed in AMOVA. AMOVA uses both information on the haplotype frequencies and the genetic distances among haplotypes (Excoffier et al., 1992). The latter were calculated based on Kimura 2-parameter model and the gamma parameter set to 0.092. Two AMOVA analyses were conducted: three-level AMOVA for the total data set and two-level AMOVA for a data set containing only the populations from Germany. In the former analysis populations were grouped according to the geographical clusters revealed by the network analyses: Germany plus Russia, Finland, Iceland plus Greenland. Population-specific F_{ST} indices were calculated for the German samples. These indices show whether some populations contribute differently to the average inter-population differentiation (ARLEQUIN ver. 3.1 manual).

Genealogical network analyses

A network is considered to be a more appropriate representation for intra-specific genealogies than a tree (Posada & Crandall, 2001 b). Many different methods have been developed for network reconstruction, and performance of some of them has been recently tested in simulation studies (Cassens et al., 2005; Wooley et al., 2008). Yet, neither method seems to be apparently superior to others, and rigorous testing with real data sets is still lacking.

Hence, following the recommendations of Wooley et al. (2008) we used several methods and then compared the inferences. We used three different network-building methods: statistical parsimony network (SPN), minimum-spanning network (MSN) and median-joining network (MJN) analyses. SPN was implemented in TCS 1.21 software (Clement et al., 2000), with the connection limit set to 95%. This method first calculates a matrix of uncorrected distances among haplotypes and defines the distance above which the parsimony principle is violated with a 95% probability, and then haplotypes are connected into a network starting with the smallest distance. MSN was constructed in ARLEQUIN ver. 3.11 (Excoffier et al., 2005). Contrary to the statistical parsimony algorithm, only sampled haplotypes are connected in MSN, and missing nodes are not inferred. MJN was constructed in the NETWORK software (Bandelt et al., 1999). A minimum spanning network was inferred, which uses an algorithm analogous to the previous method. Then, using the parsimony criterion missing nodes were inferred and added to the network.

6.3 Results

6.3.1 Natural genetic variation in *C. sphaericus* s. str. at the regional scale

We analyzed the COI sequence variation in a total of 81 individuals of *C. sphaericus* from 14 German and 8 distant European localities (Table 6.1; Fig. 6.1). The final alignment was 461 bp long and it consisted of 71 sequences. There were 33 variable sites, and a total of 21 unique COI haplotypes were detected (Table 6.2), with the maximum divergence of 5.4% (K2P distances).

All the network methods applied identified three divergent haplotype groups that corresponded to the following geographical areas: Germany + Russia, Finland and Greenland + Iceland (Fig. 6.2), hence, the studied species had pronounced phylogeographic structuring. The assignment of the haplotypes to the particular geographical groups did not vary among the network methods, however, there was a discrepancy among the methods with respect to the among-groups relationships: SPN and MSN suggested that Greenland-Iceland group was more closely related to the German haplotypes, while MJN placed the former lineage as the closest relative of the Finnish haplotypes (Fig. 6.2: the alternative placement is shown in

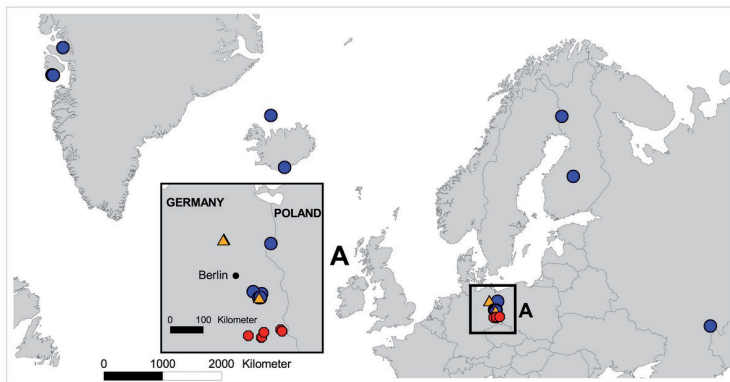


Figure 6.1 Sampling localities for the studied populations of *C. sphaericus* s. str. Habitat types: blue circles—reference lakes; red polygons—acidic mining lakes; orange triangles—bog lakes

gray). Further discrepancies among the network-building methods were in the presence of some among-haplotype connections: SPN inferred three additional connections, which were not inferred by the other methods (Fig. 6.2: dotted lines), and MSN inferred one additional connection between 19Ice and 18Gree haplotypes (not shown). In fact, these connections seemed to be homoplasy artefacts (Posada & Crandall, 2001 b), as they were loop-creating and connected rare haplotypes (Fig. 6.2).

AMOVA analysis, conducted on the data set with defined geographical groups, provided further evidence for the significant phylogeographic structure in *C. sphaericus*, as 60.87% of the variation within the data set was explained by the variation among the geographical groups (Table 6.3). On the other hand, few haplotypes were widely distributed, e.g. haplotype 3 occurred both in Germany and in Greenland, haplotype 13 occurred in Germany and in Iceland, and haplotype 15 was shared by the German and Russian populations (Fig. 6.2).

Table 6.2 COI haplotypes detected in the studied water bodies. See Table 6.1 for the abbreviations of localities' names

Haplotype#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	No. of specimens	No. of haplotypes
1a. SFB36			17				2							1								20	3
1b. SFB7			2				1				1	1	1		3							8	5
2. GB	2																					2	1
3. TIE		1				4																5	2
4. Oder							1	1			2											4	3
5. FK									1													1	1
6. SCH	1						1															1	1
7. FEL								1														1	1
8. WOL					1				1		1				1							4	4
9. LAN	3			1							1											5	3
10. MEL													2									2	1
11. KMIL												2										2	1
12. GLA							1															1	1
13. SED							1															1	1
14. MOR																				2		2	1
15. RUS														1						2		1	1
16. ICE16													1		1							1	1
17. ICE17																		3				3	1
18. FIN18						2																2	1
19. FIN19																	1					1	1
20. GRE20																		1			1	2	1
21. GRE21																			1			1	1
22. GRE22																						1	1
Haplotype frequency	4	2	21	1	1	2	4	7	1	1	1	5	4	1	4	3	1	2	3	2	1	1	1
Haplotype occurrence	2	1	3	1	1	1	1	6	1	1	1	4	3	1	2	2	1	2	1	1	1	1	1

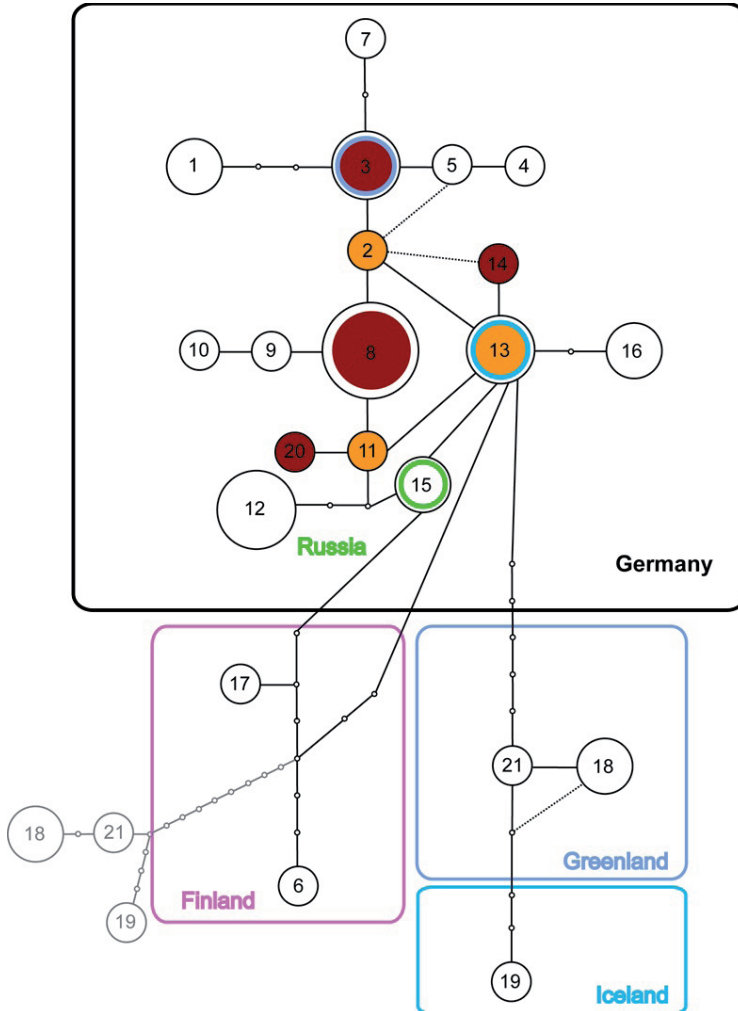


Figure 6.2 Statistical parsimony network (SPN) for the observed COI haplotypes calculated in TCS software with a 95 % connection limit. The area of the circles is proportional to the number of sampling localities, where a haplotype was detected. Lake types: red—acidic mining lakes, orange—naturally acidic bog lakes, empty—reference neutral and alkaline water bodies. Geographical regions: pink—Finland, green—Russia, purple—Greenland, blue—Iceland. Dotted lines: the connections that were present only in the SPN, but not in the MSN and MJN graphs

Table 6.3 Results of the hierarchical analyses of molecular variance (AMOVA)

Source of variation	<i>df</i>	Variance components	Percentage of variation	Fixation indices	Significance <i>p</i> -value
Europe					
Among regions	2	3.69	60.87	$\phi_{CT}=0.61$	< 0.0001
Among populations within regions	20	1.15	18.99	$\phi_{SC}=0.49$	< 0.000001
Within populations	36	1.22	20.14	$\phi_{ST}=0.80$	< 0.000001
Germany					
Among populations	14	0.66	32.73	$\phi_{ST}=0.33$	< 0.000001
Within populations	32	1.36	67.27		

6.3.2 Local population differentiation and haplotypic diversity in the German populations

The part of the haplotype network, which corresponded to Germany, comprised a rather large proportion of the total intra-specific diversity: it included 17 haplotypes, the maximum divergence among them being rather high—2.6 percent. Despite this high diversity, all German haplotypes were closely related to each other, with the maximum of two mutational steps connecting each haplotype to another German haplotype.

Hierarchical AMOVA revealed rather high among-population differentiation at the local scale (Table 6.3). Three-level AMOVA applied to the total data set revealed roughly the same amount of variation partitioned among and within populations—18.99 % and 20.14 %, respectively. When only German populations were analyzed (two-level AMOVA), the amount of variation among populations was still considerable, but it was ca. two times lower than the variation within populations (32.73 % and 67.27 %, respectively). ϕ -statistics indices were rather large and significant in all AMOVA analyses (Table 6.3), indicating low gene flow and a high degree of population differentiation. The exact test of population differentiation revealed a highly significant population structure (global test: $p < 0.000001$). Although some populations were not significantly different from the other ones (Table 6.4), the lack of significance here likely resulted from the insufficient sampling size, as a single individual was sampled in each of these localities (Table 6.1). The habitats with the most divergent haplotypic composition were the acidic part of Lake Senftenberger See and the mesotrophic reference lake Tiefer See, each with 8 significant differences to the other populations (Table 6.4).

Table 6.4 Results of the exact test for population differentiation and the population-specific F_{ST} indices for the German populations of *C. sphaericus* s. str. calculated in ARLEQUIN software. Significant differences among populations ($p < 0.05$) are indicated by '+', non-significant by '-'. Note that the negative F_{ST} values are calculation artefacts, when the real F_{ST} values are very small. See Table 6.1 for the abbreviations of the localities' names

	SFB36	SFB7	GB	TIE	Oder	FK	SCH	FEL	WOL	LAN	MEL	KMIL	GLA	SED	MOR
SFB36	-		+	+	+	-	-	-	+	+	+	+	-	-	+
SFB7	-		-	+	-	-	-	-	-	+	-	-	-	-	-
GB	+		-	+	-	-	-	-	-	-	-	-	-	-	-
TIE	+	+	+		+	-	-	-	+	-	-	+	-	-	+
Oder	+		-	+	-	-	-	-	-	-	-	-	-	-	-
FK	-		-	-	-	-	-	-	-	-	-	-	-	-	-
SCH	-		-	-	-	-	-	-	-	-	-	-	-	-	-
FEL	-		-	-	-	-	-	-	-	-	-	-	-	-	-
WOL	+		-	+	-	-	-	-	-	-	-	-	-	-	-
LAN	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
MEL	+		-	-	-	-	-	-	-	-	-	-	-	-	-
KMIL	+		-	+	-	-	-	-	-	-	-	-	-	-	-
GLA	-		-	-	-	-	-	-	-	-	-	-	-	-	-
SED	-		-	-	-	-	-	-	-	-	-	-	-	-	-
MOR	+		-	+	-	-	-	-	-	-	-	-	-	-	-
No. of different populations	8	2	2	8	2	0	0	0	2	3	1	2	0	0	2
F_{ST}	0.39	0.27	0.55	0.47	0.24	0.55	0.55	0.55	-0.05	-0.03	0.55	0.55	0.55	0.55	0.55

Roughly a half of all haplotypes occurred just in one sampling locality (Table 6.2). Several lakes, from which many individuals were analyzed, appeared to be dominated by one lake-specific haplotype, whereas in other lakes high haplotypic diversity was recorded. Interestingly, in the three localities, where sampling was repeated with a time interval of 1–5 years (Großer Barsch See, Kleiner Milasee and Moritzteich), the same haplotypes were detected on the two sampling dates. It is also noteworthy that the observed degree of differentiation among populations sampled in five interconnected reference lakes (localities 3, 6, 8, 9 and 10 in Table 6.1) was higher than it could be expected from the presumably high dispersal rates via waterways. Four of these lakes (localities 6, 8, 9, and 10) did share some but not all haplotypes, whereas the dominant haplotype in Lake Tiefer See, which was the only mesotrophic lake in the chain of otherwise eutrophied lakes, was lake-specific.

