

Sedimentary chydorid (Cladocera) ephippia in relation to lake ecological quality in the Austrian Alps

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ABSTRACT

Research on chydorid (Crustacea: Cladocera: Chydoridae) reproduction is scarce and very little is known about the dynamics of parthenogenetic (asexual) and gamogenetic (sexual) reproduction modes in these cyclical parthenogens. The paleolimnological approach has a lot to offer to this research since chydorid carapaces (indicative of asexual reproduction) and ephippia (indicative of sexual reproduction) are identifiable in lake sediments. In the current study, we examined historical changes in chydorid reproduction patterns in an Austrian Alpine lake with the objective to explore reproductive shifts under limnological variability and with the hypothesis that sexual reproduction intensifies under unfavourable environmental conditions. For this, we utilised subfossil remains from a sediment sequence covering the past ~300 years in a high Alpine lake, lake Oberer Landschützsee. The results suggested that in-lake processes likely changed as a response to post-Little Ice Age (LIA) climate warming. Indeed, the benthic quality – indicated by the chironomid-based benthic quality index (BQI) – deteriorated, and the productivity – indicated by organic matter in the sediment – increased. The chydorid community exhibited dramatic shifts in the post-LIA section of the core, around 1850-1950 AD, when the two dominant species *Alona (A.) affinis* and *A. quadrangularis* were outcompeted by *Acroporus harpae*, *Alonella excisa*, and *Chydorus (C.) sphaericus*-type and their reproduction gradually shifted towards the dominance of sexual reproduction. The community shift and prevalence of sexual reproduction coincided with the period of unfavourable limnological conditions, evidenced by low BQI values and higher organic content. Therefore, we suggest that the intensified sexual reproduction was a local response to deteriorated benthic conditions and a vigorous adaptive mechanism to ensure successful diapause and genetic variability under environmental change. The results give reason to suggest that fossil chydorid assemblages and ephippia indicate the ecological quality of lake ecosystems and, thus, they can be used as additional proxies in future assessments of the lake ecological status.

Key words: Alpine lakes, benthic quality, Chydoridae, diapause, environmental change, paleolimnology.

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INTRODUCTION

The pioneering work of David G. Frey on cladoceran sedimentary remains, particularly on chydorids (Chydoridae), laid the basis for integrating the paleoecological approach with neoecological research (Frey, 1960). Since then, subfossil remains of chydorids in surface sediments and in down-core sediment sequences have proven to be applicable for increasing knowledge on cladoceran taxa with respect to each other and their living environment. The paleoecological approach has been utilised in investigating chydorid ecology, for example distribution (Lotter *et al.*, 1997, 1998; Bjerring *et al.*, 2009; Brancelj *et al.*, 2009), habitat affinities (Binford, 1986; Kattel *et al.*, 2007; Nevalainen 2011, 2012), taxonomy (Frey, 1959, 1962; Sarmaja-Korjonen *et al.*, 2000; Nevalainen *et al.*, 2012b), and reproductive modes (Sarmaja-Korjonen, 2003; Kultti *et al.*, 2011; Nevalainen *et al.*, 2011b, 2011c).

Although the paleoecological approach on cladocerans put out findings often useful also for the neoecological research and especially for the environmental change

ecology (Manca *et al.*, 2007; Jeppesen *et al.*, 2001, 2011), a vast unexploited gap between these two research traditions still exists. Unfortunately, the paleoecological approach is not very often used to test specific ecological theories or hypotheses in aquatic ecosystems (Smol, 1991). Rather, the emphasis has been given to the use of aquatic organisms as proxies for different environmental factors. There are some advantages of using the paleoecological approach in the research of cladoceran ecology, especially with chydorids. Firstly, the sampling and sample analysis is relatively easy and effective since subfossil assemblages in single sediment samples give indications of the lake-wide community (Frey, 1960; Rautio, 2007; Nevalainen, 2010). Secondly, the down-core sequences provide a true long-term temporal perspective on the community and population dynamics that cannot be achieved even through an exhaustive neoecological monitoring (Sayer *et al.*, 2010).

Research on chydorid reproductive ecology is scarce and, thus, very little is known about the long-term dynam-

ics between parthenogenetic and gamogenetic reproduction modes in these cyclical parthenogens. In chydorids, gamogenesis – leading to diapausal resting eggs – plays an important role for the survival through periods of environmental stress, dispersal, and genetic variance within populations (Frey, 1982). In general, it is hypothesised that climatic cues primarily induce sexual reproduction in chydorids (Shan and Frey, 1968; Shan, 1974; Kubersky, 1977). However, it has been also suggested that non-climatic environmental signals, such as toxic stress (Kiser *et al.*, 1963), high predation pressure (Nevalainen, 2008), or oxygen availability (Nevalainen and Sarmaja-Korjonen, 2008) may interfere with the induction and intensity of sexual reproduction. As the intensity of gamogenesis determines the number of dormant resting eggs, the patterns in gamogenetic intensity are important for the success of diapause and survival of chydorid populations. As stated above, the paleoecological approach is useful for its long-term temporal views (Sayer *et al.*, 2010) and chydorid parthenogenetic and gamogenetic remains preserve identifiable in lake sediments. Given this, the sedimentary archives of the remains of these two reproductive modes can be employed for detecting long-term dynamics in gamogenetic intensity (Sarmaja-Korjonen, 2003; Nevalainen *et al.*, 2011b, 2011c).