6.3.3 Haplotypic diversity and composition in acidic lakes

Comparable sampling efforts in the acidic mining lakes (10 samples from 5 lakes) and in the reference German lakes (12 samples from 6 lakes) yielded considerably fewer haplotypes in the acidic mining lakes than in the reference lakes: 4 versus 10 haplotypes, respectively. Furthermore, despite the higher inter-lake connectivity among the German reference lakes, the degree of among-lake haplotype sharing was lower here as compared with the acidic mining lakes. Haplotype 8 was the most frequent one found in the acidic mining lakes, being recorded in four acidic mining lakes, and it was also found in one reference locality (River Oder flood plain).

The two groups of acidic habitats—hard-water mining lakes and soft-water bog lakes were dissimilar with respect to their haplotypic composition. Similar to some reference lakes, the two acidic and one neutralized bog lakes were dominated by lake-specific haplotypes, which were found neither in acidic mining lakes nor in reference lakes. Hence, the two groups of acidic lakes did not share any single haplotype, nevertheless, all haplotypes from German lakes were very closely related to each other (Fig. 6.2). Furthermore, haplotypes from acidic and non-acidic lakes were mixed and not in any way separated in the network, implying no long-term reproductive isolation of the acid tolerant populations.

6.3.4 Genetic diversity along the horizontal pH gradient in Lake Senftenberger See

Occurrence pattern of the haplotypes, detected along the horizontal pH gradient in Lake Senftenberger See, is depicted on Figure 6.3. One haplotype (# 3) was dominant along the entire gradient from pH 3.1 to pH 6.7, and it also occurred in the neutral part of the lake, but was less abundant there (ca. 25 % of the haplotypic composition). In the most acidic part of the gradient (pH 3.1) only haplotype 3 was detected, and haplotypic diversity increased towards the neutral end of the pH gradient. Interestingly, the two haplotypes detected at weakly acidic conditions (pH > 4.6), but not at pH 3, came from the bioassays, which tested for the pH tolerance of *C. sphaericus* collected from different points within the gradient (see Chapter 4). Test animals were transferred from pH 4.6 and 5.0 to pH 7.4 (Senftenberger See water), and the survivors were preserved for the subsequent genetic analysis. One of the haplotypes, detected at weakly acidic conditions (# 8), also occurred in three other acidic mining lakes (Sedlitz, Grünewalder Lauch and Felixsee). The second haplotype (# 14) was a singleton with a tip position in the network, and it likely derived from haplotype 13, found in the neutral part of Lake Senftenberger See.

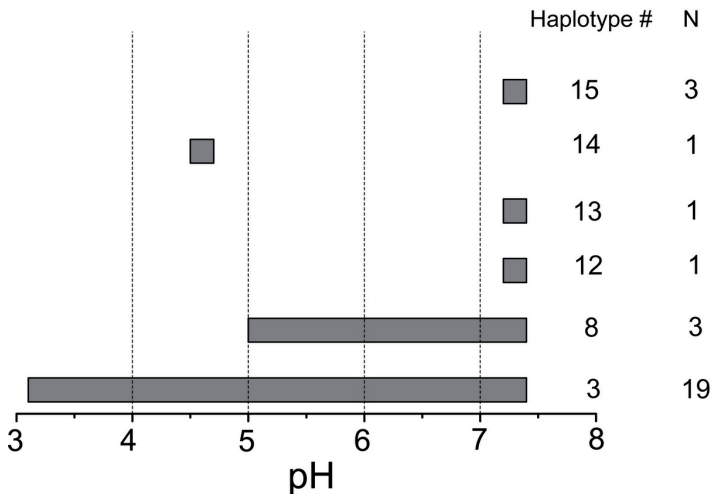


Figure 6.3 Occurrence of haplotypes along the horizontal pH gradient in Lake Senftenberger See. N—number of sequenced individuals

Despite the less intensive sampling effort in the neutral part of Lake Senftenberger See, we observed higher haplotypic diversity here as compared to the acidic part (Table 6.2; Fig. 6.3): a total of 5 haplotypes were detected versus 3 haplotypes at acidic pH. Accordingly, the ratio of haplotypes to the analyzed individuals was much higher for the neutral part, and it seems likely that more intensive sampling would reveal even more diversity. All haplotypes found in the neutral part of Lake Senftenberger See also occurred in the reference lakes (Table 6.2).

6.4 Discussion

6.4.1 Natural genetic variation and population differentiation in *C. sphaericus* s. str.

Prior to assessing the impact of extreme environmental conditions on the genetic structure of *C. sphaericus* populations, we described the natural variation at both local and regional scales (Belfiore & Anderson, 2001; Stanton et al., 2001). At the regional scale we observed strong phylogeographic structuring (Fig. 6.2; Table 6.3). Although few COI haplotypes were widely distributed across the regions, all of them had interior positions within the network (Fig. 6.2) and high frequencies of occurrence (Table 6.2), thus, according to the coalescence theory, these are ancient haplotypes (Posada & Crandall, 2001 b). Hence, it is more likely that their broad distributions resulted from ancient colonization events, rather than from recent dispersal. Overall, the divergent geographical haplotype groups, which accounted for ca. 60 % of the total genetic variation, and the absence of broadly distributed recently evolved haplotypes jointly indicate that the gene flow between North and Middle Europe should be very limited or non-existent at present.

We found that haplotypic diversity was rather high in Germany, possibly reflecting the relatively long colonization history of this region (the age of the sampled glacial lakes is ca. 18 000 years), and that selective sweeps in the mitochondrial DNA, which may reduce regional diversity and increase within-lake diversity in freshwater zooplankton (Ebert et al., 2002), seem to be uncommon in *C. sphaericus*. Furthermore, we observed a high degree of among-population differentiation at the local scale (i.e. low among-lake haplotype sharing) and low within-lake diversity. Many lakes were colonized by a single haplotype, which was often lake-specific. As such haplotypes

were often in tip positions in the network (Fig. 6.2), they likely originated within or close to the respective lakes, where they were detected (Posada & Crandall, 2001 b), pointing to a lack of recent gene flow even between adjacent lakes. The latter was further corroborated by the temporal stability of within-lake haplotypic compositions, whenever data for more than one year were available. Interestingly, the gene flow even among interconnected lakes appeared to be restricted, despite presumably higher rates of migration via connecting waterways, thus, further corroborating monopolization due to local adaptation and the rarity of selective sweeps in this species. It seems likely that persistent priority effects and the divergent lake-specific selection regimes accounted for the observed genetic differentiation among German lakes, and that they also prevented homogenization of the regional gene pool. Thus, our findings are generally consistent with the monopolization hypothesis for cyclic parthenogens (De Meester et al., 2002).

In a few localities the observed high haplotypic diversity and low degree of among-lake differentiation appeared to be at odds with the predictions of the monopolization hypothesis. However, these discrepancies could be explained by some specific characteristics of these lakes. Firstly, waterway connections among lakes, which were similar in their abiotic conditions, appeared to have caused homogenization of the gene pool within the lake chain (Table 6.2). Secondly, large lakes with putatively large carrying capacity (De Meester et al., 2002) and a heterogeneous littoral zone more often exhibited higher haplotypic diversity than small lakes. Another possible explanation for the lack of ‘monopolization’ in some lakes might be ecosystem disturbance due to anthropogenic stress. Indeed, there exists empirical evidence for the increased genetic diversity in *Daphnia* populations, exposed to intermediate stress levels (Weider, 1992; Martins et al., 2009). Anthropogenic eutrophication in three reference lakes coupled with the waterway connections, enhancing among-lake dispersal, possibly explains our observations of high within-lake haplotypic diversity and low among-lake differentiation. It is likely that changes in the local selection regimes due to eutrophication deprived the formerly resident populations of their adaptive advantages and thus disrupted the ‘monopolization’, increasing the establishment success of migrant genotypes and eventually resulting to high within-lake diversity. Two further cases of high within-lake haplotypic diversity and low degree of population differentiation—in the neutral part of Lake Senftenberger See and in the flood plain of River Oder (Table 6.2; Fig. 6.3)—were likely attributed to both ecosystem disturbance and waterway connections. Lake Senftenberger See is a large lake and it is also character-

ized by an unusual ionic composition even in its neutral part (see Chapter 4 for the water chemistry data). Thus, both anthropogenic disturbance and large habitat size explanations are applicable here as well. Besides, the inflow connection to River Schwarze Elster could possibly enhance gene flow. The observed high genetic diversity in River Oder flood plain may be due to the river connection and also to the disturbance due to the temporal character of this habitat.

We conclude that the conspicuous features of the natural genetic variation in *C. sphaericus* are strong phylogeographical structuring at a regional scale and a high degree of differentiation among local populations, both possibly being due to persistent founder effects and local adaptation, as predicted by the monopolization hypothesis. This natural 'genetic landscape' should be taken into account, when assessing the impact of extreme abiotic conditions on the genetic diversity of *C. sphaericus* populations.

6.4.2 Genetic erosion in acidic mining lakes but not in acidic bog lakes

Our results provide evidence for the genetic erosion, i.e. adverse impact of multiple abiotic stressors on the neutral genetic diversity of the *C. sphaericus* populations in acidic mining lakes, but not in naturally acidic bog lakes. The most conspicuous difference of the acidic mining lakes' populations was the frequent among-lake haplotype sharing (Table 6.2), despite the isolated character of these water bodies. Indeed, dispersal among the studied mining lakes is only possible via airborne transport of dormant eggs. Given the high diversity of the regional gene pool and the stochastic nature of the dispersal processes, extensive among-lake haplotype sharing is unlikely to have occurred by chance. Further evidence for the occurrence of genetic erosion in acidic mining lakes was provided by the decreased haplotypic diversity associated with the low pH within the continuous pH gradient in Lake Senftenberger See (Fig. 6.3). This observation indicated strong selection under acidic hard-water conditions, which was not counteracted even by presumably high migration rates through the connecting channel. Apparently, the patterns of population genetic structuring in acidic mining lakes differed from the natural genetic variation in this species, and thus they can be attributed to the effects of multiple abiotic stressors. In contrast, no differences in the population genetic structuring were observed in acidic bog lakes, as compared to non-acidic reference lakes. Derry et al. (2009), using the same molecular marker, obtained similar results on a tolerant copepod

species, whose genetic diversity was not reduced in acidic soft-water lakes.

Although we observed strong phylogeographical structuring on the regional scale (see above), on the local scale no association of the genetic structure with the geographical distances was apparent in the German populations (Figs 6.1, 6.2), thus, such an association is unlikely to be a confounding factor. Another important factor unrelated to the water chemistry conditions in the sampled water bodies that might have a strong effect on the genetic diversity is habitat age. All reference sites were natural water bodies that were colonized by cladocerans a long time ago (the age of the studied glacial lakes is ca. 18 000 years), whereas the man-made acidic mining lakes were of recent origin (30–70 years). Demographic bottlenecks are likely to occur in cladoceran populations during colonization of newly created habitats also without any abiotic stress. Indeed, for animals reproducing by cyclic parthenogenesis the monopolization hypothesis (De Meester et al., 2002) predicts strong priority effects that should cause low genetic diversity in newly originated water bodies. Hence, genetic diversity of a cladoceran species should be lower in artificial mining lakes than in old glacial reference lakes, irrespective of the physico-chemical conditions. The best way to test the effect of hard-water acidic conditions would be to compare the genetic diversity in acidic mining lakes and artificial circum-neutral lakes of the same age. Hence, neutralized mining lakes in Lusatia could be used as reference sites for a more rigorous testing of the genetic erosion hypothesis. Our observation of high genetic diversity in the neutral part of Lake Senftenberger See (Fig. 6.3) corroborates the hypothesis that young habitat age is unlikely to account for the decrease in the genetic diversity in acidic mining lakes, but it is rather due to the stressful abiotic conditions. Furthermore, enhanced founder effects in young habitats could explain the low within-lake haplotypic diversity, but not the frequent among-lake haplotype sharing in the studied acidic mining lakes.

The observed genetic erosion of the mitochondrial haplotypic diversity in acidic mining lakes could be either due to direct effects of some mitochondrial haplotypes on fitness under acidic hard-water conditions, or, what is more likely, it could be neutral and not directly related to fitness. Most population genetic studies have used mitochondrial DNA as a neutral marker, although in few cases mitochondrial genotypes were shown to be related to important physiological adaptations (see Ballard & Whitlock (2004) for a review). The direct effects of mitochondrial haplotypes on fitness can be only determined by measuring them in individuals, differing only in their mitochondrial genome (Ballard & Whitlock, 2004). We have not done such

experiments, so our data cannot distinguish between the two possibilities named above; however, the direct effects on fitness seem not to be very likely. Still, impoverishment of neutral genetic diversity has often been observed following ecosystem disturbances, and it commonly denotes considerable reductions in the genome-wide diversity and in the effective population size (van Straalen & Timmermans, 2002; Bickham, 2011). Genetically impoverished populations are likely to be more susceptible to further environmental stressors and thus have a higher risk of extinction (Willi et al., 2006; Lopes et al., 2009; Jansen et al., 2011). Therefore, the genetic erosion, observed in the populations of *C. sphaericus* from acidic mining lakes, should indicate a high level of abiotic stress in these habitats, which may cause instability of the resident populations in the long term, even though this species was shown to be highly tolerant to multiple abiotic stressors (see Chapter 4).

Overall, local selective regimes seem to play an important role in determining the population genetic structure in *C. sphaericus*. In extreme acidic environments local selection should result in particularly strong priority effects, reducing within-lake diversity (in accordance with the monopolization hypothesis). In evolutionary young extreme environments, such as acidic mining lakes, these enhanced priority effects are likely to be further superimposed by genetic erosion, resulting in greatly impoverished genetic diversity in the tolerant populations. Indeed, there were signatures of both priority effects and genetic erosion in acidic mining lakes' populations. In evolutionary old extreme environments, such as acidic bog lakes, genetic erosion was not detected in our study, supporting our initial hypothesis that long-term adaptation should increase local genetic diversity despite stressful environmental conditions. Unfortunately, our data set was too small to test whether priority effects were enhanced in acidic bog lakes due to the divergent local selection. If this were the case, the populations in evolutionary old extreme habitats would have a greater chance to eventually become reproductively isolated from the populations from adjacent non-extreme habitats. Yet, we did not observe any indications of the independent long-term evolution of acidic bog lakes' populations, and recent genetic exchange with populations from non-acidic lakes is more consistent with our observations.

6.4.3 Potential colonization sources for the acidic mining lakes' populations and the mechanisms of broad physiological tolerance in *C. sphaericus*

We aimed to determine the colonization sources for the populations in acidic lakes in order to distinguish among three alternative hypotheses, explaining how broad pH tolerance is achieved in *C. sphaericus*. The first hypothesis of inherent acid tolerance in all *C. sphaericus* individuals has to be rejected, as we did observe genetic erosion in acidic mining lakes' populations, implying that many genotypes were not tolerant and thus they were removed by selection under acid hard-water conditions. The second hypothesis that acidity tolerance, which evolved in naturally acidic bog lakes, should also enable colonization of acidic hard-water mining lakes has to be rejected as well, as no single haplotype was shared among the two types of acidic lakes. Therefore, our data most strongly supported the third hypothesis stating that colonization of acidic hard- and soft-water lakes in each case requires specific adaptations, and the evolution of the acid tolerance was independent for these two types of acidic lakes. Furthermore, our data indicated that adjacent non-acidic lakes rather than geographically distant natural acidic hard-water lakes were most likely colonization sources for the acidic mining lakes' populations, as the latter comprised no genetically divergent lineages and they also shared some haplotypes with the non-acidic reference lakes. Thus, pre-adaptation of few but not all genotypes to the acidic hard-water conditions is much more likely than colonization by adapted genotypes via long-distance dispersal. Due to the small sampling size, it remains unknown whether tolerance to acidic soft-water conditions is due to long-term adaptation or to pre-adaptation of some individuals from non-acidic populations. Haplotypes found in acidic bog lakes were not shared with non-acidic lakes, yet, they also were not separated in the network, implying that their independent evolutionary history has not been very long. Future studies should focus on the population genetic mechanisms of adaptation to acidic soft-water conditions.

Therefore, we conclude that the broad tolerance to acidic hard-water conditions in *C. sphaericus* is due to intra-specific polymorphism and it is maintained via locally adapted genotypes. Hence, the ability to colonize acidic hard-water conditions is not inherent to all individuals and populations of this species, but it is also not particularly rare. Such ecological differentiation within cyclic parthenogenetic species is likely to be accompanied by the ability for rapid adaptation, predicted by the monopolization

hypothesis (De Meester et al., 2002) and documented for daphniid Cladocera (Cousyn et al., 2001; Declerck et al., 2001). Our results indicate that as specific adaptations are probably required for successful colonization of acidic hard- and soft-water lakes, such adaptations are likely to be achieved in each case by different physiological and genetic mechanisms. In general, tolerance to low pH and also to some other stressors, associated with acidic conditions, such as toxic metal ions, is most probably related to osmotic regulation mechanisms (Potts & Fryer, 1979; Havas & Advokaat, 1995). However, detailed knowledge on the genetic basis of the specific adaptations to acidic hard- and soft-water conditions is still lacking, and further studies are clearly required.

6.4.4 Implications for the ecological quality assessment of acidic mining lakes

Genetic diversity assessment can help to evaluate the ecological quality of a habitat, depending on the extent to what it supports valuable and diverse genetic lineages (Bagley et al., 2002). It is somewhat controversial, what part of the genetic diversity should be considered as most valuable (Allendorf & Luikart, 2006). One view is that most ancient taxa and genetic lineages should be protected, because they are products of millions of years of evolution, and thus their loss would be irreplaceable. A different view advocates the primary significance of the evolutionary process itself and of the ability of species and populations to evolve and adapt to changing environmental conditions. Here young lineages with a high evolutionary potential would be the conservation targets (Allendorf & Luikart, 2006). According to the first view, the results of our genetic survey suggest rather low ecological value of acidic mining lakes, as no divergent lineages either on the species level (see Chapter 2) or on the intraspecific level (this study) have been detected, and the overall genetic diversity was impoverished compared to natural acidic and non-acidic lakes. It is still possible that genotypes colonizing acidic mining lakes may be valuable as regards their ability to rapidly adapt to stressful abiotic conditions. On the other hand, such tolerant populations also have a high extinction risk due to the negative effects of reduced population size on fitness (Willi et al., 2006) and susceptibility to further environmental stressors (Lopes et al., 2009; Jansen et al., 2011). Overall, our data indicate that acidic mining lakes are habitats of low ecological quality, and they should be subjected to restoration measure in order to achieve the neutral conditions, which are likely to support high genetic diversity. Nevertheless,

further studies are required in order to investigate the physiological and genetic costs of tolerance to extreme abiotic conditions.

Chapter 7

Synthesis and general discussion

7.1 Zooplankton in acidic hard-water mining lakes: General colonization patterns and the knowledge gaps addressed in this thesis

Despite the very harsh abiotic conditions in acidic hard-water mining lakes (Chapter 1.1), several highly tolerant zooplankton species can colonize these waters already at pH above 2.3. The literature review (Chapter 2) revealed the following characteristic features of the zooplankton communities colonizing acidic mining lakes:

1. Very low species diversity appears to be due to multiple abiotic stressors, such as low pH, and high concentrations of Al, Fe and sulphate, whereas biomass is controlled by the food availability;
2. Many tolerant zooplankton species, occurring in acidic hard-water lakes have inter-continental distributions; however, further taxonomic and genetic evidence is required;
3. Colonization exclusively by generalist species, in contrast to evolutionary old acidic soft-water lakes, where some specialists seem to have evolved;
4. Complete extirpation of pelagic taxa, including entire taxonomic groups, such as calanoid copepods;
5. Habitat shifts of benthic/littoral taxa to the pelagic zone;

6. Simple food webs and rather weak biotic interactions due to low species diversity and extinction of higher trophic levels (fish), hence, similar to acidic soft-water lakes, control by invertebrate predators.

Overall, the colonization patterns in acidic hard-water mining lakes by zooplankton generally resemble those observed in other extreme environments, at the same time, they differ considerably from the patterns found in naturally acidic soft-water lakes.

From Chapter 2 it is apparent that the published data on zooplankton diversity and ecology in acidic hard-water lakes has been very scarce up to now, and some of the identified knowledge gaps have been subsequently addressed in my thesis. Firstly, the roles of multiple abiotic factors for colonization of chemically diverse acidic lakes are poorly understood. In particular, colonization patterns by Cladocera along the pH gradient in acidic hard-water lakes were largely unknown before my thesis. As discussed below, the data on the species (Chapters 3 and 4) and genetic (Chapter 6) diversity of Cladocera in acidic mining lakes, obtained in my studies, have both theoretical and practical implications, providing a more detailed understanding of the effects of multiple abiotic factors in acidic lakes and also a basis for ecological quality assessment of acidic mining lakes. Secondly, although my review revealed many allegedly cosmopolitan species among zooplankton, colonizing acidic hard-water lakes, such cosmopolitanism is highly questionable, given the absence of detailed taxonomic comparisons and genetic data. In Chapter 5 I tackled the issues of cosmopolitanism and cryptic diversity for the *C. sphaericus* species complex—the most acid tolerant crustacean taxon, which is also one of the most frequently recorded crustaceans in acidic hard-water lakes of natural and anthropogenic origin (Chapter 2). Finally, genetic diversity assessment in acidic lakes, although not covered in my review on species diversity, deserves special attention (see Chapters 1.2.2 and 7.3). Yet, nothing is so far known about the impact of multiple abiotic stressors on the genetic diversity of locally adapted cladoceran species in acidic lakes. Available genetic studies were carried out on a copepod (Derry et al., 2009) and on daphniid Cladocera (Lopes et al., 2009; Martins et al., 2009), the latter being absent from acidic lakes at pH below 6. Addressing this knowledge gap, I described the genetic structure of field populations of a tolerant cladoceran *C. sphaericus*, which commonly occurs in acidic hard- and soft-water lakes in Germany (Chapter 6).

7.2 Species diversity and composition of littoral cladoceran communities in acidic hard- and soft-water lakes in Germany as determined by multiple abiotic stressors

The results of my studies on the species level of biodiversity of littoral Cladocera in acidic lakes were generally consistent with the currently emerging view that multiple-stressor paradigm in acidic lakes' research (Yan et al., 2008). Specifically, several lines of evidence led to the rejection of my null hypothesis that pH was the only abiotic factor, which determined cladoceran colonization patterns in the surveyed acidic lakes:

1. Species richness was significantly affected both by pH and the lake type, the latter most likely reflecting the effects of TDS-related parameters (Chapter 3).
2. Acidic hard- and soft-water lakes were colonized by highly distinct littoral cladoceran communities, both with respect to their species diversity and composition (Chapter 3), thus, pointing to the effects of other factors apart from pH.
3. Species-specific pH thresholds of species, colonizing both types of acidic lakes, were found to be consistently higher under hard-water conditions, indicating higher abiotic stress in acidic hard-water lakes, irrespective of pH (Chapter 4).

Further statistical analyses revealed significant effects of TDS-related parameters, pH and trophy on the community composition. Particularly, the ecological effects of total TDS (ionic content) and those of some of its mayor constituents, such as sulphate and Fe ions, have been virtually neglected up to now, what is probably due to the fact that previous studies were mostly based on soft-water lakes' data sets. In my studies (Chapters 3 and 4) I compared cladoceran communities along a broad TDS gradient, and I found that this factor was an important determinant of colonization patterns in acidic lakes. This finding is in agreement with physiological studies on Cladocera, suggesting that TDS should limit species occurrence at both low (soft-water) and high (hard-water) ends of the gradient (Aladin & Potts, 1995).