In the current study, we aim to use sedimentary archives of benthic chydorids to infer past intensity in their sexual reproduction in an Alpine lake and compare these results to the lake ecological quality inferred from fossil Chironomidae assemblages. We hypothesise that under a period of unfavourable environmental conditions (*i.e.* low ecological quality) chydorids reproduce with high sexual intensity to produce high amount of dormant resting eggs for survival (*i.e.* diapause and genetic diversity). With this study, we also aim to provide evidence for the usability of microbenthic communities and their resting eggs in assessments of lake ecological quality.

METHODS

Lake Oberer Landschitzsee (area 8.8 ha, max. depth 13.6 m) is located at the southern slope of the mountain range Niedere Tauern in the Eastern Alps of Austria (47°14'52"N, 13°51'40"E) (Fig. 1), at an altitude of 2076 m asl, slightly above the present tree line of *Pinus cembra*. It is the topmost basin of a chain of three lakes (Oberer, Mittlerer, and Unterer Landschitzsee). The basins are on crystalline bedrock with mica-schists and metamorphic carbonates. The basic limnological variables, measured with a multiparameter sonde 6600V2 (YSI inc., Yellow Springs, OH, USA) in summer 2010, indicated that the lake is currently oligotrophic and has good oxygen conditions during the summer. The limnological parameters as mean values from the entire water column (and hypolimnion) were as follows: dissolved oxygen 8.5 mg

L⁻¹ (9.1), pH 7.4 units (6.8), conductivity 12.8 μS cm⁻¹ (13.0), Chlorophyll a 1.5 mg L⁻¹ (3.1), and total dissolved solids 0.008 g L⁻¹ (0.009).

A short sediment core, consisting of fine detritus gyttja, was collected from a small boat with a Kajak gravity corer in July 2010 (Fig. 1). The water depth at the sampling point was 10.5 m. The retrieved 16 cm sediment core was sliced into 1-cm thick intervals in the field and the subsamples were stored in plastic bags at +4°C in a cold room for later preparation.

The sediment core was dated using the radioactive isotope of lead ²¹⁰Pb radiometric dating method. The dating was carried out in the Laboratory of Quaternary Geochronology at the Institute of Geological Sciences, Polish Academy of Sciences in Warsaw (Poland), where the time-depth model was also constructed. The ²¹⁰Pb activity was determined indirectly via alpha-spectrometry measuring the isotope of polonium ²¹⁰Po ($\alpha=5.31$ MeV, T_{1/2}=138 d) activity. As an internal yield tracer, the known amount of ²⁰⁸Po was added to the weighted sample. Polonium was separated from the sample using strong hydrochloric and nitric acid and was deposited on silver disks

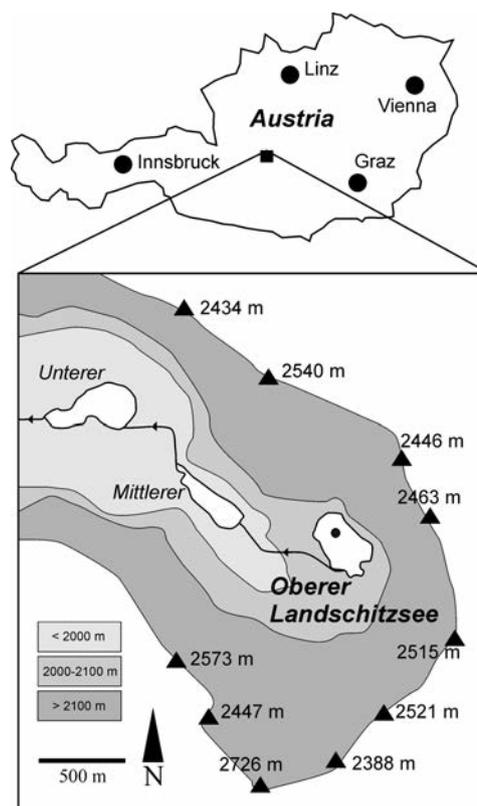


Fig. 1. Location and geographical settings of lake Oberer Landschitzsee (47°14'52"N, 13°51'40"E) in mountain range Niedere Tauern, Austria.