I found that species diversity was much more impaired in the anthropogenically acidified hard-water mining lakes compared to that in the nat-

urally acidic soft-water bog lakes. Similar to the studies on acidic streams (Dangles et al., 2004; Petrin et al., 2007, 2008; Greig et al., 2010), my results suggest both ecological and evolutionary constraints for colonization in acidic lakes. Firstly, more drastic reduction of the cladoceran species diversity with decreasing pH in acidic hard-water lakes than in soft-water lakes can be attributed to the adverse effects of water chemistry. Although organisms in both types of acidic lakes studied are subjected to multiple abiotic stressors, rather different combinations of stressors probably affect biota in acidic hard- and soft-water lakes (described in Chapters 1.1, 2 and 3). My results suggest that the resultant abiotic stress should be higher in acidic hard-water lakes, and it is likely attributed to TDS-related parameters, among them being metal ions and sulphate (Chapter 3). Secondly, apart from the ecological reasons, low species diversity in acidic hard-water mining is possibly due to the young age of these habitats. Namely, my results agreed with the predictions of the evolutionary species pool hypothesis (Pither & Aarssen, 2005) that species diversity should be particularly low in evolutionary young habitats, such as acidic hard-water mining lakes, due to a lack of time for evolution of specific adaptations. Accordingly, I found that only pre-adapted broad generalists were able to colonize these lakes. Although evolutionary old natural acidic hard-water lakes do exist, and their fauna appears to be more diverse compared to that in artificial acidic mining lakes (Uéno 1934, 1958; Chapter 2), these lakes are situated in geographically remote areas. Thus, they presumably cannot serve as colonization sources for the acidic mining lakes in Germany, as the dispersal abilities of Cladocera were shown to be rather limited (Frey, 1995; Fórró et al., 2008; see also Chapter 7.3 for the genetic evidence of limited dispersal in *C. sphaericus*). Furthermore, I found that species, associated with acidic soft-water conditions, which were regarded as 'acidity indicators' by other studies, were not able to colonize acidic mining lakes. In fact, the majority of so-called 'acidity indicators' were not acidophilic in the strict sense, as they colonized both acidic and non-acidic soft-water lakes (Chapter 3). Hence, I conclude that these are soft-water specialists (rather than acidophiles), which, in accordance with the evolutionary species pool hypothesis, have acquired these adaptations over evolutionary time scales. Based on the occurrence patterns of generalists and specialists in acidic hard- and soft-water lakes, it seems likely that colonization of these two types of acidic lakes in each case has required different physiological adaptations. It should be also noted that despite the possibility of long-term adaptive evolution in acidic soft-water lakes, many species in the region, apparently, failed to

adapt to these conditions, and multiple abiotic stressors still cause considerable impoverishment of the species diversity in these waters, compared to non-acidic lakes, although not that drastic, as that observed in acidic mining lakes (Chapter 3).

To conclude this chapter, my thesis contributes to the knowledge on the species-level biodiversity in so far poorly studied extreme habitats—acidic hard-water mining lakes—by providing detailed information on the diversity patterns of littoral Cladocera along the major environmental gradients under acidic hard-water conditions. Firstly, these results have practical relevance, as they can be used for ecological quality monitoring of acidic mining lakes (see Chapter 7.4). Secondly, my thesis advances the theoretical knowledge on the colonization patterns in acidic lakes in that it demonstrates that inclusion of acidic hard-water lakes into the research framework as well as comparisons among communities in chemically diverse acidic lakes can result in important new insights in this field.

7.3 Genetic diversity of the most acid tolerant cladoceran—*Chydorus sphaericus* in acidic versus non-acidic lakes in Germany

For the assessment of the population-level genetic consequences of multiple abiotic stressors in acidic lakes, I selected an acid tolerant cladoceran species *C. sphaericus*, which is also one of the core zooplankton species in acidic hard-water lakes (Chapter 2). Although *C. sphaericus* has been recorded from different continents, its broad geographical distribution and broad environmental tolerance strongly suggest the presence of numerous cryptic species, and it is likely that each of those species is more locally distributed and ecologically restricted than the species complex as a whole (Frey 1980; Chapter 5). Unrecognized co-existing morphologically similar, yet, ecologically distinct species can seriously confound results of ecotoxicological studies, as it would be unclear whether the observed responses are attributed to a single species or to an undefined mixture of closely related cryptic species. Therefore, I first clarified the taxonomic status of the *C. sphaericus* populations in Germany by conducting a phylogenetic study on the *C. sphaericus* species complex at the Holarctic scale (Chapter 5). All German populations, including those from acidic lakes, proved conspecific; moreover, I also obtained ecological evidence of the conspecificity of the populations, sampled within a continuous pH gradient from 3 to 7 in a

mining lake (Chapter 4).

Using the methodology (specific primers) and the phylogenetic framework for diversity, developed in Chapter 5, I then proceeded with the population genetic analysis of *C. sphaericus* s. str., colonizing acidic lakes in Germany (Chapter 6). I aimed to test for genetic erosion in acidic lakes and to determine the colonization sources for the acid tolerant populations. To my knowledge, this is the first study on the population genetic structuring in a littoral cladoceran species from the family Chydoridae, and it is also the first assessment of the population-level consequences of abiotic stress in acidic lakes for a cladoceran species, adapted to highly acidic conditions. The observed natural genetic variation at the reference sites at both regional and local scales generally agreed with the monopolization hypothesis, which predicts strong among-population differentiation in organisms with cyclic parthenogenetic life cycle (De Meester et al., 2002). Some deviations from the monopolization patterns were observed mostly in the water bodies with high levels of ecosystem disturbance (e.g. eutrophication). I then contrasted the population genetic structure in acidic lakes with the patterns of natural variation at non-acidic reference sites, and I detected genetic erosion due to abiotic stress in the studied acidic mining lakes, but not in the acidic bog lakes. In acidic mining lakes genetic erosion appeared to be superimposed on the natural reduction in within-lake genetic diversity due to monopolization effects, overall resulting in a drastic impoverishment of the genome-wide diversity and reduced effective sizes of the tolerant populations (as indicated by a neutral genetic marker, according to van Straalen & Timmermans, 2002; Bickham, 2011). Even though *C. sphaericus* is tolerant to a wide range of abiotic conditions, the observed genetic erosion in acidic mining lakes may have negative long-term consequences for the population persistence. I conclude that in extreme habitats cyclic parthenogenesis may have some disadvantages, because it naturally results in decreased within-habitat genetic diversity, and further losses due to abiotic stress may bring populations to the edge of extinction. On the other hand, the monopolization hypothesis also predicts that cyclic parthenogens should be capable of rapid adaptation to adverse abiotic conditions (De Meester et al., 2002). Such beneficial effects of the life cycle are supported by the empirical data on the zooplankton communities in acidic mining lakes. Namely, the two cyclic parthenogenetic groups—Rotifera and Cladocera clearly dominate in these lakes, whereas obligate sexual Copepoda, which are common in other water bodies, are very rare (Chapter 2).

The results of Chapter 6 suggest that the observed low genetic diversity

in acidic mining lakes is likely to be attributed to the effects of multiple abiotic stressors, but also to the insufficient time for adaptive evolution in these young extreme environments. In contrast, the abiotic stress in soft-water lakes, both naturally acidic (Chapter 6) and anthropogenically acidified (Derry et al., 2009), appears to be too weak to cause any observable effects on the neutral genetic diversity of acid tolerant zooplankton species. Overall, the genetic diversity patterns in the studied acidic hard- and soft-water lakes resembled the species diversity patterns in these lakes (see Chapter 7.2) in that diversity was more impaired in acidic mining lakes, and no specialized (acid tolerant) species and genotypes were shared by the two types of acidic lakes. My study (Chapter 6) also revealed that the most likely colonization sources for the studied acidic mining lakes were adjacent non-acidic lakes, implying that the ability of *C. sphaericus* to colonize acidic hard-water lakes is due to pre-adaptation, possessed by some but not all individuals from non-acidic lakes. Hence, the intriguing broad tolerance in this species is due to the intra-specific polymorphism and it is maintained via locally adapted genotypes. As no haplotypes were shared by acidic hard- and soft-water lakes populations, I conclude that similar to species-level adaptations (see Chapter 7.2), population-level adaptations to acidic hard- and soft-water conditions are most likely independent from each other and they are probably achieved via different physiological and genetic mechanisms.

Overall, these results provide support for my initial hypotheses that (1) the resultant abiotic stress should be higher in acidic mining lakes than in acidic bog lakes, leading to a greater biodiversity impoverishment in the former lakes at both species and population level; (2) biodiversity at both levels should increase over time in evolutionary old acidic environments, despite adverse abiotic conditions; (3) colonization of acidic hard- and soft-water lakes in each case requires specific physiological adaptations at both species and population level.

7.4 Concordant patterns in species and genetic diversity of littoral Cladocera as the basis for ecological quality assessment of acidic mining lakes

Although statistical testing for covariation between the species and genetic diversity was beyond the scope of this thesis, my investigations did reveal some concordant responses to abiotic stress in acidic lakes at these two

biodiversity levels. Specifically, I observed simultaneous and marked losses of species and genetic diversity with the increasing impact of multiple abiotic stressors towards acidic conditions in hard-water mining lakes. In contrast, in acidic bog lakes significant effects of abiotic stress were detected only at the species, but not at the genetic level. Covariation among species and genetic diversity in space and time may be due to parallel effects of locality characteristics and/or direct and sometimes reciprocal effects of one biodiversity level on the other, which is predicted by both theory and computer simulations and is also frequently observed in nature (reviewed in Vellend & Geber, 2005). On the other hand, each biodiversity component also provides information on some specific responses at different levels of biological organization, and thus species and genetic diversity are to great extent complementary indicators of environmental conditions, what often results in low correlation between them at low stress levels (Bagley et al., 2002).

I conclude that the observed strong and concordant responses at both biodiversity levels of littoral Cladocera in the studied acidic mining lakes indicated very high abiotic stress, which caused adverse effects at both community- and population-levels. In acidic bog lakes only species diversity was affected, and this also to much lesser extent, indicating rather low abiotic stress. Therefore, according to the results of my thesis, naturally acidic soft-water lakes appear to be natural undisturbed ecosystems, supporting relatively high and evolutionary unique biodiversity, which should be protected. Conversely, acidic mining lakes, at least from the perspective of the cladoceran biodiversity, appear to represent highly disturbed ecosystems, which are colonized by a small subset of tolerant species and genotypes from adjacent non-acidic lakes. Hence, these lakes do not support any unique biodiversity components, and they should be preferably subjected to restoration measures. Indeed, my results showed that neutralization measures, especially flushing with river water, appeared to be effective in restoring the biodiversity at both species and genetic levels. However, if organisms with higher dispersal abilities are considered, one may come to rather different conclusions, concerning the ecological value of acidic mining lakes. As these lakes closely resemble natural hard-waters in their water chemistry, they can be potentially colonized by specialized communities of extremophilic organisms (Nixdorf et al., 2005), given that these can disperse over large distances from natural acidic hard-water lakes. Interestingly, a new rotifer species has been recently described from acidic mining lakes in Germany and Austria, which is truly acidophilic and does not occur in nearby non-

acidic lakes (Jersabek et al., 2011). This finding supports the supposition that some small freshwater organisms, with higher dispersal potential than that of Cladocera, such as rotifers, protists and microorganisms (Finlay & Fenchel, 2004) may still colonize acidic mining lakes from naturally acidic hard-water lakes.

While ecological monitoring of acidic soft-water lakes, using littoral Cladocera, has been elaborated elsewhere (Walseng et al., 2001, 2003), the data, obtained in my thesis, on the colonization patterns of littoral Cladocera along the environmental gradients in acidic hard-water mining lakes can serve as a basis for monitoring of these waters. There are numerous large acidic mining lakes in Germany, which fall under the EC Water Framework Directive (WFD), requiring monitoring and management, hence, there is an urgent need to establish suitable bio-indicators for monitoring (Nixdorf et al., 2005). Although zooplankton was not included into the EC WFD, its utility for bio-indication, and the utility of littoral Cladocera in particular, has been proved by numerous scientific publications and practical applications in lake monitoring (e.g. De Eyto et al., 2003; Bjerring et al., 2008; Jeppesen et al., 2011). In agreement with the pioneer studies, which proposed zooplankton as a cost-effective and simple indicator of water quality in acidic mining lakes (Nixdorf et al., 1998, 2005), I found that the colonization patterns by Cladocera and other zooplankton groups (Chapters 2–4) in acidic mining lakes were largely determined by the water chemistry conditions, whereas variation due to other factors, such as lake morphometry, biotic interactions etc. proved negligible. My results on littoral Cladocera generally support the classification scheme for acidic mining lakes proposed by Nixdorf et al. (2005), and they also provide more detailed information on the ecological processes within each of the defined lake categories. Therefore, Cladocera are well-suited as ecological quality components for monitoring of these lakes.

Chapter 8

Outlook

The conclusions in this thesis are mostly based on field studies, and it should be noted that the field approach has its advantages as well as limitations. Its primary advantage is that it integrates over diverse direct and indirect effects of multiple abiotic stressors in situ, thus providing realistic and integrative estimates of biological responses to the specific combinations of multiple abiotic stressors under field conditions. However, the field approach is limited by the available lake data sets, which often suffer from the multi-collinearity problem in statistical analyses due to correlations among parameters, because of which disentangling of the effects of all ecologically relevant abiotic parameters is not always possible. My data set was designed to enable differentiation between the effects of pH from those of the TDS constituents, hence, it comprised lakes, exhibiting all combinations of low/high pH and low/high TDS. However, this data set was not appropriate to single out the effects of such ecologically relevant TDS constituents as metal ions, calcium, magnesium and sulphate. Future studies can investigate these effects by analyzing field data sets with higher variation ranges of single TDS constituents, than in the data set used in this work. Notwithstanding these limitations, given the scarcity of biological data on acidic hard-water lakes, my work represents a necessary first step, providing a basis for formulating more precise hypotheses. The next step would be to validate the observed effects under controlled laboratory conditions and to study interactions between them, thus establishing direct causal links between colonization patterns and water chemistry parameters. It is noteworthy that although it may provide direct evidence of physiological effects of particular stressors, the laboratory approach has its limitations as

well, such as the limited applicability of the results to the natural in situ conditions. Besides, not all combinations of environmental stressors can be tested, for example, due to the low solubility of metal ions at neutral conditions, combinations of high pH and high dissolved metal ions cannot be tested. Therefore, combining the field approach with the laboratory approach seems most advisable.