(Flynn, 1968). The activity of ^{210}Po and ^{208}Po was measured using the OCTETE PC alpha spectrometer (ORTEC, Oak Ridge, TN, USA). To estimate the age of sediment subsamples (Fig. 2), the constant rate of unsupported ^{210}Pb supply model (CRS) was used (Appleby, 2001). The activity of unsupported (allochthonous) ^{210}Pb was calculated by subtracting the supported (autigenic) ^{210}Pb activity. Supported ^{210}Pb was determined by measuring old sediments containing no allochthonous ^{210}Pb and assuming the constant activity of authigenic ^{210}Pb along the sediment column. The age-depth function was calculated using a randomisation method and the curve was fitted using the LOESS procedure. No terrestrial macrofossils were found from the lower sediment sequence and radiocarbon analytics from bulk sediment samples were deemed inappropriate to date the lower sediment samples because of carbonate rocks in the catchment. Thus, age estimation of the lower parts of the sediment core was obtained through extrapolation (von Gunten *et al.*, 2008).

The organic content and dry matter (DM) of the sediment was measured as loss-on-ignition (LOI) for environmental indications (*i.e.* productivity). Here, wet sediment samples (10–12 g) were first dried at 105°C for 12 h and then ignited in an oven at 550°C for 2 h (Dean, 1974). The organic content is expressed as percentages of DM (%DM).

The procedure for preparing the sediment samples for ephippium analysis followed the standard methods described in Szeroczyńska and Sarmaja-Korjonen (2007) and Sarmaja-Korjonen (2003, 2004). Known quantities (1–5 g) of wet sediment subsamples were carefully sieved through a $51\text{-}\mu\text{m}$ mesh under running tap water; the residue in the sieve was concentrated by centrifuging it for 10 min at 4000 rpm and afterwards dyed with a few drops of glycerol-safranine. A known volume of the samples were then mounted on microscopic slides and analysed for chydorid carapaces and ephippia under a light microscope with magnifications 100/200/400-times. A minimum of 100 chydorid carapaces and ephippia were enumerated from the samples and identified following Szeroczyńska and Sarmaja-Korjonen (2007). The intensity of the two reproduction modes in the community was determined by calculating the relative abundances of carapaces (parthenogenesis) and ephippia (gamogenesis) from the sum of total carapaces+ephippia (Sarmaja-Korjonen 2003, 2004). Unfortunately, ephippia of *Alona (A.) affinis* and *A. quadrangularis* are extremely difficult to separate from each another (Szeroczyńska and Sarmaja-Korjonen, 2007), thus they were here identified and clumped together as *A. affinis/quadrangularis* ephippia. Total abundances of chydorid carapaces and ephippia (per gram of sediment DM) were also determined. Then, for species-specific reproduction patterns, relative proportions of chydorid carapaces and ephippia of most common species through

the core were calculated separately, giving the relative share of species-specific sexual reproduction.

The sediment samples were further analysed for sub-fossil chironomids, prepared with standard methods (Brooks *et al.*, 2007) and analysed for species composition (Nevalainen and Luoto, 2012) following the identification guides of Wiederholm (1983) and Brooks *et al.* (2007). Indicator species of chironomids were used to infer the quality of benthic conditions via the benthic quality index (BQI) (Wiederholm, 1980). Profundal benthic macroinvertebrate communities, especially larvae of chironomids, have been often used in the ecological classification and biomonitoring of temperate lakes due to their high indicator value (Luoto, 2011). The BQI is based on a number of indicator taxa and it ranges from a score of one (eutrophic lakes with low benthic quality) to a score of five (oligotrophic lakes with high benthic quality).

Cluster analysis (unweighted pair-group average) was used to illustrate temporal changes in chydorid reproduction (proportions of chydorid carapaces and ephippia). Here, the Bray-Curtis similarity measure was chosen. Additionally, the sample clusters were further verified with the analysis of similarity (ANOSIM), which was run using Bray-Curtis similarity measure and 10,000 permutations. Spearman's rank correlation (r_s) and the level of statistical significance (P) were used to estimate relationships between proportions of ephippia of the most abundant ephippial taxa and ecological quality of the lake (BQI and LOI).

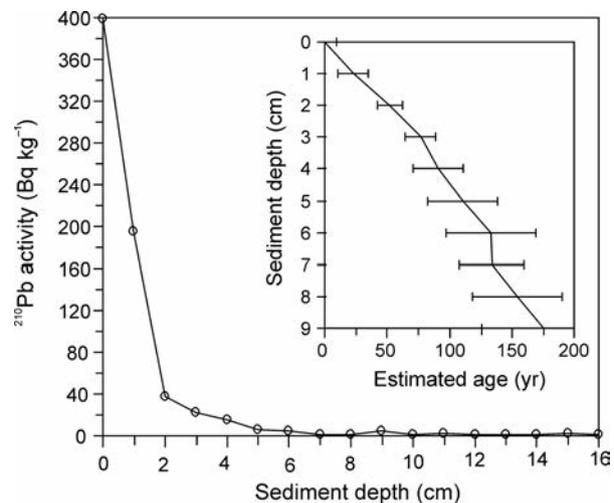


Fig. 2. Total specific ^{210}Pb activity and constant rate supply (CRS) age-depth model (right above corner) with error estimates of the sediment core from lake Oberer Landschitzsee.

RESULTS

The ^{210}Pb activity and constructed CRS age-depth model suggested that sediment section at 8 cm corresponded to ~1850 AD and horizons for 1900 AD and 1950 AD were estimated for 5 and 2 cm depths, respectively (Fig. 2). As the oldest ^{210}Pb -based age estimation was at 9 cm (~1830 AD), age estimations of the samples below this depth were determined through extrapolation and provided an age estimation of ~1700 AD for the bottom of the core (Fig. 3). As the extent of the cold climate period LIA in Central Europe and in the Alps is generally set to around mid- 19th century (Mangini *et al.*, 2005; Vincent *et al.*, 2005; Ivy-Ochs *et al.*, 2009), LIA is assumed to be represented by the lower part of the sediment sequence (16-8 cm), though the extrapolated age estimations of the lower core should be cautiously interpreted.

Cluster analysis separated the sediment assemblages into three main groups (cut-off value=0.6) (Fig. 3). Samples of 16-8 cm (until ~1850 AD), 7-1 cm (~1850-1950 AD), and 0 cm (most recent decades) were clustered on the basis of proportions of chydorid carapaces and ephippia and were further used in the zonal division of the stratigraphy (OL1-OL3) (Fig. 3). Significant differences between the sample groups were further tested with the ANOSIM, which resulted in mean ranked distances within and between groups of 33.68 and 93.62, respectively ($R=0.88$, $P<0.001$), and suggested significant dissimilarities amongst the sample groups. The *post-hoc*

pairwise comparison suggested that dissimilarities between the sample groups of 16-8 cm and 7-1 cm were significant ($P<0.001$). When only data of chydorid ephippia was used, significant mean ranked distances within and between groups were 77.37 and 56.21, respectively ($R=0.31$, $P=0.01$). The pairwise comparison here showed that groups of 16-8 cm and 7-1 cm were significant ($P=0.01$).

Carapaces of seven chydorid taxa were encountered in the core samples (Fig. 3). *Alona affinis* (Leydig) and *A. quadrangularis* (Müller) dominated the community until ~1850 AD (16-8 cm, OL1). Approximately in 1850-1900 AD (OL2), carapaces of *A. affinis* and *A. quadrangularis* markedly decreased and those of *Acroperus harpae* (Baird) increased. Simultaneously, *Alonella excisa* (Fischer) and *Chydorus (C.) sphaericus*-type (*sensu lato*) also increased. *Alona* species increased again in the most recent sediments and *Acroperus harpae*, *Alonella excisa*, and *C. sphaericus* decreased (OL3). Ephippia of all chydorid species (which were encountered as carapaces) were found, but those of *C. sphaericus*-type, *Alona guttata* (Sars), and *Alonella nana* (Baird) were very scarce through the core (Fig. 3). *Alona affinis/quadrangularis* ephippia were abundant (~10%) until 1900 AD and increased up to 20% between 1900 and 1950 AD. Ephippia of *Acroperus harpae* were constantly found in zone OL1 and they slightly increased around 1850-1950 AD (OL2). Nevertheless, they decreased again in the most recent sed-