Explicit comparisons among communities in chemically diverse acidic lakes should be performed more often in the future, as my work demonstrated that such a comparative approach can likely provide a better understanding of the effects of multiple stressors and even more generally, different environmental axes, for colonization of acidic waters. Interestingly, my findings suggest that specific adaptations are likely required for colonization of each of the studied types of acidic lakes, however, their mechanisms remain largely unknown. Future studies should focus on the physiological and genetic mechanisms of the specific adaptations to acidic hard- and soft-water conditions.

Elaboration of a practical monitoring scheme for ecological quality assessment of acidic mining lakes using Cladocera was beyond the scope of my thesis. Nevertheless, the data on the cladoceran colonization patterns along the pH gradient in these lakes, on the ecological preferences of species and their in situ pH thresholds under hard-water conditions, presented here, can be used to elaborate such a scheme. This should ideally be substantiated by data on the species-specific physiological thresholds under laboratory conditions. Apart from good correspondence of colonization patterns to the environmental gradients, another advantage of Cladocera as bio-indicators is their excellent preservation in lake sediments, hence, also in the absence of long-term data, historical changes in water chemistry, e.g. effects of neutralisation measures or re-acidifications, can be monitored by using the paleolimnological approach. Other zooplankton groups, especially Rotifers, which colonize acidic mining lakes already at pH 2.3, are potentially suitable indicators as well. Combining information on several taxonomic groups would be advantageous for integrated ecological assessment of acidic mining lakes, as already shown by many basic and applied studies on other freshwaters.

Simultaneous assessment of more than one biodiversity levels has been rarely done, when studying environmental effects on freshwater biota, because different biodiversity levels are traditionally amenable to different research fields, e.g. ecotoxicology and population genetics in case of the species and genetic diversity, respectively. Notwithstanding, my results sug-

gest that such integrative biodiversity assessment can provide valuable insights into the effects of abiotic stressors on both community- and population-levels (see Chapter 7.4 and also Geist, 2011), and it should be done more often in the future. Especially genetic diversity proved a useful indicator, providing unique information on long-term population-level effects of stressors. Here I used a neutral genetic marker, however, if non-neutral genetic diversity, i.e. variation in genes, enabling adaptations to the environmental conditions, is measured in future studies, it might reveal even more subtle responses and further advance our understanding of the consequences of multiple abiotic stress on biodiversity. I used species and genetic diversity as independent indicators of abiotic stress. Such an approach is justified, as each of these biodiversity components exhibits unique responses to stress, and thus they can complement each other in ecological quality assessment (Bagley et al., 2002). Still, statistical testing for covariance between species and genetic diversity in acidic lakes should be carried out in future studies, as it can provide valuable information on the concerted responses of these biodiversity components to stress and on interactions between them.

References

- Adamowicz, S. J. & V. Sacherová, 2006. Testing the directionality of evolution: the case of chydorid crustaceans. *Journal of Evolutionary Biology* 19: 1517–1530.
- Adamowicz, S. J., P. D. N. Hebert & M. C. Marinone, 2004. Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. *Zoological Journal of the Linnean Society* 140 (2): 171–205.
- Agra, A. R., L. Guilhermino, A. M. V. M. Soares & C. Barata, 2010. Genetic costs of tolerance to metals in *Daphnia longispina* populations historically exposed to a copper mine drainage. *Environmental Toxicology and Chemistry* 29 (4): 939–946.
- Aladin, N. V. & W. T. W. Potts, 1995. Osmoregulatory capacity of the Cladocera. *Journal of Comparative Physiology B—Biochemical Systemic and Environmental Physiology* 164 (8): 671–683.
- Allendorf, F. W. & G. Luikart, 2006. Conservation and the genetics of populations. Blackwell Publishing, New York: 644 pp.
- Alonso, M., 1996. Crustacea Branchiopoda. Fauna Iberica. Museo Nacional de Ciencias Naturales, CSIC, Madrid: 486 pp.
- Arnott, S. E. & M. J. Vanni, 1993. Zooplankton assemblages in fishless bog lakes: influence of biotic and abiotic factors. *Ecology* 74 (8): 2361–2380.
- Arvola, L., T.-R. Metsälä, A. Similä & M. Rask, 1990. Phyto- and zooplankton in relation to water pH and humic content in small lakes in Southern Finland. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 24: 688–692.
- Avise, J. C. & R. M. Ball, 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology* 7: 45–67.
- Backhaus, K., B. Erichson, W. Plinke & R. Weiber, 2003. Multivariate Analy-

- senmethoden. Springer-Verlag, Berlin: 819 pp.
- Bagley, M. J., Franson, S. E., Christ, S. A., Waits, E. R., & Toth, G. P., 2002. Genetic diversity as an indicator of ecosystem condition and sustainability: Utility for regional assessments of stream condition in the Eastern United States. U.S. Environmental Protection Agency, Cincinnati, OH: 78 pp.
- Ballard, J. W. O. & M. C. Whitlock, 2004. The incomplete natural history of mitochondria. *Molecular Ecology* 13: 729–744.
- Bandelt, H. J., P. Forster & A. Rohl, 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16 (1): 37–48.
- Banks, D., P. L. Younger, R.-T. Arnesen, E. R. Iversen & S. B. Banks, 1997. Mine-water chemistry: the good, the bad and the ugly. *Environmental Geology* 32: 157–174.
- Belfiore, N. M. & S. L. Anderson, 2001. Effects of contaminants on genetic patterns in aquatic organisms: a review. *Mutation Research* 489 (2–3): 97–122.
- Belyaeva, M., 2003. Littoral Cladocera (Crustacea: Branchiopoda) from Altai mountain lakes, with remarks on the taxonomy of *Chydorus sphaericus*. *Arthropoda Selecta* 12 (3–4): 171–182.
- Belyaeva, M. & R. Deneke, 2013. The biology and ecosystem of acidic pit lakes: Zooplankton. In W. Geller, M. Schulze, R. Kleinmann & C. Wolkersdorfer (Eds), *Acidic Pit Lakes—the Legacy of Coal and Metal Surface Mines*. Environmental Science Series, Springer-Verlag, Berlin: 117–125.
- Bērziņš, B. & B. Pejler, 1987. Rotifer occurrence in relation to pH. *Hydrobiologia* 147: 107–116.
- Bērziņš, B. & J. Bertilsson, 1990. Occurrence of limnic micro-crustaceans in relation to pH and humic content in Swedish water bodies. *Hydrobiologia* 199: 65–71.
- Bickham, J. W., 2011. The four cornerstones of Evolutionary Toxicology. *Ecotoxicology* 20 (3): 497–502.
- Bjerring, R., E. G. Bradshaw, S. L. Amsinck, L. S. Johansson, B. V. Odgaard, A. B. Nielsen & E. Jeppesen, 2008. Inferring recent changes in the ecological state of 21 Danish candidate reference lakes (EU Water Framework Directive) using palaeolimnology. *Journal of Applied Ecology* 45 (6): 1566–1575.
- Bohonak, A. J. & D. G. Jenkins, 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters* 6:

- 783–796.
- Boronat, L., M. R. Miracle & X. Armengol, 2001. Cladoceran assemblages in a mineralization gradient. *Hydrobiologia* 442: 75–88.
- Brakke, D. F., J. P. Baker, J. Böhmer, A. Hartmann, M. Havas, A. Jenkins, C. Kelly, S. J. Ormerod, T. Paces, R. Putz, B. O. Rosseland, D. W. Schindler & H. Segner, 1994. Group report: Physiological and ecological effects of acidification on aquatic biota. In C. E. W. Steinberg & R. F. Wright (Eds), *Acidification of Freshwater Ecosystems: Implications for the Future*. John Wiley & Sons Ltd.: 275–312.
- Brancelj, A., 1996. *Chydorus 'mutilus'* Kreis, 1921—a postephippial form of *Chydorus sphaericus* (O.F. Mueller, 1785). *Hydrobiologia* 323: 45–59.
- Brett, M. T., 1989. Zooplankton communities and acidification processes (a review). *Water, Air, & Soil Pollution* 44 (3): 387–414.
- Brown, D. J. A., 1983. Effect of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. *Bulletin of Environmental Contamination and Toxicology* 30 (1): 582–587.
- Burkert, U., G. Ginzel, H. D. Babenzien & R. Koschel, 2004. The hydrogeology of a catchment area and an artificially divided dystrophic lake—consequences for the limnology of Lake Fuchskuhle. *Biogeochemistry* 71 (2): 225–246.
- Cassens, I., P. Mardulyn & M. C. Milinkovitch, 2005. Evaluating intraspecific ‘network’ construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology* 54 (3): 363–372.
- Chengalath, R., 1987. The distribution of chydorid Cladocera in Canada. *Hydrobiologia* 145: 151–157.
- Christensen, M. R., M. D. Graham, R. D. Vinebrooke, D. L. Findlay, M. J. Paterson & M. A. Turner, 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. *Global Change Biology* 12 (12): 2316–2322.
- Clement, M., D. Posada & K. A. Crandall, 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.
- Colbourne, J. K., T. J. Crease, L. J. Weider, P. D. N. Hebert, F. Dufresne & A. Hobæk, 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). *Biological Journal of the Linnean Society* 65: 347–365.
- Collier, K. J., O. J. Ball, A. K. Graesser, M. R. Main & M. J. Winterbourn, 1990. Do organic and anthropogenic acidity have similar effects on aquatic fauna? *Oikos* 59 (1): 33–38.

- Colwell, R. K., 2006. EstimateS: Statistical estimation of species richness and shared species from samples. Version 8. Persistent URL: <purl.oclc.org/estimates>.
- Cousyn, C., L. De Meester, J. K. Colbourne, L. Brendonck, D. Verschuren & F. Volckaert, 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proceedings of National Academy of Sciences of the United States of America* 98 (11): 6256–6260.
- Cox, A. J. & P. D. N. Hebert, 2001. Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. *Molecular Ecology* 10: 371–386.
- Dangles, O., B. Malmqvist & H. Laudon, 2004. Naturally acid freshwater ecosystems are diverse and functional: evidence from boreal streams. *Oikos* 104 (1): 149–155.
- Davies, T. D. & K. J. Hall, 2007. Importance of calcium in modifying the acute toxicity of sodium sulphate to *Hyaella azteca* and *Daphnia magna*. *Environmental Toxicology and Chemistry* 26 (6): 1243–1247.
- De Eyto, E., 2001. *Chydorus sphaericus* as a biological indicator of water quality in lakes. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 27: 3358–3362.
- De Eyto, E. & K. Irvine, 2001. The response of three chydorid species to temperature, pH and food. *Hydrobiologia* 459: 165–172.
- De Eyto, E., K. Irvine, F. García-Criado, M. Gyllström, E. Jeppesen, R. Kornijow, M. R. Miracle, M. Nykänen, C. Bareiss, S. Cerbin, J. Salujoe, R. Franken, D. Stephens & B. Moss, 2003. The distribution of chydorids (Branchiopoda, Anomopoda) in European shallow lakes and its application to ecological quality monitoring. *Archiv für Hydrobiologie* 156 (2): 181–202.
- De Meester, L., A. Gómez, B. Okamura & K. Schwenk, 2002. The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica* 23: 121–135.
- Declerck, S., C. Cousyn & L. De Meester, 2001. Evidence for local adaptation in neighbouring *Daphnia* populations: a laboratory transplant experiment. *Freshwater Biology* 46 (2): 187–198.
- Dekker, T., G. D. Greve, T. L. Ter Laak, M. E. Boivin, B. Veuger, G. Gortzak, S. Damfries, S. M. G. Lücker, M. H. S. Kraak, W. Admiraal & H. G. van der Geest, 2006. Development and application of a sediment toxicity test using the benthic cladoceran *Chydorus sphaericus*. *Environmental Pollution* 140 (2): 231–238.