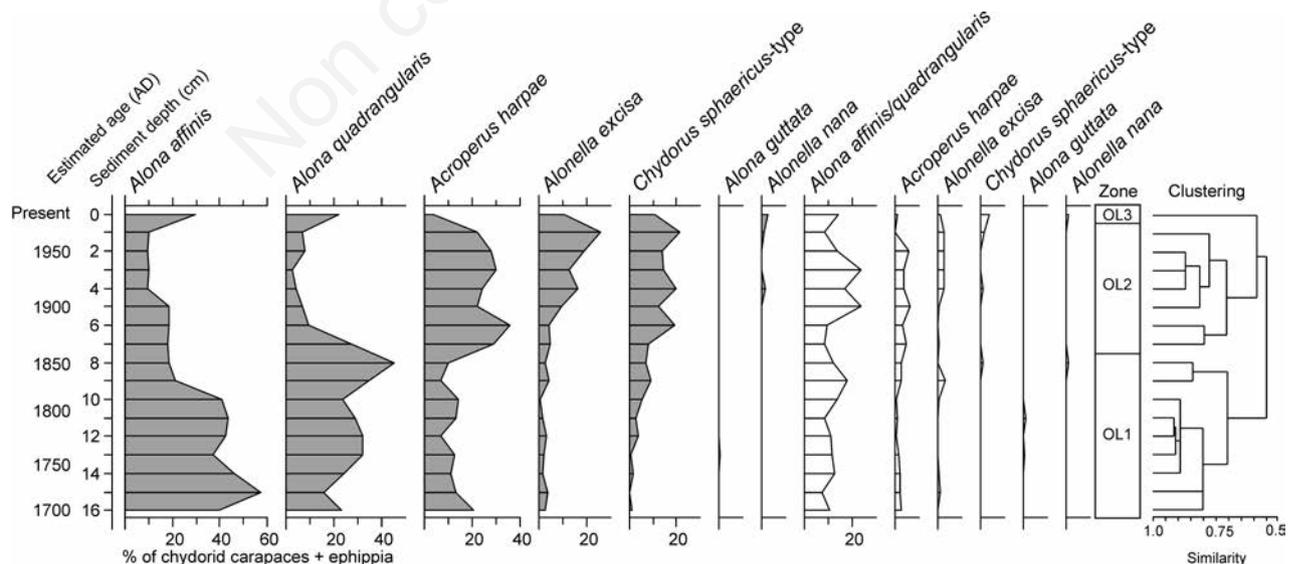


Fig. 3. Relative proportions of chydorid carapaces (gray silhouette) and ephippia (white silhouette) in the Oberer Landschitzsee core. Sample clusters (zones OL1-OL3) are based on unweighted pair-group averages and Bray-Curtis similarity measure. Age estimations of the upper core (0-9 cm) are based on the age-depth model (Fig. 2) and estimations of the lower core (10-16 cm) are based on the extrapolation of the ages from the upper sediment section.

iment layers (OL3). *Alonella excisa* ephippia occurred constantly in low proportions from ~1900 AD onward.

Chironomid-based BQI (Wiederholm, 1980) showed good benthic quality in the lower section of the core until ~1850 AD (16–8 cm, zone OL1) and decreasing quality after that until late 20th century (Figs. 3 and 4). The BQI slightly increased in the topmost sediment sample (OL3) (Figs. 3 and 4). The organic content of the sediment showed a general increasing trend from the bottom (~20%) to the top (~30%) of the core (Fig. 4). The total number of chydorid carapaces peaked in ~1850–1900 AD and decreased towards the core top. Total abundance of chydorid ephippia was highest (250–320 g⁻¹ DM) from 1850 until early 20th century (7–3 cm) but decreased toward the core top (Fig. 4). The relative proportion of total chydorid ephippia varied between 10 and 30% in the core, and the highest values observed were around ~1900 AD (Fig. 4). Proportions of ephippia were individually calculated for the species which had highest counts for carapaces/ephippia through the core: *A. affinis/quadrangularis* (14–128/8–30) and *Acroperus harpae* (4–74/0–9). Sexual reproduction of *A. affinis/quadrangularis* varied between ~10 and 25% during LIA but increased up to >60% between 1900 and 1950 AD (5–3 cm) and then decreased down to ~20% in the topmost sample (Fig. 4). Proportions of *Acroperus harpae* ephippia remained below 20% for most of the core but there was a peak at 9 cm (>25%) and no ephippia were recovered at 1 cm.

Tab. 1 illustrates the Spearman's correlations between BQI and LOI and sedimentary chydorid ephippia. Signif-

icant negative correlations were found between BQI and proportions of ephippia of *Acroperus harpae* and *Alonella excisa* (of the total community). Significant positive correlations existed between LOI and ephippia of *Acroperus harpae* (of the total community), and proportions of total chydorid ephippia (Tab. 1). Ephippia of *A. affinis/quadrangularis* (of the total community) had moderate correlations ($r_s = -0.30, 0.39$) with BQI and LOI, respectively, but these relationships were not statically significant. When using proportions of ephippia from the sum of carapaces+ephippia of individual species the relationships between BQI and LOI and *A. affinis/quadrangularis* ephippia were strong and significant ($r_s = -0.83, 0.74$; $P < 0.001$).

Tab. 1. Spearman's correlations between benthic quality index, organic matter and sedimentary chydorid ephippia (of the total community/species-specifically). Significant correlations are marked in italics.

	BQI	LOI
Total chydorid ephippia (%)	-0.44	<i>0.50*</i>
Total chydorid ephippia (No. g ⁻¹ DM)	-0.21	0.26
<i>Alona affinis/quadrangularis</i> ephippia (%)	-0.30/-0.83***	<i>0.39/0.74***</i>
<i>Acroperus harpae</i> ephippia (%)	-0.49*/-0.05	<i>0.52*/0.25</i>
<i>Alonella excisa</i> ephippia (%)	-0.59**/	0.39/

BQI, between benthic quality index; LOI, organic matter; DM, dry matter. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