- Deneke, R., 2000. Review of rotifers and crustaceans in highly acidic environments of pH values > 3. *Hydrobiologia* 433: 167–172.
- Derry, A. M., S. E. Arnott, J. A. Sheard, P. D. N. Hebert & P. T. Boag, 2009. Ecological linkages between community and genetic diversity in zooplankton among boreal shield lakes. *Ecology* 90 (8): 2275–2286.
- DeSellas, A., A. Paterson, J. Sweetman & J. Smol, 2008. Cladocera assemblages from the surface sediments of south-central Ontario (Canada) lakes and their relationships to measured environmental variables. *Hydrobiologia* 600: 105–119.
- Di Fonzo, C. & J. M. Campbell, 1988. Spartial partitioning of microhabitats in littoral cladoceran communities. *Journal of Freshwater Ecology* 4 (3): 303–313.
- Dodson, S. I., W. R. Everhart, A. K. Jandl & S. J. Krauskopf, 2007. Effect of watershed land use and lake age on zooplankton species richness. *Hydrobiologia* 579 (1): 393–399.
- Duigan, C. A., 1992. The ecology and distribution of the littoral freshwater Chydoridae (Branchiopoda, Anomopoda) of Ireland, with taxonomic comments on some species. *Hydrobiologia* 241: 1–70.
- Duigan, C. A. & D. A. Murray, 1987. A contribution to the taxonomy of *C. sphaericus* sens. lat. (Cladocera, Chydoridae). *Hydrobiologia* 145: 113–124.
- Ebert, D., C. Haag, M. Kirkpatrick, M. Riek, J. W. Hottinger & V. I. Pajunen, 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295 (5554): 485–488.
- Elías-Gutiérrez, M., A. A. Kotov & T. Garfias-Espejo, 2006. Cladocera (Crustacea: Ctenopoda, Anomopoda) from southern Mexico, Belize and northern Guatemala, with some biogeographical notes. *Zootaxa* 1119: 1–27.
- Excoffier, L., P. E. Smouse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131 (2): 479–491.
- Excoffier, L., G. Laval & S. Schneider, 2005. Arlequin ver. 3.0: An integrated software package for population genetic data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.
- Finlay, B. J. & T. Fenchel, 2004. Cosmopolitan metapopulations of free-living microbial eukaryotes. *Protist* 155 (2): 237–244.
- Flößner, D., 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publishers, Leiden: 428 pp.

- Folmer, O., M. Black, W. Hoeh, R. Lutz & R. Vrijenhoek, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Forró, L., N. Korovchinsky, A. Kotov & A. Petrusek, 2008. Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiologia* 595 (1): 177–184.
- Frey, D. G., 1960. The ecological significance of cladoceran remains in lake sediments. *Ecology* 41: 684–699.
- Frey, D. G., 1980. On the plurality of *Chydorus sphaericus* (O.F. Müller) (Cladocera, Chydoridae), and designation of a neotype from Sjælsø, Denmark. *Hydrobiologia* 69 (1–2): 83–123.
- Frey, D. G., 1985. A new species of the *Chydorus sphaericus* group (Cladocera, Chydoridae) from Western Montana. *Internationale Revue der gesamten Hydrobiologie* 70 (1): 3–20.
- Frey, D. G., 1986. The non-cosmopolitanism of chydorid Cladocera: implications for biogeography and evolution. In K. L. Heck & R. H. Gore (Eds), *Crustacean Biogeography* 4., A. A. Balkema, Rotterdam: 237–256.
- Frey, D. G., 1995. Changing attitudes toward chydorid anomopods since 1769. *Hydrobiologia* 307: 43–55.
- Friese, K., M. Hupfer & M. Schultze, 1998. Chemical characteristics of water and sediment in acid mining lakes of the Lusatian lignite district. In W. Geller, H. Klapper & W. Salomons (Eds), *Acidic mining lakes*. Springer-Verlag, Berlin: 25–45.
- Friese, K., P. Herzsprung & B. Witter, 2002. Photochemical degradation of organic carbon in acidic mining lakes. *Acta hydrochimica et hydrobiologica* 30 (2–3): 141–148.
- Friese, K., P. Herzsprung & M. Schultze, 2013. Pit lakes from coal and lignite mining: Water, sediment and pore water. In W. Geller, M. Schulze, R. Kleinmann & C. Wolkersdorfer (Eds), *Acidic Pit Lakes—the Legacy of Coal and Metal Surface Mines*. Environmental Science Series, Springer-Verlag, Berlin: 42–57.
- Frost, T. M., S. R. Carpenter, A. R. Ives & T. K. Kratz, 1995. Species compensation and complementarity in ecosystem function. In G. C. Jones & J. H. Lawton (Eds), *Linking Species and Ecosystems*. Chapman and Hall, New York: 224–239.
- Frost, T. M., P. K. Montz, T. K. Kratz, T. Badillo, P. L. Brezonik, M. J. Gonzalez, R. G. Rada, C. J. Watras, K. E. Webster, J. G. Wiener, C. E. Williamson & D. P. Morris, 1999. Multiple stresses from a single agent:

- Diverse responses to the experimental acidification of Little Rock Lake, Wisconsin. *Limnology and Oceanography* 44 (3): 784–794.
- Fryer, G., 1980. Acidity and species diversity in freshwater crustacean faunas. *Freshwater Biology* 10: 41–45.
- Fryer, G., 1993. Variation in acid tolerance of certain freshwater crustaceans in different natural waters. *Hydrobiologia* 250: 119–125.
- Fryer, G. & D. G. Frey, 1981. Two-egged ephippia in the chydorid Cladocera. *Freshwater Biology* 11: 391–394.
- Geist, J., 2011. Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators* 11 (6): 1507–1516.
- Geller, W. & M. Schultze, 2009. Acidification. In G. E. Likens (Ed.), *Encyclopedia of Inland Waters* 3. Elsevier, Oxford: 1–12.
- Geller, W., H. Klapper & M. Schultze, 1998. Natural and anthropogenic sulfuric acidification of lakes. In W. Geller, H. Klapper & W. Salomons (Eds), *Acidic mining lakes*. Springer-Verlag, Berlin: 3–14.
- Goodfellow, W. L., L. W. Ausley, D. T. Burton, D. L. Denton, P. B. Dorn, D. R. Grothe, M. A. Heber, T. J. Norberg-King & J. H. Rodgers, 2000. Major ion toxicity in effluents: A review with permitting recommendations. *Environmental Toxicology and Chemistry* 19 (1): 175–182.
- Gotelli, N. J. & R. K. Colwell, 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters* 4: 379–391.
- Gómez, A., M. Serra, G. R. Carvalho & D. H. Lunt, 2002. Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution* 56 (7): 1431–1444.
- Greig, H. S., D. K. Niyogi, K. L. Hogsden, P. G. Jellyman & J. S. Harding, 2010. Heavy metals: confounding factors in the response of New Zealand freshwater fish assemblages to natural and anthropogenic acidity. *Science of the Total Environment* 408 (16): 3240–3250.
- Guindon, S. & O. Gascuel, 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
- Gunn, J. M. & W. Keller, 1990. Biological recovery of an acid lake following reductions in industrial emissions of sulphur. *Nature* 345: 431–433.
- Hall, T., 1999. Bioedit sequence alignment editor. Department of Microbiology, North Carolina State University.
- Hämmerling, R., J. Rücker, H. Behrendt & B. Nixdorf, 2006. Development of phosphorus input in Lake Scharmützelsee, Germany, and the changes

- in phosphorus balance. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 29: 1639–1641.
- Haney, R. A. & D. J. Taylor, 2003. Testing paleolimnological predictions with molecular data: the origins of Holarctic Eubosmina. *Journal of Evolutionary Biology* 16: 871–882.
- Hann, B. J., 1975. Taxonomy of Chydoridae in Ontario and genus *Chydorus* (worldwide). Unpublished Ms. Sc. Thesis, University of Waterloo, Ontario: 234 pp.
- Hann, B. J. & M. A. Turner, 2000. Littoral microcrustacea in lake 302S in the Experimental Lakes Area of Canada: acidification and recovery. *Freshwater Biology* 43 (1): 133–146.
- Havas, M. & T. C. Hutchinson, 1983. The smoking hills: natural acidification of an aquatic ecosystem. *Nature* 301: 23–27.
- Havas, M. & G. E. Likens, 1985. Changes in ^{22}Na influx and outflux in *Daphnia magna* (Straus) as a function of elevated Al concentrations in soft-water at low pH. *Proceedings of National Academy of Sciences of the United States of America* 82 (21): 7345–7349.
- Havas, M. & E. Advokaat, 1995. Can sodium regulation be used to predict the relative acid-sensitivity of various life-stages and different species of aquatic fauna? *Water, Air, & Soil Pollution* 85 (2): 865–870.
- Havas, M. & B. O. Rosseland, 1995. Response of zooplankton, benthos, and fish to acidification: An overview. *Water, Air, & Soil Pollution* 85 (1): 51–62.
- Havens, K. E., 1993. Acid and aluminum effects on the survival of littoral macroinvertebrates during acute bioassays. *Environmental Pollution* 80 (1): 95–100.
- Havens, K. E. & R. T. Heath, 1989. Acid and aluminum effects on freshwater zooplankton: An in situ mesocosm study. *Environmental Pollution* 62: 195–211.
- Havens, K. E. & R. E. Carlson, 1998. Functional complementarity in plankton communities along a gradient of acid stress. *Environmental Pollution* 101 (3): 427–436.
- Havens, K. E., N. D. Yan & W. Keller, 1993. Lake acidification: effects on crustacean zooplankton populations. *Environmental Science & Technology* 27: 1621–1624.
- Henrikson, L. & H. G. Oscarson, 1985. Waterbugs (Corixidae, Hemiptera-Heteroptera) in acidified lakes: Habitat selection and adaptations. *Ecological Bulletins* (37): 232–238.

- Hoffmann, A. A. & Y. Willi, 2008. Detecting genetic responses to environmental change. *Nature Reviews. Genetics* 9 (6): 421–432.
- Hofmann, W., 1987. Cladocera in space and time: Analysis of lake sediments. *Hydrobiologia* 145: 315–321.
- Hortal, J., P. A. V. Borges & C. Gaspar, 2006. Evaluating the performance of species richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology* 75 (1): 274–287.
- Huelsenbeck, J. P. & F. Ronquist, 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8): 754–755.
- Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood & M. Vellend, 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11 (6): 609–623.
- Hughes, J. M., P. B. Mather, A. L. Sheldon & F. W. Allendorf, 1999. Genetic structure of the stonefly, *Yoraperla brevis*, populations: the extent of gene flow among adjacent montane streams. *Freshwater Biology* 41: 63–72.
- Hurlbert, S. H., 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 52: 577–586.
- Jackson, J. K. & V. H. Resh, 1998. Morphologically cryptic species confound ecological studies of the caddisfly genus *Gumaga* (Trichoptera: Sericostomatidae) in Northern California. *Aquatic Insects* 20: 69–84.
- Jansen, M., L. De Meester, A. Cielen, C. C. Buser & R. Stoks, 2011. The interplay of past and current stress exposure on the water flea *Daphnia*. *Functional Ecology* 25 (5): 974–982.
- Jeppesen, E., P. Leavitt, L. De Meester & J. P. Jensen, 2001. Functional ecology and palaeolimnology: Using cladoceran remains to reconstruct anthropogenic impact. *Trends in Ecology and Evolution* 16: 191–198.
- Jeppesen, E., P. Nöges, T. A. Davidson, J. Haberman, T. Nöges, K. Blank, T. L. Lauridsen, M. Søndergaard, C. Sayer, R. Laugaste, L. S. Johansson, R. Bjerring & S. L. Amsinck, 2011. Zooplankton as indicators in lakes: a scientific-based plea for including zooplankton in the ecological quality assessment of lakes according to the European Water Framework Directive (WFD). *Hydrobiologia* 676 (1): 279–297.
- Jersabek, C. D., G. Weithoff & T. Weisse, 2011. *Cephalodella acidophila* n. sp. (Monogononta: Notommatidae), a new rotifer species from highly acidic mining lakes. *Zootaxa* 2939: 50–58.
- Jeziorski, A., N. D. Yan, A. M. Paterson, A. M. DeSellas, M. A. Turner, D. S. Jeffries, B. Keller, R. C. Weeber, D. K. McNicol, M. E. Palmer, K. McIver, K. Arseneau, B. K. Ginn, B. F. Cumming & J. P. Smol, 2008.