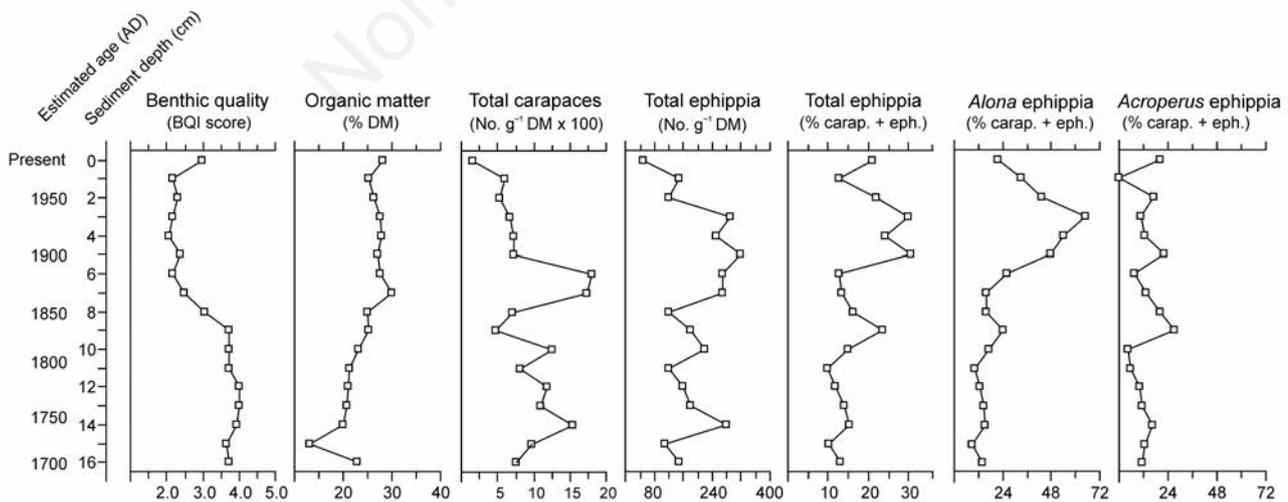


Fig. 4. Benthic quality index (BQI), sediment organic matter, total number of chydorid carapaces and ephippia, percentage of total chydorid ephippia, and proportions of ephippia of *Alona affinis/quadrangularis* and *Acroperus harpae* in the Oberer Landschitzsee core. Age estimations of the upper core (0–9 cm) are based on the age–depth model (Fig. 2) and estimations of the lower core (10–16 cm) are based on the extrapolation of the ages from the upper sediment section.

DISCUSSION

Little Ice Age: high benthic quality, until ~1850 AD

In the current study, the fossil chironomid assemblages and organic content of the sediment were utilised to infer long-term variability in the ecological status (*i.e.* benthic quality and productivity) of lake Oberer Landschitzsee. There exists a long tradition in using macrobenthic invertebrate communities as indicators for lake benthic quality (Brundin, 1949; Sæther, 1979; Wiederholm, 1980), and, accordingly, the chironomid-based BQI values showed that the benthic quality of lake Oberer Landschitzsee was good during the LIA (until ~1850 AD), but started to decline from 1850 AD onward and resulted in poor benthic quality at early 20th century (Fig. 4). In support of this, organic matter of the sediment showed corresponding trends as it was lower during the LIA than in the *post*-LIA section of the core (Fig. 4). As lake Oberer Landschitzsee is located remotely at high altitude in the Austrian Alps (Fig. 1) and, thus, not subjected to direct anthropogenic influence, its in-lake processes have likely been governed by direct atmospheric forcing. Accordingly, previous paleo-limnological and -climatological investigations on the lake indicated that warm climate periods in the past have been coupled with increased productivity, onset of stratification, and hypolimnetic oxygen deficit (Schmidt *et al.*, 2008). These processes are also closely related to Alpine land-use, which has extended at higher altitudes through periods of ameliorated climate. Thus, the long-term ecological quality of the lake has likely been driven by past climate variability and the high benthic quality and low productivity during the LIA until ~1850 AD may have been related to short open-water season under cold climate conditions (Nevalainen and Luoto, 2012).

The current results (proportions of chydorid carapaces) (Fig. 3) indicated that two benthic *Alona* species – *A. affinis* and *A. quadrangularis* – were abundant in lake Oberer Landschitzsee prior to ~1850 AD when the lake had good benthic quality (Fig. 4). This is in accordance with previous knowledge on their high-altitude distribution and ecological preferences in the Alps (Lotter *et al.*, 1997; Bigler *et al.*, 2006; Manca and Armiraglio, 2002; Kamenik *et al.*, 2007; Nevalainen *et al.*, 2011a). Some chydorid species, such as *A. affinis* and *A. quadrangularis*, can be defined as true bottom-dwellers inhabiting sediments down to the profundal zone (Fryer, 1968; Flössner, 2000), and thus, they are easily exposed to environmental conditions of their benthic habitats, *e.g.*, oxygen availability. Therefore, it is possible that the prevalence of the two *Alonas* chydorid in Oberer Landschitzsee was also indicative of their preference for good benthic conditions.