- The widespread threat of calcium decline in fresh waters. *Science* 322 (5906): 1374–1377.
- Kappes, H. & U. Sinsch, 2005. Tolerance of *Ceriodaphnia quadrangula* and *Diaphanosoma brachyurum* (Crustacea: Cladocera) to experimental soft water acidification. *Hydrobiologia* 534 (1): 109–115.
- Kasprzak, P., 1993. The use of an artificially divided bog lake in food-web studies. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 25: 652–656.
- Keller, W., 2009. Limnology in northeastern Ontario: from acidification to multiple stressors. *Canadian Journal of Fisheries and Aquatic Sciences* 66 (7): 1189–1198.
- Kennedy, A., D. Cherry & C. Zipper, 2005. Evaluation of ionic contribution to the toxicity of a coal-mine effluent using *Ceriodaphnia dubia*. *Archives of Environmental Contamination and Toxicology* 49 (2): 155–162.
- Knowlton, N. & L. A. Weigt, 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London B: Biological Sciences* 265: 2257–2263.
- Korosi, J. & J. Smol, 2011. Distribution of cladoceran assemblages across environmental gradients in Nova Scotia (Canada) lakes. *Hydrobiologia* 663 (1): 83–99.
- Korosi, J. & J. Smol, 2012. A comparison of present-day and pre-industrial cladoceran assemblages from softwater Nova Scotia (Canada) lakes with different regional acidification histories. *Journal of Paleolimnology* 47 (1): 43–54.
- Korovchinsky, N. M., 1992. Sididae and Holopediidae. *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*. SBF Academic Publishing, The Hague: 82 pp.
- Korovchinsky, N. M., 1996. How many species of Cladocera are there? *Hydrobiologia* 321: 191–204.
- Korovchinsky, N. M., 2000. Trends in Cladocera and Copepoda taxonomy. *Arthropoda Selecta* 9 (3): 153–158.
- Korovchinsky, N. M., 2006. The Cladocera (Crustacea: Branchiopoda) as a relict group. *Zoological Journal of the Linnean Society* 147: 109–124.
- Kosakovsky Pond, S. L., S. D. W. Frost & S. V. Muse, 2005. HyPhy: hypothesis testing using phylogenies. *Bioinformatics* 21 (5): 676–679.
- Kosakovsky Pond, S. L., D. Posada, M. B. Gravenor, C. H. Woelk & S. D. W. Frost, 2006 a. GARD: a genetic algorithm for recombination detection. *Bioinformatics* 22 (24): 3096–3098.

- Kosakovsky Pond, S. L., D. Posada, M. B. Gravenor, C. H. Woelk & S. D. W. Frost, 2006 b. Automated phylogenetic detection of recombination using a genetic algorithm. *Molecular Biology and Evolution* 23 (10): 1891–1901.
- Koschel, R., 1995. Manipulation of whole-lake ecosystems and long-term limnological observations in the Brandenburg-Mecklenburg Lake District, Germany. *Internationale Revue der gesamten Hydrobiologie* 80 (4): 507–518.
- Koste, W., 1978. Rotatoria: Die Rädertiere Mitteleuropas. Gebrüder Borntraeger, Berlin: 673 pp.
- Krause-Dellin, D. & C. Steinberg, 1986. Cladoceran remains as indicators of lake acidification. *Hydrobiologia* 143: 129–134.
- Kullberg, A., 1992. Benthic macroinvertebrate community structure in 20 streams of varying pH and humic content. *Environmental Pollution* 78 (1–3): 103–106.
- Kullberg, A., K. H. Bishop, A. Hargeby, M. Jansson & R. C. Petersen, 1993. The ecological significance of dissolved organic carbon in acidified waters. *Ambio* 22: 331–337.
- Kurek, J., R. C. Weeber & J. P. Smol, 2011. Environment trumps predation and spatial factors in structuring cladoceran communities from Boreal Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 68 (8): 1408–1419.
- Lackey, J. B., 1939. Aquatic life on waters polluted by acid mine waste. *Public Health Reports* 54: 740–746.
- Lefébure, T., C. J. Douady, M. Gouy, P. Trontelj, J. Briolay & J. Gibert, 2006. Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. *Molecular Ecology* 15: 1797–1806.
- Lemke, A. M. & A. C. Benke, 2009. Spatial and temporal patterns of microcrustacean assemblage structure and secondary production in a wetland ecosystem. *Freshwater Biology* 54 (7): 1406–1426.
- Lessmann, D., R. Deneke, R. Ender, M. Hemm, M. Kapfer, H. Krumbeck, K. Wollmann & B. Nixdorf, 1999. Lake Plessa 107 (Lusatia, Germany)—an extremely acidic shallow mining lake. *Hydrobiologia* 408/409: 293–299.
- Lieder, U., 1996. Crustacea: Cladocera: Bosminidae. Die Süßwasserfauna von Mitteleuropa. Gustav Fischer Verlag, Stuttgart: 80 pp.
- Locke, A. & W. G. Sprules, 2000. Effects of acidic pH and phytoplankton on survival and condition of *Bosmina longirostris* and *Daphnia pulex*.

- Hydrobiologia 437: 187–196.
- Lopes, I., F. Gonçalves, A. M. Soares & R. Ribeiro, 1999. Discriminating the ecotoxicity due to metals and to low pH in acid mine drainage. *Ecotoxicology and Environmental Safety* 44 (2): 207–214.
- Lopes, I., N. Martins, D. J. Baird & R. Ribeiro, 2009. Genetic erosion and population resilience in *Daphnia longispina* O.F. Müller under simulated predation and metal pressures. *Environmental Toxicology and Chemistry* 28 (9): 1912–1919.
- Louette, G., L. D. Meester & S. Declerck, 2008. Assembly of zooplankton communities in newly created ponds. *Freshwater Biology* 53 (11): 2309–2320.
- Martins, N., C. Bollinger, R. M. Harper & R. Ribeiro, 2009. Effects of acid mine drainage on the genetic diversity and structure of a natural population of *Daphnia longispina*. *Aquatic Toxicology* 92 (2): 104–112.
- Matveev, V., 1986. Long-term changes in the community of planktonic crustaceans in Lake Glubokoe in relation to predation and competition. *Hydrobiologia* 141 (1): 33–43.
- McConathy, J. R. & J. B. Stahl, 1982. Rotifera in the plankton and among filamentous algal clumps in 16 acid strip-mine lakes. *Transactions of Illinois Academy of Sciences* 75 (1 and 2): 85–90.
- Muysen, B. T. A., B. T. A. Bossuyt & C. R. Janssen, 2005. Inter- and intra-species variation in acute zinc tolerance of field-collected cladoceran populations. *Chemosphere* 61 (8): 1159–1167.
- Nevalainen, L., 2010. Evaluation of microcrustacean (Cladocera, Chydoridae) biodiversity based on sweep net and surface sediment samples. *Ecoscience* 17 (4): 356–364.
- Nierzwicki-Bauer, S. A., C. W. Boylen, L. W. Eichler, J. P. Harrison, J. W. Sutherland, W. Shaw, R. A. Daniels, D. F. Charles, F. W. Acker, T. J. Sullivan, B. Momen & P. Bukaveckas, 2010. Acidification in the Adirondacks: Defining the biota in trophic levels of 30 chemically diverse acid-impacted lakes. *Environmental Science & Technology* 44 (15): 5721–5727.
- Nilssen, J. P., 1980. Acidification of a small watershed in southern Norway and some characteristics of acidic aquatic environments. *Internationale Revue der gesamten Hydrobiologie* 65: 177–207.
- Nilssen, J. P. & S. Sandøy, 1990. Recent lake acidification and cladoceran dynamics: surface sediment and core analyses from lakes in Norway, Scotland and Sweden. *Philosophical Transactions of the Royal Society B: Biological Sciences* 327: 299–309.

- Nilssen, J. P. & S. B. Wærvågen, 2003. Ecological distribution of pelagic copepods and species relationship to acidification, liming and natural recovery in a boreal area. *Journal of Limnology* 62 (1): 97–114.
- Nixdorf, B., K. Wollmann & R. Deneke, 1998. Ecological potentials for planktonic development and food web interactions in extremely acidic mining lakes in Lusatia. In W. Geller, H. Klapper & W. Salomons (Eds), *Acidic mining lakes*. Springer-Verlag, Berlin: 147–167.
- Nixdorf, B., D. Lessmann & C. E. W. Steinberg, 2003. The importance of chemical buffering for pelagic and benthic colonization in acidic waters. *Water, Air, & Soil Pollution* 3: 27–46.
- Nixdorf, B., D. Lessmann & R. Deneke, 2005. Mining lakes in a disturbed landscape: Application of the EC Water Framework Directive and future management strategies. *Ecological Engineering* 24: 67–73.
- Nixdorf, B., M. Hemm, A. Schlundt, M. Kapfer & H. Krumbek, 2001. Braunkohlentagebauseen in Deutschland—Gegenwärtiger Kenntnisstand über wasserwirtschaftliche Belange von Braunkohlentagebau-restlöchern. Umweltbundesamt, Berlin: 519 pp.
- Omilian, A. R. & D. J. Taylor, 2001. Rate acceleration and long-branch attraction in a conserved gene of cryptic daphniid (Crustacea) species. *Molecular Biology and Evolution* 18 (12): 2201–2212.
- Orlova-Bienkowskaja, M. Y., 2001. Cladocera: Anomopoda. Daphniidae: Genus *Simocephalus*. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. Backhuys Publishers, Leiden: 130 pp.
- Ormerod, S. J., M. Dobson, A. G. Hildrew & C. R. Townsend, 2010. Multiple stressors in freshwater ecosystems. *Freshwater Biology* 55: 1–4.
- Petrin, Z., H. Laudon & B. Malmqvist, 2007. Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology* 52 (11): 2172–2183.
- Petrin, Z., G. Englund & B. Malmqvist, 2008. Contrasting effects of anthropogenic and natural acidity in streams: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences* 275 (1639): 1143–1148.
- Pietsch, W., 1998. Colonization and development of vegetation in mining lakes of the Lusatian lignite area depending on water genesis. In W. Geller, H. Klapper & W. Salomons (Eds), *Acidic mining lakes*. Springer-Verlag, Berlin: 169–193.
- Pither, J. & L. W. Aarssen, 2005. The evolutionary species pool hypothesis and patterns of freshwater diatom diversity along a pH gradient. *Journal of Biogeography* 32 (3): 503–513.

- Posada, D. & K. A. Crandall, 2001 a. Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proceedings of National Academy of Sciences of the United States of America* 98 (24): 13757–13762.
- Posada, D. & K. A. Crandall, 2001 b. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* 16 (1): 37–45.
- Posada, D., 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25 (7): 1253–1256.
- Potts, W. T. W. & G. Fryer, 1979. The effects of pH and salt content on sodium balance in *Daphnia magna* and *Acantholeberis curvirostris* (Crustacea: Cladocera). *Journal of Comparative Physiology, B* 129: 289–294.
- Purvis, A. & A. Hector, 2000. Getting the measure of biodiversity. *Nature* 405 (6783): 212–219.
- Rahel, F. J., 1984. Factors structuring fish assemblages along a bog lake successional gradient. *Ecology* 65 (4): 1276–1289.
- Raymond, M. & F. Rousset, 1995. An exact test for population differentiation. *Evolution* 49 (6): 1280–1283.
- Roff, J. C. & R. E. Kwiatkowski, 1977. Zooplankton and zoobenthos communities of selected northern Ontario lakes of different acidities. *Canadian Journal of Zoology* 55: 899–911.
- Rothschild, L. J. & R. L. Mancinelli, 2001. Life in extreme environments. *Nature* 409 (6823): 1092–1101.
- Rousselet, C. F. R., 1907. *On Brachionus sericus*, n. sp., a new variety of *Brachionus quadratus*, and remarks on *Brachionus rubens*, of Ehrenberg. *Journal of the Quekett Microscopical Club* 10 (61): 147–154.
- Rybak, M., J. I. Rybak & K. Tarwid, 1964. Differences in Crustacea plankton based on the morphological character of the littoral of the lakes. *Ekologia Polska* 12 (11): 159–171.
- Sacherová, V. & P. D. N. Hebert, 2003. The evolutionary history of the Chydoridae (Crustacea: Cladocera). *Biological Journal of the Linnean Society* 79: 629–643.
- Sandøy, S. & J. P. Nilssen, 1986. A geographical survey of littoral Crustacea in Norway and their use in paleolimnology. *Hydrobiologia* 143: 277–286.
- Sarmaja-Korjonen, K., 2004. Chydorid ephippia as indicators of past environmental changes—a new method. *Hydrobiologia* 526: 129–136.
- Sawyer, S. A., 1989. Statistical tests for detecting conversion. *Molecular Biology and Evolution* 6: 526–538.