Post-Little Ice Age: declined benthic quality, ~1850 to present

The benthic quality of lake Oberer Landschitzsee started to decline from 1850 AD onward and resulted in

poor status during the early 20th century (Fig. 4). Correspondingly, organic matter of the sediment has increased from the LIA-section up to now (Fig. 4), which can be attributed to lake productivity (Willemsse and Törnqvist, 1999; Nesje and Dahl, 2001; Shuman, 2003). The documented *post*-LIA climate warming in Austrian Alps (Auer *et al.*, 2007) likely resulted in a longer open-water season causing higher production (increased LOI) (Fig. 4) and possibly reduced profundal oxygen availability due to lake stratification (decreased BQI) (Fig. 4) and increased organic matter (Nevalainen and Luoto, 2012). Following the declining trend in BQI and increasing trend in LOI (Fig. 4), the abundance of the two *Alona* species in the community declined severely (Fig. 3) and were replaced in the *post*-LIA period by *Acroperus harpae*, *Alonella excisa*, and *C. sphaericus*-type, thus giving evidence for a recent and dramatic community shift in chydorids. This change was further supported by the cluster analysis (Fig. 3) and ANOSIM results that suggested a period of change in assemblages (~1850 AD). The occurrence of *Acroperus harpae* is usually related to the presence of aquatic vegetation (Fryer, 1968), though it is very frequent in cold lakes across Europe (Brancelj *et al.*, 2009). Hence, its increase in lake Oberer Landschitzsee may be related to higher productivity and development of some aquatic macrophytes (Nevalainen and Luoto, 2012). Additionally, although recognised as counterparts in alpine lakes (Manca and Armiraglio, 2002; Catalan *et al.*, 2009; Nevalainen *et al.*, 2011a), the success of *C. sphaericus* and *Alonella excisa* may be associated to *post*-LIA climate warming and increased productivity. *C. sphaericus* is known to thrive under nutrient enrichment (Guilizzoni *et al.*, 2012) and *Alonella excisa* is reportedly associated to milder and more productive conditions (Manca and Armiraglio, 2002; Catalan *et al.*, 2009), which is in accordance with BQI and LOI developments (Fig. 4).

Even though diapause in aquatic invertebrates – including cladocerans – has generally received much attention and is fairly well understood (Aleksseev *et al.*, 2007), Frey's (1982) assumption that induction and control factors of gamogenesis are only partially comprehended, pertains even today. The environmental control of gamogenesis and diapause in cladocerans has been investigated since the early 20th century (*e.g.*, Banta, 1925; Banta and Brown, 1929). Subsequently, induction of gamogenesis and formation of diapausing resting eggs in *Daphnia* were shown to be mainly controlled by climatic factors, such as water temperature and photoperiod length, but also by some density-related factors, such as starvation (Stross and Hill, 1965, 1968; Stross, 1969a, 1969b). More recently, many stressors other than climate have been shown to easily and quickly interact with the induction of gamogenesis, as the influences of food limitation, crowding and pressure from fish predation (Hobæk and

Larsson, 1990; Larsson, 1991; Kleiven *et al.*, 1992; Ślusarczyk, 1995, 2001; Pijanowska and Stolpe, 1996). Much less attention has been paid to diapause in Chydoridae. However, fragmental studies on natural populations (Kubersky, 1977, Koksvik, 1995, Nevalainen, 2008; Nevalainen and Sarmaja-Korjonen, 2008) and laboratory traits (Shan and Frey, 1968; Shan, 1974) suggest that multiple environmental stressors control the induction, timing, and intensity of gamogenesis and dormant stage.

The paleolimnological approach has offered some valuable information on the patterns and the environmental control of chydorid gamogenesis through the use of total chydorid ephippia (TCE), *i.e.* the relative proportion of chydorid ephippia from the sum of chydorid carapaces+ephippia in lake sediments (Sarmaja-Korjonen, 2003, 2004). In general, TCE has been interpreted to be related to climate variables, such as length of the open-water season (Sarmaja-Korjonen, 2007; Sarmaja-Korjonen and Seppä, 2007; Luoto *et al.*, 2008) and summer temperatures (Kultti *et al.*, 2011; Nevalainen *et al.*, 2012a). Precisely, it has been assumed to be higher during short open-water season and cold summers. This interpretation is based on the relative length and intensity of both parthenogenetic and gamogenetic reproduction periods because under harsh climate conditions the relative importance of gamogenesis increases and that of parthenogenesis decreases. However, TCE can greatly vary due to increases in ephippia of individual species and, thus, its use in paleoclimatic implications is hampered by other environmental stimuli, *e.g.* pollution, which may affect gamogenesis of specific species (Nevalainen *et al.*, 2011b, 2011c). The current results from lake Oberer Landschitzsee show that TCE remained relatively stable during LIA (10-15%), but exhibited two peaks: ~1850 AD (20%) and ~1900-1950 AD (~25-30%), which were mainly driven by increased ephippia of *A. affinis/quadrangularis* (Fig. 3). Thus, it can be suggested that the increased TCE in the *post*-LIA section of the Oberer Landschitzsee core was impacted by a species-specific response to environmental stressor not attributable to decreased open-water season or summer temperatures, such as benthic quality (Fig. 4; Tab. 1), because general *post*-1850 AD trends in regional temperatures stand for climate warming (Auer *et al.*, 2007). Cluster analysis identified a significant threshold in proportions of carapaces and ephippia at around 1850 AD that was further evidenced by ANOSIM, which indicated significant differences in assemblages of chydorid ephippia between zones OL1 and OL2 (16-8 and 7-1 cm) (Fig. 3).

In addition to ephippia of *A. affinis/quadrangularis*, those of *Acroperus harpae* and *Alonella excisa* increased in the *post*-LIA period (Fig. 3) and correlated with the ecological quality of lake Oberer Landschitzsee (Tab. 1). However, as *Acroperus harpae* and *Alonella excisa* in-

creased in the community (as indicated by their carapaces), we can assume that the possibility of encountering their ephippia also increased and, accordingly, their ephippia became more abundant in the core. This development strongly contradicts the abundance of carapaces of the two *Alonas* as they dramatically decreased from 1850 AD onward, but yet their ephippia increased. Thus, apparently, *A. affinis* and *A. quadrangularis* were most impacted by the environmental change. Therefore, the current results suggest that the sexual reproduction of the two declining *Alona* species exhibited an increase in the *post*-LIA sediment section around 1900-1950 AD, which was indicated by a markedly increased proportion (from 10-20 up to 60%) of their ephippia (Fig. 4). This change strongly corresponded with the period of declined benthic quality and increased organic matter in the sediment (Fig. 4, Tab. 1). This implies that the increased productivity and deteriorated benthic quality in lake Oberer Landschitzsee may have resulted in intensified sexual reproduction of the two *Alona* species. As diapause is used as a mechanism to avoid unfavourable environmental conditions (Frey 1982), low benthic quality (*e.g.*, low oxygen availability in summer) might result in intensified gamogenesis. This in turn results in high abundance of diapausing resting eggs since oxygen availability is crucial for the survival of benthic animals. Accordingly, the current results appear to support our hypothesis that a population with low probability for survival as active individuals through a period of unfavourable environmental conditions reproduce with high sexual intensity in order to lay a considerable amount of dormant resting eggs.

Clustering of samples showed that the most recent assemblage (zone OL3) of chydorid carapaces and ephippia diverged from previous samples (Fig. 3) and this divergence was supported by ANOSIM, although the pairwise comparison between the sample groups 7-1 cm and 0 cm did not result in significant differences. The topmost sediment sample suggested that the community bounced back toward LIA conditions and that ephippia of *A. affinis/quadrangularis* decreased to values comparable to those of the LIA period (Figs. 3 and 4). The community and reproductive shift occurred again alongside with improved benthic quality (Figs. 3 and 4; Tab. 1). Thus, it is possible that the late 20th century stepwise and high-magnitude increase in temperatures, which is documented in a regional instrumental temperature series (Auer *et al.*, 2007), caused a breakage of summer stratification, thus improving benthic conditions (Nevalainen and Luoto, 2012) and, accordingly, the observed community and reproduction rebound. Currently, the water column of lake Oberer Landschitzsee during summer time is not strongly stratified and the hypolimnion is well oxygenated (see Methods section above).

Since chydorid gamogenesis has not been studied intensively, earlier documentations on gamogenetic behav-

four of individual chydorid species are almost non-existing. The paleolimnological records from Finland have however caught up signals of increased gamogenesis in *A. affinis* during periods of environmental stress related to altered limnological conditions and food-web changes (Sarmaja-Korjonen, 2003; Nevalainen *et al.*, 2011c). In addition, increased gamogenesis of *Alonella nana* has been detected from sedimentary archives and has been related to eutrophication (Nevalainen *et al.*, 2011b). On the basis of the current results, it seems appropriate to suggest that benthic *A. affinis* and *A. quadrangularis* may intensify their gamogenesis to guarantee successful diapause and/or increase genetic variability for survival under altered limnological ranges. As the intensity of gamogenesis may vary considerably among populations of *Daphnia* (Cáceres and Tessier, 2004) and chydorids (Frey, 1982; Nevalainen and Sarmaja-Korjonen, 2008), it is likely that the archive of subfossil ephippia in lake Oberer Landschitzsee is indicative of an intensified sexual reproduction of local populations to meet and adapt to new environmental conditions.

CONCLUSIONS

The present results show that paleolimnological records of chydorid microfossils may be utilised to seek answers to particular ecological phenomena. The results also imply that subfossil chydorid assemblages and ephippia may give indications on the ecological quality of the lake ecosystems and, thus, may be used as additional proxies in future assessments of lake ecological status. Furthermore, some ecological phenomena, such as reproductive behaviour of cladocerans which are difficult and demanding to detect and quantify via the neolimnological approach, can be reliably distinguished from the paleolimnological archives. Thus, the paleolimnological approach should not be segregated from aquatic ecology, but rather seen as a promising tool for detecting long-term ecological trends for understanding general ecological phenomena.

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