- Schartau, A. K. L., B. Walseng, T. Nøst & G. Halvorsen, 2000. Freshwater crustaceans as monitors of long-range transported air pollutants. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 27: 2484–2487.
- Schindler, D. W., 1988. Effects of acid rain on freshwater ecosystems. *Science* 239 (4836): 149–157.
- Schindler, D. W., K. H. Mills, D. F. Malley, D. L. Findlay, J. A. Shearer, I. J. Davies, M. A. Turner, G. A. Linsey & D. R. Cruikshank, 1985. Long-term ecosystem stress: The effects of years of experimental acidification on a small lake. *Science* 228 (4706): 1395–1401.
- Schlötterer, C., M.-T. Hauser, A. von Haeseler & D. Tautz, 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophilla*. *Molecular Biology and Evolution* 11 (3): 513–522.
- Schubart, C. D., R. Diesel & S. B. Hedges, 1998. Rapid evolution to terrestrial life in Jamaican crabs. *Nature* 393: 363–365.
- Schwenk, K. & P. Spaak, 1995. Evolutionary and ecological consequences of interspecific hybridization in cladocerans. *Experientia* 51: 465–481.
- Schwenk, K., D. Posada & P. D. N. Hebert, 2000. Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. *Proceedings of the Royal Society of London B: Biological Sciences* 267: 1833–1842.
- Shan, R. K. & D. G. Frey, 1983. *Pleuroxus denticulatus* and *P. procurvus* (Crustacea: Chydoridae) in North America: distribution, experimental hybridization and the possibility of natural hybridization. *Canadian Journal of Zoology* 61: 1605–1617.
- Simon, J., 1997. *Resampling: The new statistics*, 2nd ed. Textbook to the Resampling Stats software.
- Simon, T. P., 2002. *Biological response signatures: Patterns in biological indicators for assessing freshwater aquatic assemblages*. CRC Press, Boca Raton, FL: 600 pp.
- Sinev, A. Y., 2009. Discrimination between two sibling species of *Acroperus* (Baird, 1843) from the Palearctic (Cladocera: Anomopoda: Chydoridae). *Zootaxa* 2176: 1–21.
- Sinev, A. Y. & M. M. Atroschenko, 2011. Revision of the genus *Alonopsis* Sars, 1862 and its position within Aloninae (Cladocera: Anomopoda: Chydoridae). *Zootaxa* 2800: 1–17.
- Smirnov, N. N., 1971. [Chydoridae of the World Fauna] in Russian. *Fauna SSSR. Rakoobraznye*. Nauka, Leningrad: 531 pp.
- Smirnov, N. N., 1992. *The Macrothricidae of the world*. Guides to the

- Identification of the Microinvertebrates of the Continental Waters of the World. SPB Academic Publishing, The Hague: 143 pp.
- Smirnov, N. N., 1996. The Chydorinae and Sayciinae (Chydoridae) of the world. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. SPB Academic Publishing, Amsterdam.
- Soucek, D. J., D. S. Cherry & C. E. Zipper, 2001. Aluminum-dominated acute toxicity to the cladoceran *Ceriodaphnia dubia* in neutral waters downstream of an acid mine drainage discharge. *Canadian Journal of Fisheries and Aquatic Sciences* 58 (12): 2396–2404.
- Sprules, W. G., 1975. Midsummer crustacean zooplankton communities in acid-stressed lakes. *Journal of the Fisheries Research Board of Canada* 32 (3): 389–395.
- Staton, J. L., N. V. Schizas, G. T. Chandler, B. C. Coull & J. M. Quattro, 2001. Ecotoxicology and population genetics: The emergence of phylogeographic and evolutionary ecotoxicology. *Ecotoxicology* 10 (4): 217–222.
- Steinberg, C. E. W., 2003. Ecology of humic substances in freshwaters. Springer-Verlag, Berlin: 440 pp.
- Steinberg, C. E. W., H. Schäfer, J. Tittel & W. Beisker, 1998. Phytoplankton composition and biomass spectra created by flow cytometry and zooplankton composition in mining lakes of different states of acidification. In W. Geller, H. Klapper & W. Salomons (Eds), *Acidic mining lakes*. Springer-Verlag, Berlin: 127–145.
- Steinberg, C. E. W., A. Paul, S. Pflugmacher, T. Meinelt, R. Klöcking & C. Wiegand, 2003. Pure humic substances have the potential to act as xenobiotic chemicals—a review. *Fresenius Environmental Bulletin* 12: 391–401.
- Steinberg, C. E. W., S. Kamara, V. Y. Prokhotskaya, L. Manusadzianas, T. A. Karasyova, M. A. Timofeyev, Z. Jie, A. Paul, T. Meinelt, V. F. Farjalla, A. Y. O. Matsuo, B. K. Burnison & R. Menzel, 2006. Dissolved humic substances—ecological driving forces from the individual to the ecosystem level? *Freshwater Biology* 51 (7): 1189–1210.
- Stendera, S. & R. K. Johnson, 2008. Tracking recovery trends of boreal lakes: use of multiple indicators and habitats. *Journal of the North American Benthological Society* 27 (3): 529–540.
- Swofford, D. L., 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland, Massachusetts, Sinauer Associates.
- Tait, R. D., R. J. Shiel & W. Koste, 1984. Structure and dynamics of zoo-

- plankton communities, Alligator Rivers Region, N.T., Australia. *Hydrobiologia* 113 (1): 1–131.
- Tamura, K., J. Dudley, M. Nei & S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24 (8): 1596–1599.
- Taylor, D. J., P. D. N. Hebert & J. K. Colbourne, 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. *Molecular Phylogenetics and Evolution* 5 (3): 495–510.
- Taylor, D. J., T. L. Finston & P. D. N. Hebert, 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution* 52 (6): 1648–1670.
- Taylor, D. J., C. R. Ishikane & R. A. Haney, 2002. The systematics of Holarctic bosminids and a revision that reconciles molecular and morphological evolution. *Limnology and Oceanography* 47 (5): 1486–1495.
- ter Braak, C. J. F. & I. C. Prentice, 1988. A theory of gradient analysis. *Advances in Ecological Research* 18: 271–317.
- ter Braak, C. J. F. & P. Šmilauer, 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Ithaca NY, USA, Microcomputer Power.
- Thienemann, A., 1931. Tropische Seen und Seetypenlehre. *Archiv für Hydrobiologie Suppl.* 9: 205–231.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins, 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25 (24): 4876–4882.
- Tremel, B., S. E. Frey, N. D. Jan, K. M. Somers & T. W. Pawson, 2000. Habitat specificity of littoral Chydoridae (Crustacea, Branchiopoda, Anomopoda) in Plastic Lake, Ontario, Canada. *Hydrobiologia* 432: 195–205.
- Uéno, M., 1934. Acid water lakes in North Shinano. *Archiv für Hydrobiologie* 27: 571–584.
- Uéno, M., 1958. The disharmonious lakes of Japan. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 13: 217–226.
- Uimonen-Simola, P. & K. Tolonen, 1987. Effects of recent acidification on Cladocera in small clear-water lakes studied by means of sedimentary remains. *Hydrobiologia* 145: 343–351.

- Vallin, S., 1953. Zwei azidotrophe Seen im Küstengebiet von Nordschweden. Reports of the Institute of Freshwater Research, Drottningholm 34: 167–189.
- Valois, A. E., W. B. Keller & C. W. Ramcharan, 2011. Recovery in a multiple stressor environment: using the reference condition approach to examine zooplankton community change along opposing gradients. *Journal of Plankton Research* 33 (9): 1417–1429.
- Van Damme, K. & H. J. Dumont, 2006. Sex in a cyclical parthenogen: mating behaviour of *Chydorus sphaericus* (Crustacea; Branchiopoda; Anomopoda). *Freshwater Biology* 51 (12): 2334–2346.
- van de Weyer, K., J. Neumann, W. Pietsch, J. Pätzolt & P. Tigges, 2009. Die Makrophyten des Senftenberger Sees. *Naturschutz und Landschaftspflege in Brandenburg* 18 (3): 88–95.
- van Straalen, N. M. & M. J. T. N. Timmermans, 2002. Genetic variation in toxicant-stressed populations: An evaluation of the ‘genetic erosion’ hypothesis. *Human and Ecological Risk Assessment* 8 (5): 983–1002.
- Vellend, M. & M. A. Geber, 2005. Connections between species diversity and genetic diversity. *Ecology Letters* 8 (7): 767–781.
- Walseng, B. & A. K. L. Schartau, 2001. Crustacean communities in Canada and Norway: Comparison of species along a pH gradient. *Water, Air, & Soil Pollution* 130 (1–4): 1319–1324.
- Walseng, B., G. Halvorsen & S. E. Storeid, 2001. Littoral microcrustaceans (Cladocera and Copepoda) as indices of recovery of a limed water system. *Hydrobiologia* 450 (1–3): 159–172.
- Walseng, B., N. D. Yan & A. K. Schartau, 2003. Littoral microcrustacean (Cladocera and Copepoda) indicators of acidification in Canadian Shield lakes. *Ambio* 32 (3): 208–213.
- Walseng, B., D. O. Hessen, G. Halvorsen & A. K. Schartau, 2006. Major contribution from littoral crustaceans to zooplankton species richness in lakes. *Limnology and Oceanography* 51 (6): 2600–2606.
- Walseng, B., N. D. Yan, T. W. Pawson & O. Skarpaas, 2008. Acidity versus habitat structure as regulators of littoral microcrustacean assemblages. *Freshwater Biology* 53 (2): 290–303.
- Weatherley, N. S., G. P. Rutt, S. P. Thomas & S. J. Ormerod, 1991. Liming acid streams: Aluminium toxicity to fish in mixing zones. *Water, Air, & Soil Pollution* 55 (3): 345–353.
- Weider, L. J., 1992. Disturbance, competition and the maintenance of clonal diversity in *Daphnia pulex*. *Journal of Evolutionary Biology* 5 (3): 505–522.

- Weithoff, G., 2004. Vertical niche separation of two consumers (Rotatoria) in an extreme habitat. *Oecologia* 139 (4): 594–603.
- Weithoff, G., 2005. On the ecology of the rotifer *Cephalodella hoodi* from an extremely acidic lake. *Freshwater Biology* 50 (9): 1464–1473.
- Weithoff, G., E. Spijkerman, N. Kamjunke & J. Tittel, 2013. The biology and ecosystem of acidic pit lakes: Trophic interactions and energy flow. In W. Geller, M. Schulze, R. Kleinmann & C. Wolkersdorfer (Eds), *Acidic Pit Lakes—the Legacy of Coal and Metal Surface Mines*. Environmental Science Series, Springer-Verlag, Berlin: 135–149.
- Whipple, A. V. & W. A. Dunson, 1993. Amelioration of the toxicity of H⁺ to larval stoneflies by metals found in coal mine effluent. *Archives of Environmental Contamination and Toxicology* 24 (2): 194–200.
- Willi, Y., J. Van Buskirk & A. A. Hoffmann, 2006. Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* 37 (1): 433–458.
- Williamson, C. E., D. P. Morris, M. L. Pace & O. G. Olson, 1999. Dissolved organic carbon and nutrients as regulators of lake ecosystems: Resurrection of a more integrated paradigm. *Limnology and Oceanography* 44 (3): 795–803.
- Wishart, M. J. & J. M. Hughes, 2003. Genetic population structure of the net-winged midge, *Elporia barnardi* (Diptera: Blephariceridae) in streams of the south-western Cape, South Africa: implications for dispersal. *Freshwater Biology* 48: 28–38.
- Wöflf, S., B. Zippel & G. Packroff, 1998. Planktongesellschaften der mitteleuropäischen Tagebaurestseen. Deutsche Gesellschaft für Limnologie. Erweiterte Zusammenfassung der Jahrestagung 1997 in Frankfurt am Main, p. 376–380.
- Wollmann, K. & R. Deneke, 2004. Nahrungsnetze in sauren Tagebauseen: Die Rolle der Konsumenten und die Entwicklung der Biodiversität im Zuge der Neutralisierung. In B. Nixdorf & R. Deneke (Hrsg.), *Grundlagen und Maßnahmen zur biogenen Alkalinisierung von sauren Tagebauseen*. Weißensee Verlag, Berlin: 199–218.
- Wollmann, K., R. Deneke, B. Nixdorf & G. Packroff, 2000. Dynamics of planktonic food webs in three mining lakes across a pH gradient (pH 2–4). *Hydrobiologia* 433: 3–14.
- Woolley, S. M., D. Posada & K. A. Crandall, 2008. A comparison of phylogenetic network methods using computer simulation. *PLoS ONE* 3 (4): e1913.
- Yan, N. D. & R. Strus, 1980. Crustacean zooplankton communities of acidic,

- metal-contaminated lakes near Sudbury, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 2282–2293.
- Yan, N. D., W. Keller, K. M. Somers, T. W. Pawson & R. E. Girard, 1996. Recovery of crustacean zooplankton communities from acid and metal contamination: Comparing manipulated and reference lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 53 (6): 1301–1327.
- Yan, N. D., K. M. Somers, R. E. Girard, A. M. Paterson, W. Keller, C. W. Ramcharan, J. A. Rusak, R. Ingram, G. E. Morgan & J. M. Gunn, 2008. Long-term trends in zooplankton of Dorset, Ontario, lakes: the probable interactive effects of changes in pH, total phosphorus, dissolved organic carbon, and predators. *Canadian Journal of Fisheries and Aquatic Sciences* 65 (5): 862–877.
- Yim, J. H., K. W. Kim & S. D. Kim, 2006. Effect of hardness on acute toxicity of metal mixtures using *Daphnia magna*: Prediction of acid mine drainage toxicity. *Journal of Hazardous Materials* 138 (1): 16–21.

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